INVESTIGATOR'S BROCHURE

Sponsor:	NeuroRx, Inc.
Product No.:	RLF-100
Other Names:	Vasoactive Intestinal Peptide (VIP) Aviptadil
Version No.:	1.0
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Replaces:	None

SPONSOR SIGNATURE PAGE

Sponsor Signatory:

Jonathan Javitt, M.D., MPH Chief Executive Officer NeuroRx, Inc.	**Date**

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1 SUMMARY

RLF-100 (Aviptadil) is a synthetic form of Vasoactive Intestinal Polypeptide (VIP), a ubiquitous, naturally synthesized human peptide with extensively documented anti-inflammatory, anti-cytokine cascade properties. Relief Therapeutics has been granted investigational new drug (IND) status in the US and Europe, along with Orphan Drug Designation for the use of Aviptadil in Acute Respiratory Distress Syndrome (ARDS), Acute Lung Injury (ALI), Pulmonary Fibrosis, and Sarcoidosis. In conjunction with phentolamine, Aviptadil has been marketed in Europe for 20 years by Evolan SE for the treatment of erectile dysfunction (ED) with no record of serious adverse events (SAE). At least five well-controlled studies have been conducted for the use of Aviptadil in chronic lung conditions. A phase 1 safety trial of Aviptadil was conducted in seven patients with ARDS, who had an expected 50% mortality rate. All seven patients demonstrated clinical benefit based on improved blood oxygenation. Six patients survived and were successfully withdrawn from advanced life support. One patient died when the family demanded that advanced life support be withdrawn. These results demonstrate in a small sample size that Aviptadil significantly reduced expected mortality in patients with ARDS (mortality reduction of $p<.01$ vs historical controls). We propose RLF-100 (Aviptadil) for the treatment of ARDS in the setting of corona virus 2019 (COVID-19) infection. RLF-100 is known to have potent anti-cytokine activity in numerous non-clinical models of ARDS and ALI and has shown early evidence of efficacy in preserving life in patients with ARDS.

2 INTRODUCTION

Acute Respiratory Distress Syndrome is a severe pulmonary parenchymal injury to most or all of both lungs. ARDS is the rapid onset of progressive malfunction of the lungs, especially with regard to the ability to take in oxygen, usually associated with the malfunction of other organs. The condition is associated with extensive lung inflammation and accumulation of fluid in the alveoli (air sacs) that leads to low oxygen levels in the lungs. ARDS is characterized by diffuse pulmonary microvascular injury resulting in increased permeability and, thus, non-cardiogenic pulmonary edema. ARDS is well-known to occur in patients with novel COVID-19 infection and is the primary cause of death in those who die of COVID-19 related complications with a mortality rate of 50% or higher. An American-European consensus conference in 1994 established criteria for ARDS, alveolar damage which results in altered pulmonary gas exchange. The criteria state that both ALI and ARDS are associated with an acute onset, bilateral infiltrates on chest X-ray, and low pulmonary artery wedge pressure, an indirect measure of the pressure in the left atrium of the heart and/or the absence of left atrial hypertension. This latter clinical feature indicates that the cause of the pulmonary edema is not cardiac-related. The one feature that distinguishes ARDS from ALI is a worsened state of oxygenation. This is seen in the PaO2/FIO2 ratio, which reflects the status of oxygenation. The PaO2/FIO2 ratio can be calculated from knowing the amount of oxygen a patient is breathing (FIO2) and the PaO2 value from an arterial blood gas. A normal PaO2/FIO2 ratio is 500 mmHg. In ALI, the PaO2/FIO2

ratio is <300 mmHg. In ARDS, the PaO2/FIO2 ratio is <200 mmHg. The need for increasing oxygen requirements with persistently low levels of oxygen saturation and arterial oxygen content on an arterial blood gas is a sign that ALI is progressing to ARDS. This is also known as "refractory hypoxemia," meaning that although increasing amounts of oxygen are provided, the level of hypoxemia continues or even worsens, due in part to the lung injury that occurs in ALI/ARDS.

ARDS is diagnosed based on signs and symptoms indicating progressively worsening respiratory functioning. All patients with ARDS have ALI, yet, as mentioned, differentiating when ALI has progressed to ARDS is often challenging. Using the clinical parameters of the definitions of ALI and ARDS, i.e., the PaO2/FIO2 ratio, assists clinicians to determine whether a patient has ALI or ARDS and to distinguish when ALI has progressed to ARDS. The pathologic hallmark of the disease is diffuse alveolar damage, vascular endothelium damage, and damage to the surfactant-producing type II cells which results in loss of the integrity of the alveolar-capillary barrier, transudation of protein-rich fluid across the barrier, pulmonary edema, and hypoxemia from intrapulmonary shunting. ARDS has a diversity of predisposing conditions. Prior to the outbreak of COVID-19, sepsis and the systemic inflammatory response syndrome were the most common predisposing factors associated with development of ARDS (up to 40% of cases). These conditions may result from the indirect toxic effects of neutrophil-derived inflammatory mediators in the lungs. ARDS is also well known to occur in the setting of a cytokine storm triggered by viral infections, such as Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and COVID-19.

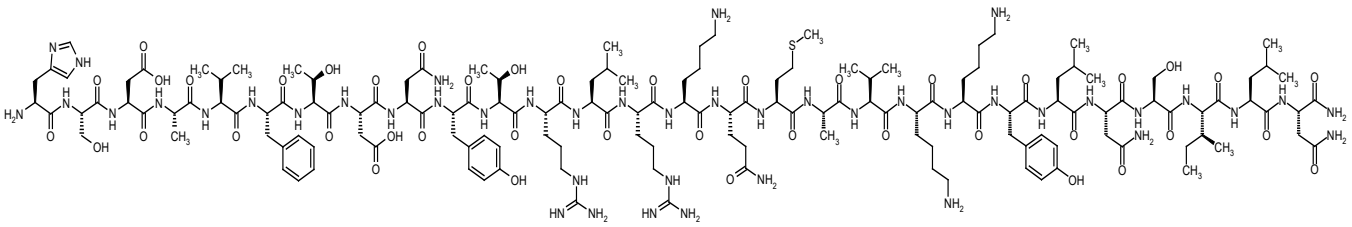
Frequently, ARDS develops in association with other organ dysfunction. ARDS was first described in 1967 as a syndrome of severe respiratory failure associated with pulmonary infiltrates, similar to infant hyaline membrane disease. ARDS occurs in children as well as adults. The condition originates from a number of insults involving damage to the alveolocapillary membrane with subsequent fluid accumulation within the airspaces of the lung. Histologically, these changes have been termed diffuse alveolar damage. ARDS is a medical emergency. Typically, patients require care in an intensive care unit (ICU). Symptoms usually develop within 24 to 48 hours of the original injury or illness. The mortality rate is approximately 50%. Deaths usually result from multisystem organ failure rather than lung failure alone.

3 PHYSICAL, CHEMICAL, PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 DRUG SUBSTANCE

Intern. nonproprietary name (INN):	Aviptadil
Other name:	VIP (Vasoactive Intestinal Peptide)

CAS registry number:	40077-57-4 (net)
Bachem product number:	4055854
Chemical structure	Aviptadil (VIP) is a linear, 28-AA peptide with an amidated C-terminus. All amino acids of Aviptadil are in L-configuration.



Abbreviated chemical name:	H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2
Molecular formula:	$C_{147}H_{238}N_{44}O_{42}S$ (net)
Molecular mass:	3325.8 g/mol (net)

3.2 DRUG PRODUCT

The drug product is a solution for infusion. The hospital research pharmacy will prepare a solution of 350 pmol/ml in sterile saline and tailor the infusion by body weight in achieve 12 hour infusions of 50, 100, or 150 pmol/kg/hour

4 NONCLINICAL STUDIES

4.1 NONCLINICAL PHARMACOLOGY

4.1.1 BRIEF SUMMARY

Aviptadil (VIP, vasoactive intestinal peptide) for injection is approved in combination with phentolamine for treatment of erectile dysfunction in the United Kingdom, Denmark and New Zealand. It is an endogenous 28 aminoacid peptide with a molecular weight of 3326 Dalton and has been recognized as a widely distributed neuropeptide, acting as a neurotransmitter in the heart, lung, thyroid gland, kidney, immune system, urinary tract and genital organs.

The physiological activities of Aviptadil include smooth muscle relaxation which leads to systemic vasodilation, increased cardiac output, bronchodilation, some differential effects on secretory processes in the gastrointestinal tract and gastric motility, hyperglycemia, inhibition of smooth muscle cell proliferation, hormonal regulation, analgesia, hyperthermia, neurotropic effects, learning and behaviour, and bone metabolism. VIP is also one of the signal molecules of the neuroendocrine-immune network inducing anti-proliferative, anti-inflammatory, and immunoregulatory features, especially in the lung where it is predominantly localized.

4.1.2 PRIMARY PHARMACODYNAMICS

4.1.2.1 Introduction

Aviptadil (VIP, vasoactive intestinal peptide) has been used in the United States and in European countries for more than 4 decades in controlled experiments involving humans and animals. Aviptadil for injection is approved in combination with phentolamine, and is sold under the brand name of Procivni for treatment of erectile dysfunction in the United Kingdom, Denmark and New Zealand. Pharmacology and toxicology of the endogenous peptide Aviptadil have been described since its discovery in the early 1970's in a large number of peer-reviewed publications.

Endogenous Aviptadil is a 28-amino acid peptide with a molecular weight of 3326 Dalton. Conformational analysis of Aviptadil by two-dimensional NMR and circular dichroism spectroscopy has shown an initial disordered N-terminus sequence of eight amino acid residues with two beta-turns, followed by two helical segments at residues 7-15 and 19-27 connected by a region of undefined structure that confers mobility to the peptide molecule. Endogenous Aviptadil is synthesized from a precursor molecule, which contains 170 amino acids and is processed to its biologically active form via a signal peptidase in the endoplasmic reticulum and finally cleaved by prohormone convertases and by carboxypeptidase-B like enzymes to Aviptadil.

Aviptadil was isolated from the intestine. Several years later Aviptadil was identified in the central and peripheral nervous system, and has since been recognized as a widely distributed neuropeptide, acting as a neurotransmitter or neuromodulator in many organs and tissues, including heart, lung, thyroid gland, kidney, immune system, urinary tract and genital organs. The anatomical distribution of 125I-labeled Aviptadil binding sites was studied in peripheral tissues by in vitro autoradiography in rats and guinea pigs. Dense binding occurred within the gastrointestinal, and genital tracts, in respiratory epithelium, smooth muscle of airway and blood vessels, and alveolar walls.

The widespread distribution of Aviptadil is correlated with its involvement in a wide variety of physiological activities including smooth muscle relaxation which leads to systemic vasodilation, increased cardiac output, bronchodilation, regulation of immune-response by tolerigenic activites on monocyte populations, some differential effects on secretory processes in the gastrointestinal tract and gastric motility, hyperglycemia, inhibition of smooth muscle cell proliferation, hormonal regulation, analgesia, hyperthermia, neurotropic effects, learning and behaviour, and bone metabolism.

Aviptadil acts via G-protein-coupled receptors VPAC1 and VPAC2 that are expressed on the cell membrane of normal and various malignant tissues. Regarding the lungs VPAC receptors were detected on airway epithelia, on macrophages surrounding the capillaries, and in the subintima of veins and arteries of the pulmonary circulation.

Circulating Aviptadil in the plasma of normal individuals (~ 40 pg/mL) mainly originates from nerve fibres in the gastrointestinal tract and also reflects peptide overflow from vascular nerves. With gastrointestinal stimulation, the plasma concentration of the peptide may more than double. Although plasma concentrations are low, Aviptadil released in tissues can produce a physiological effect without significantly increased plasma concentrations.

Single agent tolerability and efficacy have been demonstrated in asthmatic patients, patients suffering from pulmonary hypertension, acute lung injury, and pulmonary sarcoidosis. Despite of significant achievements in their management these diseases are still not curable with a poor prognosis for many of the affected patients. This constitutes a large unmet medical need to be challenged by Aviptadil.

The current and future program is designed to confirm the therapeutic benefit conveyed by Aviptadil in sarcoidosis, cystic fibrosis, chronic obstructive pulmonary disease, pulmonary arterial hypertension, chronic thromboembolic pulmonary hypertension, acute lung injury and chronic fatigue syndrome as a single therapeutic inhaled agent.

4.1.2.2 The nervous system

VIP release in the body is stimulated by high frequency (10-20 Hz) nerve stimulation and by cholinergic agonists, serotonin, dopaminergic agonists, prostaglandins and nerve growth factor. VIP has been localized to neuronal cell bodies, axons and dendrites, and presynaptic nerve terminals from which VIP is released as a neurotransmitter. In the peripheral nervous system, VIP is present on sympathetic ganglia, the vagus, some motor nerves that supply exocrine glands, vascular and nonvascular smooth muscles and ganglion-like clusters of neuronal cell bodies that provides intrinsic organ innervation. Many systemic blood vessels and also pulmonary blood vessels are innervated by VIP immunoreactive nerve fibers, which cause vascular smooth muscle dilation. In addition, VIP facilitates the secretory response to acetylcholine in glandular epithelium and is involved in the control of exocrine as well as endocrine secretion in the gastrointestinal, respiratory and urogenital tracts.

4.1.2.3 VIP receptors and function

VIP functions through specific receptors which belong to family B or group II of the G-protein-coupled receptors (GPCRs). So far, two receptors that display high affinity for VIP have been cloned and according to the established IUPHAR nomenclature are named as VPAC1 and VPAC2. These receptors share a common molecular architecture, made of seven transmembrane domains (7TM), three extracellular loops (EC1, EC2, and EC3), three intracellular loops (IC1, IC2, and IC3), a long amino-terminal extracellular domain and an intracellular carboxyl terminus. VPAC receptors couple to (1) stimulation of adenylyl cyclase triggering a protein kinase A (PKA)-cAMP transduction pathway, and (2) activation of phospholipase C (PLC) and phospholipase D (PLD). VPAC receptors induce responses by activating transduction systems

that involve different G-proteins, with Gα as the best characterized in different tissues and cell lines expressing recombinant receptors. Other G-proteins that have been shown to be coupled to VPAC receptors belong to Gi/Go and Gq families. Genes encoding VPAC receptors have been cloned from frog, fish, chicken, rat, mouse, and human. The human Vpr1 gene is located on region p22 of chromosome 3. The human Vpr2 gene is located in region q36.3 of chromosome 7. There are presently no reported isoforms for the Vpr1 or Vpr2 genes.

VIP binds to its receptor and dose-dependently activates adenylyl cyclase as demonstrated in various blood vessels. The degree of VIP induced activation of adenylyl cylase varies with the species and also the organ or tissue. The dose-dependent increase in adenylyl cyclase activity and cAMP concentration corresponds well with VIP's ability to produce vasodilation in isolated arteries. In cerebral microvessels, the effects of VIP on adenylyl cyclase activity are additive with the effects of isoproterenol, 2-chloroadenosine, and prostaglandin E1. This suggests compartmentalization of effects of VIP on adenylyl cylase activity, possibly involving different receptors, G-proteins, and adenylyl cyclase isoenzyms. In the rat mesenteric artery, VIP is 100 fold more potent than isoproterenol and prostaglandin E1 in enhancing adenylyl cylase activity.

After VIP binds to its receptor, the peptide is rapidly internalized, probably by receptor mediated endocytosis. This internalization decreases the cell surface receptor density. Most of the receptors are recycled back to the cell surface, but some receptors are degraded in lysosomes (Henning and Sawmiller, 2001) .

Mice lacking the vasoactive intestinal peptide gene develop pulmonary arterial hypertension

The deletion of the VIP gene in mice lead to spontaneous expression of moderately severe PAH during air breathing; The VIP knockout (VIP -/-) mice were examined for evidence of pulmonary arterial hypertension (PAH), right ventricular (RV) hypertrophy, and pulmonary vascular remodelling. Relative to wild-type (WT) control mice, VIP -/- mice showed: moderate RV hypertension; RV hypertrophy, confirmed by increased RV/ (LV + septum) weight ratio; and enlarged, thickened pulmonary arteries and smaller branches, with increased muscularization and narrowed lumen. Lung sections also showed perivascular inflammatory cell infiltrates. There was no systemic hypertension, and no arterial hypoxemia to explain the PAH. The condition was associated with lower body weight, hypothermia, and pro-inflammatory milieu resulting in increased mortality (Szema and Hamidi 2014). Both the vascular and RV remodeling were attenuated following 4-week treatment with VIP (Said et al., 2007).

The effects of Aviptadil on monocrotaline induced pulmonary hypertensive rabbits following caridiopulmonary bypass

This study was performed in male Japanese white rabbits model (10 rabbits received Aviptadil) of monocrotaline induced pulmonary hypertension. Aviptadil (10-5 M or 10-6 M) was perfused intra-arterially for 60 min. During infusion, heart rate increased and the increased heart rate was

maintained throughout the procedure. Mean arterial pressure increased significantly throughout the procedure. Pulmonary blood flow increased significantly. Pulmonary artery pressure and left atrium pressure decreased significantly. Pulmonary resistance decreased. Aviptadil has dose responsive, positive inotropic and pulmonary vasodilatory effects in whole body cardiopulmonary bypass rabbit model acting via calcium channels (Gunaydin et al., 2002). In rats, Aviptadil benefit on monocrotaline-induced pulmonary hypertension was enhanced by co-administration with bosentan, an endothelin (ET) receptor antagonist (Hamidi et al., 2011). More recently Koga et al, (2014) demonstrated that VPAC2 but not VPAC1 agonist could mimic VIP action on this rodent model. Currently, Phasebio Pharmaceuticals is developing Vasomera, alias PB1046, a long acting VIP analogue with preferential VPAC2 activity for patients with hypertension.

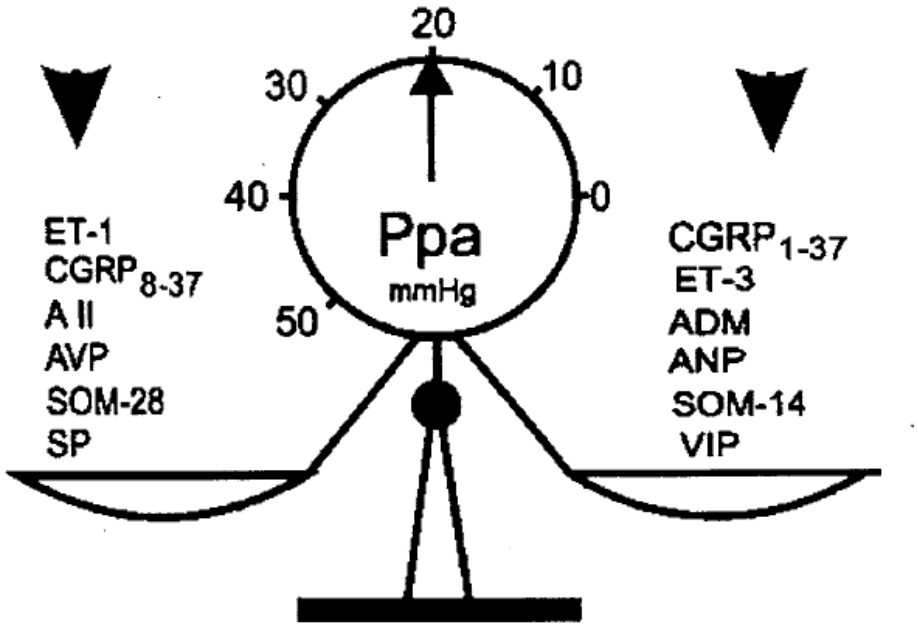
Downregulation of VIP and upregultion of the VIP receptor VPAC-1 in overcirculation-induced pulmonary hypertension in piglets

The expressions of VIP, the related peptide PACAP, and the receptors VPAC-1,VPAC-2 and PAC-1, in piglets with shunt induced pulmonary hypertension as an early-stage PAH model was investigated (Rondelet et al., 2003). Seventeen 3-week old piglets were included in the study, 9 with an anastomosis of the right subclavian artery to the pulmonary arterial trunk (modified Blalock Taussig operation), and 8 after a sham operation. After 3 months, the animals underwent a hemodynamic evaluation, and lung tissue was sampled for morphometric and biological studies. Three months on shunting increased pulmonary vascular resistance from 3.7 ± 0.4 to7.5 ± 0.5 mmHg x l-1 x min x m2) and increased small resistive arterioles medial thickness by 85%. The expressions of VIP and VPAC-1 were respectively decreased and increased (RTQ-PCR). The expression of VPAC-1 was positively correlated to PVR and to arteriolar medial thickness ($P<0.05$). There was a positive immunostaining for VPAC-2 and PAC-1 in pulmonary arterial smooth muscle cells and bronchial epithelial cells and a positive immunostaining for VIP and PACAP in nerve fibers around pulmonary arterial smooth muscle cells. These results are compatible with the notion that abnormal VIP signalling participates to early stage PAH pathobiology (Vuckovic et al., 2006).

Vasodilating activity of VIP

Vascular resistance in the mammalian pulmonary circulation is influenced by many endogenous agents (such as VIP), artrial natriuretc peptide (ANP), endothelin-3 (ET-3), calcitonin gene related peptide (CGRP), andrenomedullin (ADM), somatostatin-14 (SOM-14) on one side which reduce pulmonary pressure (Ppa) and endothelin 1 (ET-1), substance P (SP), arginine vasopressin (AVP), somatostatin-28 (SOM-28) or angiotensin II (A II) on the other side which increase pulmonary pressure. When the balance of these agents is disturbed pulmonary hypertension may occur as characterized by elevated pulmonary pressure (PPa) (see Figure 1), (Keith, 2000).

Figure 1: Balance of Endogenous Agents and Pulmonary Pressure



Petkov et al. (Petkov et al., 2003) demonstrated that VIP concentrations in the serum of patients having PPH were very low at the detection threshold (10 pg/ml). In addition, VIP could not be detected in nerve fibers in the median layer of pulmonary arteries of these patients. In contrast to VIP, the VIP receptors VPAC1 and VPAC2 were overexpressed in the lung tissue of PPH patients, indicating a counter regulation to compensate the reduced VIP levels. The findings of Petkov et al. (Petkov et al., 2003) are in agreement with the proposed model of (Keith, 2000),(Figure 5.1.2.3-1) that a lack of endogenous agents acting as vasodilators reducing pulmonary pressure as shown on the right side of the balance leads to a pathophysiological increase of PPa. The proposed model is also supported by the fact that an enhanced level of endogenous agents e.g. ET-1 having vasocontrictive properties as shown on the left side of the balance were also found in PPH patients (see Figure 5.1.2.3-1).

In vascular smooth muscle, a VIP-induced increase in cAMP concentration can activate protein kinase A, which phosphorylates phospholamban, and thereby increases the sequestration of Ca2+ by the sarcoplasmic reticulum. Cyclic AMP can also increase the activity of the sarcolemmal Ca2+ pump ATPase, thereby increasing the extrusion of Ca2+ into the extracellular space. In addition, cAMP decreases the affinity of myosin light chain kinase for the Ca2+ calmodulin complex, thereby reducing myosin phosphorylation and decreasing actin–myosin affinity. These processes when activated by VIP can produce smooth muscle relaxation and vasodilation. The vasodilatory effect of VIP in different vascular tissues or species is not solely due to an increase in cAMP. The vasorelaxant effect of VIP is dependent on the endothelium in the rat aorta, the bovine intrapulmonary artery, and the human uterine artery, and is mediated by activation of

lipoxygenase in the rat aorta and by nitric oxide and activation of guanylyl cyclase in the human uterine artery (Henning and Sawmiller, 2001).

In the bovine intrapulmonary artery, endothelial-dependent vasorelaxation in response to VIP involves activation of guanylyl cyclase and cyclooxygenase through two pathways, which are probably mediated by nitric oxide and prostacyclin. In this system cAMP and cGMP may interact synergistically to initiate and sustain the vasodilatory response to VIP. The vasorelaxant effect of VIP is independent of the endothelium in the feline middle cerebral artery, canine carotid artery, canine hepatic artery, porcine coronary artery and the rat portal vein. Moreover, the vasorelaxant effect of VIP in some species may ultimately involve hyperpolarization of the vascular smooth muscle membrane, which reduces calcium influx and the intracellular calcium concentration. The precise contributions of cAMP, cGMP, nitric oxide and other signalling agents to the vasodilation elicited by VIP in different vascular beds is not known. One possible mechanism for the interaction between these mediators of vasodilation is present in gastrointestinal smooth muscle, in which VIP elicits relaxation via activation of both cAMP and cGMP-dependent pathways. The increase in cAMP is due to activation of VPAC receptors, whereas the increase in nitric oxide may be due to activation of natriuretic peptide clearance receptors (NPR-C) coupled to a membrane-bound endothelial nitric oxide synthase. Whether a similar interaction exists in vascular smooth muscle or in the heart is presently not known and is under investigation. After VIP binds to its receptor, the peptide is rapidly internalized, probably by receptor mediated endocytosis. This internalization decreases the cell surface receptor density. Most of the receptors are recycled back to the cell surface, but some receptors are degraded in lysosomes (Henning and Sawmiller, 2001).

In the lung VIP is found in perivascular nerves and VIP receptors were predominately found in smooth muscles of pulmonary arteries and lung membranes. Consequently, VIP causes both bronchial and pulmonary artery vasodilation. Elevated levels of VIP were found in acutely hypoxic dogs having decreased PaO2 and increased PPA. Thus, VIP release both systemically and from the lung during hypoxia might be a compensatory regulatory response to elevated PPA (Keith, 2000). In cerebral microvessels, the effects of VIP on adenylyl cyclase activity are additive with the effects of isoproterenol, 2-chloroadenosine, and prostaglandin E1(Huang and Rorstad, 1983).

4.1.2.4 Inhibition of smooth muscle cell proliferation

Hyperproliferative pulmonary arterial smooth muscle cells and a thickened vessel wall are hallmarks of the pathology of PAH. VIP is in vitro able to affect the proliferation of pulmonary arterial smooth muscle cells (PASMC) and the mobilization of intracellular free calcium concentration in these cells. VIP inhibits basal proliferation of PASMC from PAH patients. This inhibitory effect is dose-dependent (Petkov et al., 2003). Further studies suggested that VIP suppresses vascular smooth muscle cell proliferation primarily by reducing intracellular Ca2+ via activation of the cAMP/protein kinase A (PKA) pathway (St Hilaire et al., 2009) and by

inhibiting NFATc3, a transcription factor linked to pulmonary arterial smooth muscle (PASMC) hyperplasia and hypertrophy in chronic hypoxia-induced PH (Szema et al.,2017).

4.1.2.5 Inhibition of endothelin (ET-1) receptor expression

Preproendothelin, endothelin receptors and endothelin converting enzyme have been shown to be upregulated followed by hypoxia for 60 min in a hypoxic pulmonary hypertension animal model using fawn-hooded rats. VIP attenuates the upregulation of endothelin receptors A and B whereas endothelin expression and endothelin converting enzyme remain unchanged. As a consequence endothelin can not target its receptors and signal transduction does not occur. Moreover, endothelin induced contraction of pulmonary artery in guinea pigs could be attenuated by VIP indicating that VIP directly inhibits endothelin signalling pathway (Sami Said, personal communication). Indeed, VIP attenuated endothelin-induced NFATc3 nuclear translocation in cultured human PASMC (Szema et al., 2017). Interestingly, combining VIP treatment with an endothelin receptor antagonist treatment gives a very effective combination against monocrotaline-induced pulmonary arterial hypertension in rats, suggesting that VIP alone is not sufficient to fully sppress endothelin signaling pathway (Hamidi et al., 2011).

4.1.2.6 Inhibition of myofibroblast proliferation

Proliferation of primary cultures of human colonic myofibroblasts can be induced by platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor, interleukin-1beta (IL-1β), and tumor-necrosis-factor alpha (TNFα). VIP elevates cAMP in these cells and inhibited proliferation induced by platelet-derived growth factor in a dose-dependent manner (Jobson et al., 1998).)

4.1.2.7 Attenuation of matrix metalloproteinase 9 expression

VIP has been shown to attenuate lipopolisaccharide (LPS)-induced MMP-9 expression by alveolar macrophages (AM) in rats. The MMP-9 activity and expression of LPS-induced AMs were significantly higher than those in the control group. VIP (10-9 to approximately 10-6 mol/L) down-regulated LPS-induced MMP-9 activity and its expression (Liu et al., 2005). Moreover, exposure of L2 cells to CSE at a concentration of 0.25% resulted in a 50% increase of caspase-3 and matrix metalloproteinase (MMP) activities. Specific inhibitors for caspases and MMPs attenuated the cytotoxicity of CSE. VIP at concentrations higher than 10–13 M, produced a concentration-dependent inhibition of CSE-induced cell death in L2 cells. VIP, at 10–7 M, reduced CSE-stimulated MMP activity and caspase-3 activation (Onoue et al., 2004).

4.1.2.8 Increase in nitric oxide synthase expression

Nitric oxide is a potent endothelium-derived vasorelaxant substance and an inhibitor of smooth-muscle-cell growth. Nitric oxide is produced in various cell types by the action of an enzyme, nitric oxide synthase. The expression of endothelial nitric oxide synthase in the lungs of control

subjects with that in the lungs of patients with pulmonary hypertension was compared. In the lungs of the control subjects, nitric oxide synthase was expressed at a high level in the vascular endothelium of all types of vessels and in the pulmonary epithelium. In contrast, little or no expression of the enzyme was found in the vascular endothelium of pulmonary arteries with severe histologic abnormalities (i.e., plexiform lesions) in patients with pulmonary hypertension. The intensity of the enzyme immunoreactivity correlated inversely with the severity of histologic changes. There was an inverse correlation between the arterial expression of the enzyme and total pulmonary resistance in patients with plexogenic pulmonary arteriopathy ($r = -0.766$, $P = 0.004$), (Giaid and Saleh, 1995).

Nitric oxide synthase expression in endothelial cells prepared from pulmonary arteries of control subjects after 96 hours of incubation. Cells were incubated with VIP (10-7 M) under normoxic and hypoxic conditions for various times. Western blots reveal constitutive expression under normoxic conditions without VIP. Under hypoxic conditions in the absence of Aviptadil the expression of Nitric oxide synthase is completely downregulated. In contrary, the addition of Aviptadil leads to increased expression of Nitric oxide synthase above constitutional level both under normoxic and hypoxic condition (Patent No. PCT/EP01/13590).

Anti-inflammatory / Immunomodulatory activities of VIP

VIP belongs to the VIP/secretin/glucagon family of peptides. VIP is present and released from both innervation and immune cells, particularly Th2 cells, and exerts a wide spectrum of immunological functions controlling the homeostasis of immune system through different receptors expressed in various immunocompetent cells (Figure 2), adapted from Ganea et al., 2015). VIP has a general anti-inflammatory effect, both in innate and adaptive immunity. In innate immunity, VIP inhibits the production of pro-inflammatory cytokines and chemokines from macrophages, microglia and dendritic cells. Furthermore, VIP reduces the expression of co-stimulatory molecules (particularly CD80 and CD86) on antigen-presenting cells, and therefore reduces stimulation of antigen-specific CD4(+) T cells. In terms of adaptive immunity, VIP promotes Th2-type responses, and reduces the pro-inflammatory Th1-type responses. The molecular mechanisms involved result in the inhibition of cytokine and chemokine expression, and in the preferential development and/or survival of Th2 effectors. Therefore, VIP has been proposed as very promising candidate for treating acute and chronic inflammatory and autoimmune diseases (Gonzalez-Rey et al., 2007, Ganea et al., 2015).

Figure 2: VIP effects on innate and adaptive immunity (Ganea et al., 2015)

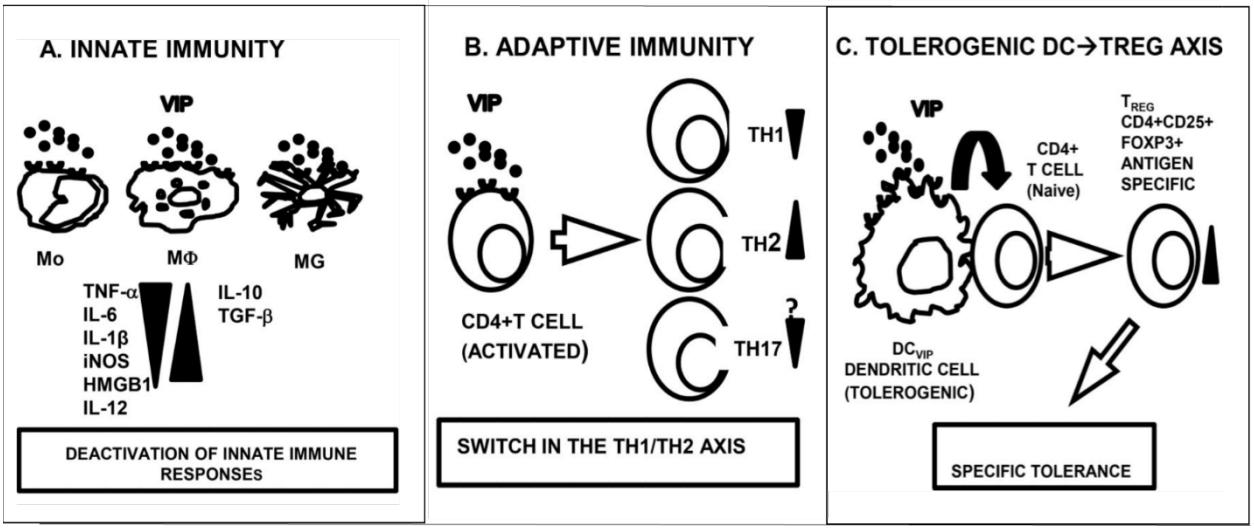


Figure 4.2: A. VIP inhibits production of pro-inflammatory and promotes production of anti-inflammatory factors in activated innate immune cells; B. VIP inhibits Th1 and promotes Th2 differentiation. Inhibitory effects on Th17 were observed in vivo but not in vitro. C. Bone marrow DC generated in the presence of VIP (DCVIP) are tolerogenic and induce Ag-specific Treg.

The role of VIP as an immunotolerogenic agent has been supported by direct and indirect evidences. In vivo administration of Aviptadil together with specific antigens to T-cell receptors results in the expansion of CD4+/CD25+ Treg cells (Delgado et al., 2005a, b). Similarly, DC transduced with lentiviral vectors expressing VIP differentiate into VIP-secreting tolerogenic-like DCs (tDC), that once inoculated could alleviate proinflammatory cytokine secretion and increase interleukin-10 (IL-10) production in animal models of experimental autoimmune encephalomyelitis (EAE) and cecal ligation and puncture (CLP) sepsis (Toscano et al., 2010). Conversely, the use of VIP-receptors antagonist in cytomegalovirus-infected mice, resulted in enhanced cellular antiviral immunity with increased levels of type-I cytokines such as IFN-g and TNF-a (Li et al., 2013). VIP plays a major role in the maternal tolerogenic response to the embryo, especially during the early trophoblast stage (Fraccaroli et al., 2009; Fraccaroli et al., 2012).

VIP is also involved in the maintenance of the bronchial system, as VIP knock-out mice display airway hyper-responsiveness to the cholinergic agonist methacholine, as well as peribronchial and perivascular inflammation, which is partially reversible by exogenous Aviptadil (Szema et al., 2006). Aviptadil inhalation also improves clinical respiratory outcome in ovalbumin-induced asthma in mice. This benefit is accompanied by a decrease of IL-17 and IL-10 levels in the bronchoalveolar lavage fluid (BALF), suggesting that VIP can improve airway inflammation by regulating the Th17/Treg imbalance in asthmatic mice (Ke et al., 2017). This modulation of IL-17 expression by macrophages leading to Th17 switch has already been reported in BALF from

acute lung injury (ALI) murine model (Ran et al., 2015). Of note, exaggerated IL-17 release is a pivotal feature of sarcoid pathogenesis.

These set of data comfort those obtained on VIP deficient mice, unravelling the role of VIP not only on inflammatory cells but also on Treg (Szema et al., 2011). Furthermore they provide a mechanistic explanation of the anti-asthma effects published by Barnes and Dixon in 1984 on six atopic patients. Overall these observations support a potential benefit of Aviptadil in the treatment of pulmonary dysfunctions and inflammation characterizing the Pulmonary Sarcoidosis condition.

4.1.3 SECONDARY PHARMACODYNAMICS

4.1.3.1 Inhibition of TNF- α secretion

An important role of TNF-α in interstitial fibrosis has been established using transgenic mice, which either overexpress or display a deficiency of this cytokine. Mice transgenically modified to overexpress TNF-α develop lung fibrosis. In contrast, null for TNF-α show marked resistance to bleomycin induced fibrosis (Allen and Spiteri, 2002; Lasky and Brody, 2000). TNF-α can stimulate fibroblast replication and collagen synthesis in vitro, and pulmonary TNF-α gene expression rises after administration of bleomycin in mice. Soluble TNF-α receptors reduce lung fibrosis in murine models and pulmonary overexpression of TNF-α in transgenic mice is characterized by lung fibrosis. In patients with CFA or asbestosis, bronchoalveolar lavage fluid-derived macrophages release increased amounts of TNF-α compared with controls.

VIP has been shown in a variety of publications to inhibit the expression of TNF-α by immune cells and consequently to potentially inhibit fibrosis and collagen synthesis. In detail, VIP protects mice from endotoxic shock presumably through the inhibition of TNF and IL-6 production. This study was performed in a high endotoxemia murine model (male BALB/c mice), with different Aviptadil dosages (0-10 nmol/animal injected intraperitoneally, 36 mice), monitoring the survival over 3-7 days. Under toxicity conditions associated with high bacterial LPS doses, Aviptadil could act as protective mediator that regulates the excessive release of TNF-α and IL-6 to reduce inflammation or shock, suggesting treatment of human septic shock syndrome (Beutler and Cerami, 1989). VIP was also reported to suppress TNF production in human peripheral blood cells (Dewit et al., 1998; Hernanz et al., 1996). Furthermore VIP inhibits LPS-induced TNF production in Raw 264.7 murine macrophages. The inhibitory effect is dose-dependent within a wide range of neuropeptide concentrations (10-7-10-11 M), with the maximum effect being observed at 10-8 M (Delgado et al., 1998).

4.1.3.2 Inhibition of TGF-b1 Production

Aviptadil does not affect the baseline TGF-b1 production by unstimulated macrophages, but reduces dramatically TGF-b1 production by LPS-stimulated macrophages. The effects are

mediated through the specific receptors VPAC1, VPAC2, and PAC1. The effect of VIP is mediated primarily through the cAMP pathway. VIP reduces the TGF-b1 steady-state mRNA levels in both peritoneal macrophages and Raw 264.7 cells treated with LPS. A similar effect is observed upon the in vivo administration of VIP. This report adds VIP together with PACAP to the only other neuropeptide, substance P, known to regulate TGF-b1 production in immune cells (Sun et al., 2000).

4.1.3.3 Prevention of experimental arthritis by down-regulating both autoimmune and inflammatory components of the disease

This study was performed in a murine model of collagen-induced arthritis (immunization with type II collagen of male DBA/1J mice) resembling immunologic features of human rheumatoid arthritis. Aviptadil was administered at different dosages (1-10 nmol/animal) daily or on alternate days for up to 30 days intraperitoneally. The therapeutic effect of Aviptadil decreased the incidence and severity of collagen-induced arthritis, and showed down-regulation of both, inflammatory and autoimmune components of the disease, indicating Aviptadil as a viable candidate for the development of treatments for rheumatoid arthritis in humans (Delgado et al., 2001).

4.1.3.4 Bronchodilating effects

Aviptadil inhibits basal and histamine-stimulated proliferation of human airway smooth muscle (ASM) cells. ASM cell proliferation contributes to increased airway resistance in bronchial asthma. The modulation of ASM proliferation by Aviptadil was examined. Human ASM cells were grown in culture as a monolayer. Aviptadil (10-9M – 10-6M) inhibited proliferation in a dose-dependent manner by up to 82% on day 2, but the related peptide glucagon had no effect. Histamine (100 nM-100 μM) increased cell counts by 66%, but in the presence of Aviptadil, cell counts and [3H]thymidine incorporation were reduced by up to 55%. KT-5720 (10-9M – 10-6M), a selective inhibitor of cAMP-dependent protein kinase A (PKA), abolished the inhibitory effect of Aviptadil. The results show that Aviptadil selectively and potently inhibits human ASM cell growth and multiplication, and nullifies the mitogenic effect of histamine, by a PKA-mediated mechanism. A deficiency of Aviptadil may lead to ASM hyperplasia due to unopposed stimulation by endogenous mitogens (Maruno et al., 1995).

A study was performed in 18 cats of both sexes to examine acute pharmacologic effects of Aviptadil in airways, administered intravenously or by inhalation. Aviptadil (0.1-10 mg/kg) was administered intravenously in a bolus injection, or 10-100 mg/ml Aviptadil in aerosol formulation. Intravenously applied Aviptadil produced dose-dependent reversal of induced brochoconstriction, whereas inhaled Aviptadil did not. The Aviptadil-mediated bronchodilatation occurs independent of prostaglandin production or beta adrenergic receptor activation (Diamond et al., 1983).

A study was performed in 13 mongrel dogs via infusion of Aviptadil (10 or 100 pmol) into the bronchial artery. Bronchial artery flow increased over 20 – 30 s and then levelled off to a stable plateau. Bronchial vascular conductance increased significantly during infusion. No significant effect on systemic arterial pressure was observed (Pisarri et al., 1999).

A study was performed to detect effects of inhaled Aviptadil against bronchoconstriction induced by inhaled histamine. Ten guinea pigs were investigated. Animals were treated with 120 mg inhaled Aviptadil and 100 mg aerosolized histamine. Inhaled Aviptadil provided substantial protection against histamine-induced bronchoconstriction in awake, spontaneously breathing guinea pig (Cox et al., 1983).

Three guinea pigs were treated with inhaled Aviptadil (0.1 mM, 20 breaths). The increase in total pulmonary resistance caused by histamine was significantly inhibited. No effects on heart rate or blood pressure were observed (Linden et al., 1998).

In this study 30 guinea pigs received intravenous Aviptadil (1, 3 and 10 µg/kg bodyweight) after a histamine challenge. Administration of Aviptadil resulted in a rapid inhibition of increased respiratory resistance

(1 min). Respiratory resistance returned to control levels at 15 min. (Saguchi et al., 1997).

4.1.3.5 Protective effects in acute respiratory distress syndrome animal models

A study was performed in the rat model of acute, diffuse lung injury. Anesthetized Sprague-Dawley rats received for 60 min. 0.2 M HCl and 1 mg/kg/min Aviptadil. Aviptadil protects against HCl-induced pulmonary edema, pulmonary hypertension, and increased airway pressure in perfused rat lungs, suggesting the usefulness of Aviptadil in the prevention and management of acute, diffuse lung injury in humans (Foda et al., 1988).

Aviptadil has been shown to inhibit NF-kB activation in the following animal models of acute lung injury, while preventing or attenuating the injury:

1) In the rat model of NMDA-induced acute lung injury in perfused lungs, in the presence of L-arginine, the acute condition is manifested in high-permeability pulmonary edema by sharp increases in the peak airway pressure, as well as by the leakage of protein into the airspaces. In this model, Aviptadil inhibited NF-kB activation and totally prevented all manifestations of NMDA injury (Said et al., 1996; Said and Dickman, 2000).

2) In the rat model of xantine/xanthine oxidase-induced acute lung injury in perfused lungs, the acute condition is manifested by increase in the peak airway pressure and strong increase of arachidonic acid metabolites thromboxane B2, and 6-ketoprostaglandin. In this model, Aviptadil inhibited NF-kB activation and greatly reduced or totally abolished the increased production of cyclooxygenase metabolites provoked by the oxidant injury (Said and Dickman, 2000; Berisha et

al., 1990). In addition, Aviptadil is reported to be a singlet oxygen quencher (Misra and Misra, 1990), providing direct confirmation of its antioxidant activity.

3) In the guinea pig model of perfused lungs, paraquat (methyl violagen) was used to induce acute lung injury. Ingestion of the herbicide paraquat leads to acute high-permeability pulmonary edema due to endothelial and epithelial damage. The injury is mediated by the generation of several reactive oxygen radicals, leading to membrane lipid peroxidation. In this model, Aviptadil inhibited NF-kB activation and effectively reduced this acute lung injury due to infused paraquat (Pakbaz et al., 1993; Said and Dickman, 2000).

In addition to the above described models of acute lung injury with experimental evidence for modulation of NF-kB activity by Aviptadil, there are additional lung injury models, where the injury was either reduced or completely prevented by treatment with Aviptadil:

Sheep animal model of acute lung injury by intravenous infusion of platelet-activating factor – Aviptadil protects unanesthetized sheep against pulmonary and systemic effects of platelet-activating factor (Abstract, Pakbaz et al., 1988).

Dog animal model of acute lung injury by intravenous infusion of platelet-activating factor – Aviptadil protects anesthetized dogs against pulmonary and systemic effects of platelet-activating factor (Abstract, Pakbaz et al., 1988).

Guinea pig model of acute lung injury by phospholipase C as this enzyme activates proinflammatory pathways – Aviptadil attenuates the phospholipase C-induced lung injury (Abstract, Pakbaz et al., 1991).

Rat animal model of acute lung injury by intravenous infusion of cobra venom factor (Mulligan et al., 1992), which leads to systemic hypotension followed by a neutrophil- and free radical-dependent hemorrhagic that mimics septic shock-induced injury – Aviptadil attenuates this septic shock-induced injury (Said and Dickman, 2000).

Guinea pig model of acute lung anaphylaxis with animals being sensitized immunologically with ovalbumin and challenged afterwards intra-tracheally again with ovalbumin – Aviptadil protects against the increased airway pressure and the release of eicosanoids triggered by anaphylaxis in lungs (Abstract, Takamatsu et al., 1991).

Sepsis is the most common predisposing condition that precipitates ALI/ARDS (Matthay et al., 2003). Therefore, evidence for medical plausibility of administering Aviptadil in animal models of sepsis is presented.

Mice were injected intraperitoneally with lethal doses of Salmonella enteridis derived LPS in absence or presence of Aviptadil – Aviptadil protects the mice from high-dose endotoxin shock.

The protective effect seems to be associated with decreased expression of TNF-a and IL-6 (Delgado et al., 1999).

Rats were injected intraperitoneally with Escherichia coli derived LPS in absence or presence of Aviptadil – Aviptadil increased the survival rate of septic rats by reduced production of superoxide anions, enhanced inactivation of histamine, inhibition of mast cell degranulation, and regulation of mediator content of mast cells (Tuncel et al., 2000).

In another study, mice were treated with trinitrobenzene sulfonic acid (TNBS) to induce experimental colitis in presence or absence of Aviptadil. In this animal model, TNBS induced strongly the expression of Toll-like receptors 2 and 4 (TLR2 and TLR4). TLR2 and TLR4 mediate signals from a great variety of bacterial products (LPS, LAM, Lipoproteins) as sensors for microbial recognition. Overexpression of TLR2 and TLR4 leads to perpetuation of inflammatory conditions. Aviptadil time-dependently inhibits the overexpression of TLR2 and TLR4 at mRNA and protein levels. In addition, Aviptadil acts at a systemic level in lymph nodes regulating the cellular traffic, reducing significantly the TNBS-increased proportion of dendritic cells and macrophages restoring the cell number to the level of control animals. TNBS-induced inflammation leads to a massive infiltration of neutrophils and macrophages to the sites of inflammation, where they produce high levels of proinflammatory cytokines. Aviptadil selectively modulates cell recruitment into lymph nodes which may be the reason for prevention of the pathological effects of TNBS (Gomariz et al., 2005).

In yet another study, mice were sensitized with dinitrofluorobenzene (DNFB) and later challenged again with DNFB to elicit a delayed type hypersensitivity (DTH) response in presence or absence of Aviptadil. Aviptadil dramatically reduced the inflammation-dependent cellular infiltration into the affected tissues. Aviptadil administration resulted in complete abrogation of blood mononuclear cell migration in response to the chemokines CCL2, CCL3, CCL4, CCL19, CXCL10, and CXCL12 (Grimm et al., 2003).

A pathologic hallmark of the ALI/ARDS is the damage to the surfactant-producing type II cells. Investigations were performed to look for effects of Aviptadil on those cells. There are high density Aviptadil binding sites on rat type II cells. Rat lung explants were cultured with in presence or absence of Aviptadil. Aviptadil dose-dependently increased the incorporation of methyl-choline into phosphatidylcholine, the major component of the pulmonary surfactants by enhancing the activity of the enzyme choline-phosphate cytidylyltransferase (Li et al., 2004).

4.1.3.6 Protective role on isolated and transplanted lung models

a) The effects of Aviptadil in pneumoplegic solution were tested in isolated rat lungs. The outcome of lung transplantation often depends on the quality of the donor lungs. Rat lungs were isolated, flushed and stored for 24 hours in presence or absence of Aviptadil in the restoration solution. Electron microscopy showed that lungs stored in Aviptadil-containing solutions had

significantly more normal shaped mitochondria, less mitochondrial edema, less distortion of mitochondrial cristae, thinner basal lamina, and less aggregation of nuclear chromatin than control solutions (Alessandrini et al., 1993).

b) The effects of Aviptadil were tested in the model of prolonged perfusion of isolated rat lungs. The perfusion was continued until overt lung injury, defined by the appearance of foam in tracheostomy tube. Aviptadil significantly delayed the onset of edematous lung injury in isolated perfused rat lungs – in control solution after 213 minutes, in Aviptadil solution after 349 minutes (Li et al., 2004; Pakbaz et al., 1994).

c) This study was performed in pulmonary allograft reperfusion injury rat model of lung transplantation. Male Fisher rats were lung transplanted and treated with Aviptadil for 18 hours via bolus intravenous injection (3 mg/kg). Aviptadil ameliorates reperfusion injury which is associated with improved pulmonary function suggesting a potential use in human lung transplantations (Nagahiro et al., 1998).

4.1.3.7 Stimulation of prolactin secretion in Rhesus monkeys

In this study 16 female Rhesus monkeys were investigated. Aviptadil was administered in a bolus intravenous injection of 20 µg/kg bodyweight. Twenty minutes after injection, Aviptadil caused a profound stimulation of prolactin secretion that leveled off after 2 -3 hours (Frawley and Neill, 1981).

4.1.3.8 Effects in the eye and on regional blood flows

A study was performed in 41 albino rabbits of both sexes to examine the acute (10-20 min after injection) effect of Aviptadil (500 ng/kg bodyweight) on regional blood flows. The mean arterial blood pressure was not significantly changed by the Aviptadil injections. The most pronounced vasodilation was observed in the choroids plexus, pancreas, heart muscle, the thyroid gland and the parotid gland. This study shows that Aviptadil is a potent vasodilator in many tissues at doses hardly affecting the arterial blood pressure and the blood flow in the intestine, and supports the suggestion that Aviptadil is responsible for the non-cholinergic vasodilation in the eye caused by facial nerve stimulation (Nilsson and Bill, 1984).

4.1.4 SAFETY PHARMACOLOGY

4.1.4.1 Effects on the cardiovascular system

Influence on cardiovascular parameters in vitro

The human ether-à-go-go related gene KCNH2 codes for the voltage-gated potassium channel that participates in the repolarisation phase of the human cardiac action potential. Its physiological functional role is described as to mediate the slow repolarisation current in the

human cardiac action potential. Therefore, a blockade of this channel may lead to a prolongation of the QT-interval.

To assess the influence of Aviptadil on the HERG-channel mediated potassium current, the following concentrations have been tested in vitro on HERG-transfected CHO cells: 10 µM, 100 µM and 300 µM. Negative controls were DMSO and water and 100 nM Haloperidol serves as a positive control. Results form the tests are given in Table 2.2.1.4-1 below.

Table 2.6.2 1: Results of Aviptadil effects on the HERG- mediated potassium content in HERG-mediated CHO cells

Test Item Concentration Relative remaining current ± SD
Aviptadil 10 µM (n=3)
100 µM (n=3)
300 µM (n=3) 0.91 ± 0.06
0.88 ± 0.05
0.82 ± 0.04
Vehicle control (DMSO)
Vehicle control (water) 0.1% (n=1)
0.1% (n=3) 0.92
0.95 ± 0.06
Haloperidol (positive control) 100 nM (n=3) 0.15 ± 0.02

A very minor effect was observed upon application of 300 µM Aviptadil which was reversible [Study No.GEP_LPT_646_08, 08] .

Influence on cardiovascular parameters and the respiration in anaesthetised Beagle dogs

A safety pharmacology study according to 'Good Laboratory Practice' Regulations has been performed to assess the influence of Aviptadil on several cardiovascular parameters and the respiration in anaesthetized Beagle dogs following intravenous administration. The following parameters were assessed: peripheral arterial blood pressure, pulmonary arterial blood pressure, heart rate, electrocardiography, cardiac output and cardiac stroke volume, left ventricular pressure, dp/dt max, central venous pressure, respiration, blood gas analysis.

The test item was dissolved in sterile 0.9% NaCl solution. Escalating dose levels of vehicle and 0.1, 0.3 and 1.0 mg Aviptadil/kg b.w. were administered intravenously. Due to strong cardiovascular reactions the dose levels were reduced for the last 3 dogs to 0.01, 0.03, 0.1 and 0.3 mg Aviptadil/kg b.w. The vehicle was administered at the same experimental conditions as the test item. An interval of 60 minutes was employed between each administration with a 30-minutes observation period and a 30-minutes stabilisation period. The results were compared to the corresponding predose values. Aviptadil is a potent vasodilator. Hence, a dose-related

pronounced decrease in peripheral arterial blood pressure was noted starting at the lowest tested dose of 0.01 mg Aviptadil/kg b.w. followed by a compensatory increase. Compensatory effects were also an increase in pulmonal arterial blood pressure, capillary blood pressure, systolic left ventricular pressure and central venous pressure as well as in heart rate, cardiac output, stroke volume, respiratory rate and respiratory volume. No test item-related influence was noted on any of the following cardiovascular parameters: QRS-interval, QT-interval, QTc-interval, P-interval, PQ-interval, blood pH value and blood gases. No evidence was noted for a prolongation of the QT-interval or QTc-interval. The expected cardiovascular reactions to noradrenaline and isoproterenol were not influenced (LPT Report No.19296/05).

The following publications in peer-reviewed journals regarding the cardiovascular system are available:

A study was performed to examine the effects of Aviptadil on the cardiovascular system in 12 mixed-breed dogs. Aviptadil was infused intravenously at 0.02 and 0.05 mg/kg/min. Baseline Aviptadil concentration in blood was < 50 pg/ml. After low dose Aviptadil infusion, Aviptadil levels in blood rose to 540 pg/ml, after high dose Aviptadil infusion to 1200 pg/ml. Aviptadil decreases aortic systolic, diastolic, and mean pressure and left ventricular end diastolic pressure. There was an increase of the cardiac index and an increase of heart rate. Aviptadil significantly decreases total systemic resistance and coronary vascular resistance. Aviptadil dilates coronary arteries after contractions with potassium. After Aviptadil infusion, regional blood flow increased in all cardiac chambers, esophagus and pancreas, decreased in cerebellum and mid-brain, and remained unchanged in gastrointestinal tract, stomach, liver, spleen, cerebrum and eye (Unverferth et al., 1985).

A study was performed in mongrel dogs, 11 of them being exposed to bolus injection of 150 pmol Aviptadil into the sinus node artery. Aviptadil augmented heart rate in the dogs reaching maximum heart rate increase after 36 sec. After 5 min, the heart beat recovered by 80% compared to baseline levels (Rigel, 1988).

In this study 24 mongrel dogs were investigated. Aviptadil (5.2 ng/kg bodyweight) was administered into the sinus node artery of the dogs. Heart beat rate significantly increased in all dogs tested (Hill et al., 1995).

Twenty-three mongrel dogs received intravenously 10 mg Aviptadil. The heart rate increased significantly, and the mean arterial blood pressure significantly decreased (Roossien et al., 1997).

4.1.4.2 Coronary vasodilating effects

A study was performed in dogs to analyze acute cardiovascular effects by intravenous infusion of Aviptadil over a dose range of 0.25-5 mg/kg bodyweight. Aviptadil is a potent coronary

vasodilator. The effects of Aviptadil are not mediated via prostaglandins, and are not dependent on intact endothelium (Smitherman et al., 1989).

Forty-four mongrel dogs received various Aviptadil amounts (90 pmol – 2.1 nmol) directly into the left circumflex coronary artery. Aviptadil significantly increased the coronary blood flow without significant change in mean aortic pressure, left ventricular systolic or end-diastolic pressures, or heart rate. Aviptadil has a direct vasodilator effect on the coronary arteries. Aviptadil-induced increase in cAMP most likely contributes to coronary smooth muscle relaxation. Aviptadil increases the coronary artery cross-section area, and decreases the coronary vascular resistance (Feliciano and Henning, 1998).

A study was performed in neonatal pigs 1-5 days of age to examine acute cardiopulmonary response to Aviptadil. Hearts were excised and underwent an aortic perfusion. Aviptadil decreased in doses of 0.1 and 0.5 nmol the pulmonary vascular resistance and had significant vasodilator activity in the isolated heart (Champion et al., 1996).

4.1.4.3 Effects on cardiovascular parameters

A study was performed in 26 cats of both sexes to examine acute cardiopulmonary response to Aviptadil. Bolus injections of 10 mg Aviptadil into the lobar artery decrease lobar artery pressure without changing left atrial pressure. Systemic vascular resistance decreased. Responses to Aviptadil are not dependent on a muscarine or b-adrenergic mechanism or release of a dilator prostaglandin (Nandiwada et al., 1985).

Another study was performed in 75 cats of both sexes to examine the acute cardiovascular responses to Aviptadil. Aviptadil was administered intravenously at doses of 0.1, 0.3, 1, and 3 nmol/kg bodyweight. Arterial pressure decreased, systemic vascular resistance decreased, heart rate remained unchanged, central venous pressure and cardiac output remained almost unchanged. Pulmonary arterial pressure, pulmonary vascular resistance and left atrial pressure remained unchanged (Minkes et al., 1992).

A further study was performed in cats of both sexes to examine acute cardiopulmonary response to Aviptadil. Intravenous injection of Aviptadil in doses up to 3 nmol/kg caused a dose-related decrease in systemic arterial pressure (Champion et al., 1996).

In 5 anesthetized Sprague-Dawley rats, 1 μM intravenously administered Aviptadil caused a significant reduction of the blood pressure, which remained for a mean of 6 min. (Absood et al., 1992).

Six cats were intra-arterially infused with 10-14 to 10-11 mol/min Aviptadil. Aviptadil caused a dose-dependent increase in local blood flow and vasodilatory response. Blood flow returned to normal within 3-5 min after the Aviptadil infusions (Lundberg et al., 1982).

4.1.4.4 Effects on the renal system

Stimulation of renin secretion

A study was performed in 13 mongrel dogs to analyze the effects of Aviptadil on the renal system by intraarterial infusions of the peptide (33 ng/kg/min). Blood samples were collected until 30 min after start of infusion. Renal blood flow and clearence of exogenous creatinine increased, as well as plasma renin activity. Plasma potassium concentration decreased and diastolic blood pressure slightly decreased. No change in sodium or potassium excretion was observed. Aviptadil seems to act directly on the juxtaglomerular cells of the kidneys (Porter et al., 1982).

Effects on renal function and plasma renin activity

A study was performed in 4 male Sandy Half-lop conscious rabbits for acute testing of renal function. Each rabbit underwent four separate infusions at intervals of 7 days. Aviptadil was infused intravenously over 115 min at rates of 1, 10, or 25 pmol/kg/min. Urine flow and cardiovascular effects were measured over a period of 6 hours. Systemic blood pressure remained constant except for a rise of 10mmHg during infusion of the middle dose. There was a dose-related increase in heart beats. Results indicate a role for Aviptadil in the regulation of renal function (increase of fractional excretion of sodium, potassium and chloride), including increase of plasma renin activity (Dimaline et al., 1983).

4.1.4.5 Effects on the Gastrointestinal Tract

Effects on colon motility

A study was performed in perfused mongrel dogs to examine the role of Aviptadil as inhibitory transmitter to gut musculature. Perfusion of canine proximal colon after Aviptadil administration to perfusate (100 nmol – 1 mmol) markedly reduced the spontaneous rhythmic contractions of the colon in a dose-dependent manner. Aviptadil is a regulator of the pacemaker activities that controls the circular muscle layer in the proximal colon (Suzuki et al., 1996).

Effects on lower esophageal sphincter

Aviptadil was administered intravenously by constant infusion for 15-min periods, beginning at 1 µg/kg/hour and doubling each subsequent infusion rate until 16 µg/kg/hour in awake baboons is reached. Aviptadil causes dose-dependent reduction of lower esophageal sphincter pressure (Siegel et al., 1979).

Effects on stress-induced gastric ulceration

This study was performed in a rat model (52 animals) for induction of acute gastric stress ulcers. Sprague-Dawley rats of either sex received 25 ng/kg Aviptadil intraperitoneally for up to 3 days. When Aviptadil was used after induction of stress ulcer in rats, it was therapeutically beneficial. Aviptadil prevented stress-induced ulcers and mast cell degranulation and protected gastric tissue from lipid peroxidation. Due to its antioxidant and anti-inflammatory activity, Aviptadil can be valuable in the prevention of gastric mucosal damage induced by cold-restraint stress (Tuncel et al., 1998).

Effects on ileal enteric absorptive physiology

A study was performed with 7 mongrel dogs by intravenous infusion of 500 pmol/kg/hour Aviptadil for 90 min. Aviptadil markedly decreased net ileal fluxes of water and electrolytes. Mean systolic blood pressure and hematocrit remained unchanged (Oishi and Sarr, 1995).

4.1.4.6 Effects on the nervous system

The influence of Aviptadil on the central nervous system has been investigated in two studies:

1. On the spontaneous motility of rats (Study No. 21327)

2. On Neuropharmacological parameters according to Irwin (Study No. 21328)

Examination of the influence of Aviptadil on the spontaneous motility of rats (CD/ Crl: CD(SD) following intravenous administration

One vehicle control group (NaCl solution), one positive control group treated with 2.5 mg

d-amphetamine/kg p.o. and 3 dose level groups of 100, 300, and 1000 µg/kg i.v. were employed. The results were compared to those obtained for the vehicle control group. The animals were observed by a motility evaluation system with microprocessor control. Changes of an electrical field generated in the chamber were monitored on active locomotion and on static movements of the animals in 10 minutes intervals throughout 130 minutes after administration of the test item or controls. The active locomotion of the animals treated with Aviptadil were decreased in a dose-related way compared with the control animals through the whole observations period and all dose levels (significantly at 300 µg/kg, period 30-40 min and 120-130 minutes) and at 1000 µg/kg at intervals 0-10, 30-40, 60-70, and 120-130 min after administration). The effects noted are considered to be due to the pharmacodynamic effects as Aviptadil is a potent vasodilator.

Static movements were increased during the whole observation period at all dose levels (significant $p \leq 0.01$ in the 1000 µg/kg group){Study No.21327, 2008 1043 /id}.

Neuropharmacological screening test of rats according to Irwin following intravenous administration of Aviptadil

Forty parameters (behavioural reactions, motor activity, CNS, posture, motor co-ordination, muscle tone, reflexes) were examined in CD/Crl:CD(SD) rats (n=8 rats /group) after i.v injection of either vehicle (0.9% NaCL) or Aviptadil in the following concentrations 100, 300, and 1000 µg/kg. Compared to the vehicle control, no influence was observed on neuropharmacological parameters of the test item treated animals at any dose level and at any time point (5, 15, 30, and 60 minutes after administration){Study No.21328, 2008 1044 /id}.

Literature data on the influence of Aviptadil in the CNS

In the central nervous system, Aviptadil contributes to the regulation of cerebral blood flow, energy metabolism and enzymatic activity and is twenty times more potent than norepinephrin in stimulating the enzymatic breakdown of glycogen to glucose. Aviptadil is also involved in the release of corticotrophin-releasing hormone, prolactin, oxytocin and vasopressin (Henning and Sawmiller, 2001).

In 5 baboons, effects of Aviptadil on cerebral blood flow, cerebral oxygen consumption and the electroencephalogram were investigated following injection of 10-11 mol into the carotid artery. No effect was seen under these conditions. When the same dose was administered following the hypertonic opening of the blood-brain barrier, cerebral blood flow and cerebral oxygen consumption were increased (Edvinsson and McCulloch, 1979).

Concommitantly to its effects on prolactin secretion VIP plays a role in social behaviour such as parental care, social recognition and agrgression (Kingsbury MA, 2015). Meanwhile (VIP) neurons and fibers are present in virtually every brain area that is important for social behavior including all nodes of the core "social behavior network", some hotspots with specific actions have been identified. The lateral septum, medial extended amygdala, arcopallium, and medial nidopallium, correlate with species and/or seasonal differences in flocking behaviour. The anterior hypothalamus-caudocentral septal circuit relate positively to aggression and negatively to parental care while VIP elements in the mediobasal hypothalamus relate negatively to aggression and positively to parental care. (Kingsbury MA and Wilson LC, 2016).

A study was performed in REM sleep-deprived cats searching for factors suggested to have a role in regulating sleep. Aviptadil (200 ng) was injected into cats and showed to increase the frequency of REM periods, confirming and extending the observations of small increase of REM sleep in normal cats (Drucker-Colin et al., 1984).

4.1.4.7 Discussion and conclusion

In line with the finding that the same Aviptadil peptide sequence is also endogenously present in dogs, cows, pigs, rabbits, rats, and man, the same effects as seen in humans are seen in these species. Systemic administration of exogenous Aviptadil reveals beyond the beneficial effects also some side effects comprising symptoms of tachycardia and vasodilatation.

Cardiovascular effects of Aviptadil have also been extensively described based on studies in healthy subjects, to whom Aviptadil was given via infusion in high doses. Typical effects of Aviptadil were a moderate decrease of mean, systolic and diastolic arterial pressure, a moderate decrease of stroke volume, a moderate decrease of total peripheral resistance and a moderate increase in heart rate, which were all observed following infusion in healthy subject (Frase et al., 1987). In humans these effects are diminished or could not be detected when applying Aviptadil by inhalation.

4.1.5 PHARMACODYNAMIC INTERACTIONS

INVICORP™ is an approved medicinal product containing Aviptadil (in combination with phentolamine mesylate for intracavernosal injection for the treatment of erectily dysfunction). In the experience of the use of INVICORP™ with antihypertensives or other cardiovascular drugs, no clinically relevant interactions have been observed. Aviptadil is not known to be incompatible with any medicinal products (INVICORP™ Datasheet, Douglas Pharmaceuticals LTD).

In the planned clinical trials in the indication pulmonary arterial hypertension, Aviptadil will be used on top of oral treatments approved for this indication (i.e. bosentan and/or sildenafil). Although Aviptadil has a different mechanism of action compared to bosentan or sildenafil, both Aviptadil and bosentan or sildenafil will finally lead to an increase in cAMP and/or cGMP levels of the smooth muscle cells of the pulmonary vascular system.

Dysfunctional pulmonary arterial endothelial cells have a decreased production of prostacycline and nitric oxide, with an increased production of endothelin-1 promoting vasoconstriction and proliferation of pulmonary arterial smooth muscle cells. The effects on the smooth muscle cells are induced by either decreased cAMP levels and/or decreased cGMP levels. The endothelin-1 receptor antagonists block the action of endothelin-1 and lead to an increased level of cAMP. Phosphodiesterase type 5 inhibitors increase the cGMP levels in smooth muscle cells. Aviptadil mainly increases the cAMP levels and partly the cGMP levels of smooth muscle cells and this may lead to an increase in the overall cAMP and cGMP levels, respectively. As a consequence, the overall effect of the combination of Aviptadil with sildenafil and/or bosentan is expected to exceed the effect produced by the single agents. A combination of prostacyclin (which also increases cAMP levels) and an endothelin-1 receptor antagonist has already shown an add-on effect compared to the single agents in a clinical trial. Such an add-on effect has also been clinically demonstrated for the combination of bosentan and sildenafil.

For these reasons, it is not expected that pharmacodynamic interactions would lead to an increased risk for the patients participating in the PAH clinical development program with Aviptadil. Based on this, it is justified that no specific nonclinical studies investigating pharmacodynamic interactions have been performed.

4.1.5.1 Discussion and Conclusion

An overwhelming number of beneficial pharmacological effects of Aviptadil in the pulmonary system indicate that the peptide functions in the lung to maintain normal homeostatic conditions. Evidence suggests that improper lung functions ensue when the endogenous peptide is absent or no longer functioning. In contrast to the pulmonary application, systemic administration of exogeneous Aviptadil reveals beyond the beneficial effects also some side effects comprising symptoms of tachycardia and vasodilation. In line with the finding that the same Aviptadil peptide sequence is also endogenously present in dogs, cows, pigs, rabbits, rats and man, the same effects as seen in humans are seen in these species.

The cardiovascular effects of Aviptadil have been extensively described based on studies in healthy subjects, to whom Aviptadil was given via infusion in high doses (Module 2.7). A moderate decrease of mean, systolic and diastolic arterial pressure, a moderate decrease of stroke volume, a moderate decrease of total peripheral resistance and a moderate increase in heart rate were typical effects of Aviptadil, which were observed following infusion in healthy subjects (Frase et al., 1987). It is concluded that systemic doses of Aviptadil, which are much higher than those to be administered by inhalation, cause moderate changes of parameters of the cardiovascular system.

4.1.6 PHARMACOKINETICS

4.1.6.1 Brief Summary

Aviptadil is an endogenous peptide having identical amino acid sequence in man, cow, sheep, goat, dog, rabbit and pig. Pharmacokinetics within the different species seems also similar. After IV (bolus or infusion) administration to rats, dogs, pigs and man, the plasma half-life of Aviptadil is approximately 1 min.

Absorption of Aviptadil to the blood circulation is poor following the inhalative route of administration.

The anatomical distribution of ^{125}I-labeled Aviptadil binding sites was studied in peripheral tissues by *in vitro* autoradiography in rats and guinea pigs. Dense binding occurred within the gastrointestinal, and genital tracts, in respiratory epithelium, smooth muscle of airway and blood vessels, and alveolar walls.

4.1.6.2 Methods of Analysis

Aviptadil concentrations in solutions and on filters were determined by a HPLC method (CRM Study Report No. 181342). Different antibody-based test were used to determine levels of Aviptadil in animals' plasma. An antibody based electrochemilumenscence assay was used for plasma samples derived from rats (Appendix 14 to CRM Study Report No. 79349), a validated

radio immunoassay (Study Report No. 320193) for plasma samples of cynomolgus monkeys (Appendix 8 to CRM Study Report No. 79350), and a commercially available and validated enzyme immunoassay EIA (Peninsula Laboratories Inc., CA) for the determination of Aviptadil in dog plasma (Amendment 1 to LPT Study Report No.19219/05). The method of *in vitro* autoradiography was used detecting Aviptadil distribution on tissue sections (Hassan et al., 1994). This method permits the labelling of tissue sections mounted onto microscope slides. Conditions of binding can be controlled and modified and the metabolism of the ligand can be controlled.

4.1.6.3 Absorption

Aviptadil binding to its receptors occurs in discrete locations within the gastrointestinal, respiratory, and genital tracts. Aviptadil is localized on respiratory epithelium, smooth muscles of the airways, blood vessels and alveolar walls.

Toxicokinetic analysis performed in rats and monkeys following administration of Aviptadil by inhalation and in dogs following the intravenous route of administration are presented under individual study summaries in Section 4.2 Toxicology.

Briefly, inhalation of single doses, even at high concentrations did not produce Aviptadil levels in the plasma of animals. Repeated dose inhalation of Aviptadil for 14-days may lead to elevated plasma concentrations in monkeys. However, the measured Aviptadil- level in monkeys may contain mainly not-pharmacologically active metabolites of Aviptadil which are detected by the used radio immune assay RIA in addition to the entire molecule. Reason for this assumption is that the high measured concentration did not cause any expected pharmacodynamic effect.

4.1.6.4 Distribution

Aviptadil binding to its receptors occurs in discrete locations within the gastrointestinal, respiratory, and genital tracts. Aviptadil is localized on respiratory epithelium, smooth muscles of the airways, blood vessels and alveolar walls (Henning and Sawmiller, 2001).

The *in vivo* distribution of Aviptadil was studied using a rat model in combination with labeled VIP (^{131}I-VIP) and a gamma-camera. A dynamic scan showed that ^{131}I-VIP was cleared rapidly from the blood circulation. The radioactivity was taken up and accumulated in the lungs during the first minute. During the next 15 min, the radioactivity was slowly removed from the lungs and redistributed into the kidneys, gastric mucosa, liver and small intestine. However, the radioactivity extracted by the lungs was about 6-fold lower during the first minute when a large amount of the non iodinated VIP was coinjected with the ^{131}I-VIP. ^{131}I-VIP was eliminated rapidly from the blood with a half-life of 0.44 +/- 0.05 (min +/- SD) while in lung the elimination half-life was determined to 2.3 +/- 0.8 (min +/- SD). Of the radioactivity in the lungs, 2% was found to be intact ^{131}I-VIP after 20 min. In all other organs the radioactivity found was assumed

to be low molecular weight fragments of ^{131}I-VIP. We suggest that lungs play an important role to extract Aviptadil from the circulation after an i.v. administration. ^{131}I-VIP degradation products are redistributed mostly to the kidneys and to the gastric mucosa to be excreted through urine and stomach contents, respectively (Hassan et al., 1994).

4.1.6.5 Metabolism

The major sites of the peptide metabolism are the lungs, the liver, and the kidneys. The peptide is degraded by neutral endopeptidase, mast cell tryptase and mast cell chymase (Gourlet et al., 1997; Lilly et al., 1993; Lilly et al., 1994).

The entire Aviptadil sequence is necessary for recognition by the Aviptadil receptors. Aviptadil is degraded by lung enzymes into the major products Aviptadil (1-25) and Aviptadil (26-28) at either the Ser25-Ile26 site, or into the minor products Aviptadil (1-7) and Aviptadil (8-28) at the Thr7-Asp8 site. Aviptadil (8-28) may be further cleaved at Ser25-Ile26 site to yield Aviptadil (8-25) and Aviptadil (26-28). The fragments Aviptadil (1-25) and Aviptadil (8-25) were found to be weak agonists with EC50 values of 2500 nM and 4200 nM, respectively, and Aviptadil (1-7) and Aviptadil (26-28) were inactive on guinea pig tracheal rings as smooth muscle relaxants (Bolin et al., 1995). This indicates that Aviptadil is effectively inactivated in the lung. The degradation reaction is an enzymatic reaction rather than a simple thermal decomposition. Common peptidase inhibitors were tested for their effect on the rate of Aviptadil degradation. Phosphoramidon (100 µM) showed a twofold inhibition of the degradation rate. Bacitracin (0.1%) and EDTA (1 mM) both significantly inhibited the reaction, slowing the degradation half-time by about 10-fold. The degradative activity could also be heat-inactivated. These results suggest metalloendopeptidases responsible for Aviptadil degradation in the lungs (Bolin et al., 1995).

After IV (bolus or infusion) administration to rats, dogs, pigs and man, the plasma half life of Aviptadil is approximately 1 min (see Table 1).

Table 1: Plasma half-life of Aviptadil after IV (bolus or infusion) administration to rats, dogs, pigs and man

Species	route/dose	half-life (min)
Rat	IV bolus / 20 MBq 131I-VIP	0.62 ± 0.05 (Refai et al., 1999) 3.18 ± 0.22 in lung tissues (Refai et al., 1999)
Dog	Infusion, 60 min 0.8, 1.8, 2.3, 3.6 pmoles/kg/min	1.0 ± 0.04 (Mitchell et al., 1982)
Pig	Infusion, 30 min / 2.3 pmoles/kg/min	0.85 ± 0.12 systemic adm. 1.0 ± 0.05 portal adm. (Mitchell et al., 1982)
Man	Infusion, 30 min, 3.3 pmoles/kg/min IV bolus (Invicorp)	0.96 ± 0.1 (Domschke et al., 1978b) 1.7

In dogs and pigs, the very short half-life is similar to the circulation time (Mitchell et al., 1982), in agreement with the high extraction rate on passage through the lungs. In particular, the percent of first pass uptake in pigs, after continuous infusion (30 min, 3 pmoles $Kg^{-1}min^{-1}$) or IV bolus (0.9 or 9.0 pmoles Kg^{-1}), was the same, i.e. 36% (no saturation (Hvidsten et al., 1989)). Data on first pass uptake in dogs has not been found, but dog's first pass uptake could be not too dissimilar from that of the pig.

In the rat all radiolabelled Aviptadil seems be removed during a single pass (Barrowcliffe et al., 1986) and the half-life of unchanged Aviptadil in lung tissue was approximately 3 minutes (Refai et al., 1999). Furthermore, in the rat, after intratracheal administration of ^{125}I-VIP, the radioactivity was cleared from the lungs with a mean half-life of 19 minutes (Barrowcliffe et al., 1986). The greatest proportion of released radioactivity was linked to VIP metabolites (Barrowcliffe et al., 1986) which indicates that VIP is completely degradaded by pulmonary proteases.

Aviptadil, administered intravenously, can easily cross the endothelial lung capillary barrier and accumulate in lung tissue, in particular into smooth muscle cells bearing the Aviptadil trans-membrane receptors. The receptor binding is followed by internalization and lysosomal degradation.

Aviptadil, administered by aerosol, can less-easily cross the alveolar epithelium, resulting in a longer residence time in lung tissue, compared to that after IV administration. Aviptadil metabolisation can take place also in the alveolar surfactant.

In man, 30 minutes after IV administration of 1 μg ^{123}I-VIP to 79 subjects, 45% (32-56%) of radioactivity was found in the (Virgolini et al., 1994). Man's uptake seems similar to that of the pig (and that of the dog). The amount of radioactivity in the lung decreased to 25% at 4 hours and to 10% after 24 hours. Radioactivity was excreted in the urine: 35% after 4 hours and 90% after 24 hours.

4.1.6.6 Excretion

In man, 30 minutes after IV administration of 1 μg ^{123}I-VIP to 79 subjects, 45% (32-56%) of radioactivity was found in the lungs. Man's uptake seems similar to that of the pig (and that of the dog). The amount of radioactivity in the lung decreased to 25% at 4 hours and to 10% after 24 hours. Radioactivity was excreted in the urine: 35% after 4 hours and 90% after 24 hours of the injected dose (Virgolini et al., 1994) .

4.1.6.7 Discussion and Conclusions Including Evaluation of Toxicokinetics

Aviptadil is rapidly cleared from the circulation, primarily into the lungs. There the peptide is metabolized by proteases into inactive metabolites. Aviptadil metabolites are excreted via urine.

No accumulation of the peptide was observed in any organ. In terms of clinical application, inhaled administration of Aviptadil to the lungs seems very much feasible and safe.

Due to the fact of being a natural important peptide hormone of the body expressed at different quantities at various timepoints in development and different tissues, it is unlikely that Aviptadil affects liver mitochondrial function, nor does it bind in non-physiological ways to plasma proteins, nor should it have capacity to inhibit the major P450 enzymes in liver microsomes. The major sites of the peptide metabolism are the lungs, the liver, and the kidneys. The peptide is degraded by neutral endopeptidase, mast cell tryptase and mast cell chymase. Metabolism does not occur in the heart.

4.2 TOXICOLOGY

4.2.1.1 Brief Summary

The toxicology of Aviptadil has been tested in animals using the intravenous route and the inhalation route of administration. In addition intratracheal administration was performed to test the local effects of Aviptadil in rats.

Intravenous administration of Aviptadil at high dosages causes tachycardia, watery diarrhea, and decrease in blood pressure which all resolves shortly upon termination of application. Administration of Aviptadil by inhalation did not show changes in clinical signs but may produce findings in the respiratory tract of animals. Intratracheal administration of Aviptadil revealed no pathological changes in the trachea or the lung of rats.

4.2.1.2 Single Dose Toxicity

Acute toxicity of Aviptadil was tested by inhalation as well as by intravenous route of administration in mice and rats according to GLP requirements.

4.2.1.2.1 Acute toxicity by single intravenous administration in mice

Aviptadil in 0.9% NaCl solution was applied via intravenous bolus injection into a tail vein. The injection speed was dose/15 sec using 1, 3 and 10 mg/kg. No-effect dose level was1 mg/kg b.w., i.v. Dose level with first intolerance reactions 3 mg/kg b.w., i.v. Lowest lethal dose level was >10 mg/kg b.w., i.v. LD_{50} of Aviptadil was >10 mg/kg b.w., i.v in males, females and male and female combined after 24 hours and 14 days. Under the present test conditions, a single intravenous administration of 3 mg Aviptadil/kg b.w. to mice caused slightly reduced motility, slight ataxia and slight dyspnoea in all 5 male and 5 female animals 15 to 30 minutes after administration. At 10 mg Aviptadil/kg b.w. the animals revealed slightly reduced motility, slight ataxia, and slight dyspnoea 15 to 60 minutes and slightly reduced muscle tone 15 to 30 minutes after administration, respectively, in all male and female animals.

A single intravenous administration of 1 mg Aviptadil/kg b.w. to mice revealed no toxic symptoms. No mortality occurred. All animals gained the expected body weight throughout the whole study period. There were no macroscopic findings during necropsy (Study Report No. 19275/05).

4.2.1.2.2 Acute toxicity by single intravenous administration in rats

Aviptadil in 0.9% NaCl solution was applied via intravenous bolus injection into a tail vein. The injection speed was dose/15 sec using 1, 3 and 10 mg/kg. No-effect dose level was1 mg/kg b.w., i.v.Dose level with first intolerance reactions 3 mg/kg b.w., i.v. Lowest lethal dose level was >10 mg/kg b.w., i.v. LD_{50} of Aviptadil was >10 mg/kg b.w., i.v in males, females and male and female combined after 24 hours and 14 days.

Under the present test conditions, a single intravenous administration of 3 mg Aviptadil/kg b.w. to rats caused slightly reduced motility, slight ataxia, slightly reduced muscle tone and slight dyspnoea in all 5 male and 5 female animals 15 to 60 minutes after administration. At 10 mg Aviptadil/kg b.w. the animals revealed slightly to moderately reduced motility, slight to moderate ataxia, slightly to moderately reduced muscle tone and slight to moderate dyspnoea 5 to 60 minutes and slight ptosis 30 minutes after administration, respectively, in all male and female animals. A single intravenous administration of 1 mg Aviptadil/kg b.w. to rats revealed no toxic symptoms (Study Report No.19274/05).

4.2.1.2.3 Acute and 10-day inhalation toxicity study in mice

A nebulized aerosol formulation of Aviptadil was administered to CD1 mice. The study was designed in two phases, a daily dose escalation phase and a 10-day repeated dose phase. Five groups of mice (n=3 per group and gender during the escalation phase and 5 during the 10-day repeated dosing phase) were treated with a nebulized formulation of Aviptadil. Theoretical dosing was as follows: Group 1: escalating dose 100µg/kg/day; Group 2: escalating dose 1000 µg/kg/day; Group 3: escalating dose 3000 µg/kg/day; Group 4: escalating dose 5800 µg/kg/ day; Group 5: repeated dose (four daily inhalation treatments of 10 min/interval on 10 consecutive days): 2500 µg/kg/ day.

No preterminal deaths were seen during the course of the study. No clinical signs, no changes in body weights, no treatment-related changes in hematology or clinical biochemistry could clearly be attributed to treatment with Aviptadil. There were no macroscopic findings that were considered to be related to the administration of Aviptadil.

Detailed histopathological evaluation was performed only in animals from group 5, the repeated dose phase. Eosinophilic globules were seen in the nasal epithelium of 3/5 male and 1/5 female animals of group 5. These globules are occasionally seen spontaneously but are a common

treatment-related change in studies utilizing the inhalation route of administration. In the absence of animals for control in this study a treatment association can not be ruled out.

Some epithelial hyperplasia observed in a single male animal was not seen in other individuals and was considered to probably have been a spontaneous occurrence. All other histopathological changes seen were considered to have been spontaneous in origin and not to be associated with treatment.

In conclusion, nose-only inhalation exposure of CD1 mice to aerosolized Aviptadil at a dose of 1546 µg/kg/day was well tolerated and produced no apparent changes in any of the parameters evaluated. In addition, no changes were observed after a single dose administration as high as 3920 µg/kg/day. The no-observable-adverse-effect level (NOAEL) was considered to be at least 3920 µg/kg/day fore an acute exposure and 1546 µg/kg/day for a 10 day repeated exposure. A dose formulation of 5.5 mg/mL was considered the maximum concentration suitable for use for treatment based upon precipitation and gelification of the substance observed at higher concentrations (Study Report No. 79354).

4.2.1.2.4 Acute and 10-day inhalation toxicity study in rats

Five groups of Sprague-Dawley CD, Crl:CD (SD)BR rats (n=3 per group and gender during the escalation phase and 5 during the 10-day repeated dosing phase) were treated with a nebulized formulation of Aviptadil. Theoretical dosing was as follows: Group 1: escalating dose 100µg/kg/day; Group 2: escalating dose 1000 µg/kg/day; Group 3: escalating dose 3000 µg/kg/day; Group 4: escalating dose 3900 µg/kg/day; Group 5: repeated dose (four daily inhalation treatments of 10 min/interval on 10 consecutive days): 1000 µg/kg/ day. Corresponding calculated achieved doses were as follows: Group 1 to 5: 162.7, 1170, 1885, 2630, and 1017 µg/kg/day in the repeated dose phase.

All animals survived to termination of the study. There were no clinical signs that were considered related to treatment with Aviptadil. No changes in body weight occurred that could clearly be attributed to drug exposure. There were no treatment-related changes in haematology parameters. Slight decrease (5-10%) in red blood cell counts, haemoglobin and hematocrit were observed in the repeated dose female animals when compared to historical normal ranges. However, this could be related to the blood volume that was collected the day prior to necropsy for drug plasma level analysis. Also no treatment-related changes in biochemical parameters could be observed. Aviptadil plasma concentrations analysis performed by RIA at PCS-MTL revealed presence of Aviptadil at levels from 37.5 to 116.9 pg/ml in 3 out of 10 samples. All other levels were below the limit of quantification of 25.3 pg/ml. In a second analysis of Aviptadil plasma levels evaluated at Tandem Labs, the Aviptadil plasma levels was 10-85 pg/ml and were in generally good agreement with the RIA results and with literature data. None of the values obtained after the last inhalation of Aviptadil on Day 10 exceed what is reported as baseline peripheral level of VIP in rats, and therefore, peripheral exposure to Aviptadil following

inhalation appears unlikely. There were no gross pathology changes that were considered treatment related.

Histopatholocical findings: Eosinophilic granules were seen in the nasal olfactory epithelium of 2/5 female animals, in the laryngeal ventrolateral epithelium of 3/5 male and 3/5 female animals and in the tracheal epithelium of a single female animal. These globules are occasionally seen during treatment via the inhalation route of administration. However, a treatment association can not been ruled out because the study did not contain control animals. All other histopathology changes were considered to be of spontaneous origin and not to be associated to drug treatment.

In conclusion, nose-only inhalation exposure of Sprague-Dawley rats to nebulized formulation of Aviptadil at a dose of 1017 µg/kg/day for 10 days was well tolerated and produced no apparent changes in any of the parameters evaluated. In addition, no changes were observed after a single dose administration as high as 2630 µg/kg/day.

As no changes could be unequivocally associated with treatment were evident in this study, the acute non-observable-adverse-effect-level (NOAEL) was considered to be at least 2630 µg/kg/day and 1017 µg/kg/day for a 10 day repeated dose. A dose formulation of 5.5 mg/ml was considered to be the maximum concentration suitable for us for treatment due to precipitation and gelification of the drug at higher concentrations (Study Report No. 79349).

4.2.1.3 Repeated Dose Toxicity

Pharmacology and toxicology of the endogenous peptide Aviptadil are described in a large number of peer-reviewed publications. Importantly, inhaled Aviptadil has been tested in several clinical trials cumulating 68 patients with various pulmonary diseases, at doses ranging from 4 x 50 µg to 3 x 100 µg VIP/ day, for treatment periods up to six months. Overall, in these studies inhaled Aviptadil was very well tolerated.

4.2.1.3.1 Repeated Dose Inhalation Toxicity of Aviptadil

Two 14-day and one 28-day repeated dose inhalation toxicity studies were conducted in rats and in cynomolgus monkeys according to GLP guidelines.

14-day inhalation toxicity study in rats

The purpose of this study was to evaluate the potential systemic toxicity of a nebulized aerosol formulation of Aviptadil following daily "nose-only" inhalation administration (up to 4 hours/day) for 14 consecutive days in Sprague-Dawley CD® (Crl:CD® (SD)) rats. Five animals per group male and female each were randomized into the following four groups and dosed as described in Table 2 below.

Table 2: Target and achieved concentrations of Aviptadil or placebo applied to four dosing groups (5 males and 5 females/ group) of rats

Group Number Identification	Target Dose Level (μg/kg/day)	Solution Concentration (mg/mL)	Active Concentration (μg/L)	Exposure Duration (min)	Mean (♂&♀) Achieved Dose (μg/kg/day)
1/ Control	0	0	0	240	0
2/ Low Dose	100	0.5	3.55	30	72.2
3/ Mid Dose	600	5.5*	27.1	30	549
4/ High Dose	6000	5.5*	19.9	240	3228

* Aviptadil in a concentration of 5.5 mg/ mL is the maximal feasible dose due to the limitation of aerosolization of the drug.

All animals survived to termination of the study. There were no clinical signs observed that were considered related to treatment with Aviptadil.

There were no treatment-related effects on body weight or food consumption during the treatment period. There were no treatment-related changes in hematology, clinical biochemistry or urinalysis parameters that could clearly be attributed to treatment with Aviptadil.

Daily inhalation administration of Aviptadil at dose levels up to and including the MFD in[1] this study, did not elicit an inhalation dose-related increase in circulating Aviptadil levels. Plasma Aviptadil levels were on all but 1 occasion within endogenous levels from literature (Hang, C.-H., et al., 2004). No treatment-related ocular changes were observed at Week 2. There were no changes that could be attributed to Aviptadil treatment in relation to organ weight, gross pathology or histopathology.

In conclusion, nose-only inhalation exposure of Sprague-Dawley rats to aerosolized Aviptadil at the maximal feasible dose of 3228 μg/kg/day for 14 days was well tolerated and produced no apparent changes in any of the parameters evaluated. The maximal feasible dose corresponds to approximately 250 times the foreseen maximal dose of Aviptadil in clinical use.

As no changes that could be unequivocally associated with treatment were evident in this study, the acute no observable adverse effect level (NOAEL) was considered to be at least 3228 μg/kg/day for a 14 day repeat dose (Charles River Study Report No. 79426).

14-day inhalation toxicity study in cynomolgus monkeys

A range finding and 14-day inhalation toxicity of a nebulized aerosol formulation of Aviptadil has been performed in Cynomolgus monkeys according to GLP guidelines.

Aviptadil or vehicle was administered oronasal by four separate applications with a recovery period of approximately 90 minutes between the administrations to reach the daily dose. The mean overall achieved doses were as follows in the dose escalation phase: 1st Escalation Dose: 338 µg/kg/day; 2nd Escalation Dose: 959 µg/kg/day; 3rd Escalation Dose: 2139 µg/kg/day; 4th Escalation Dose: 3102 µg/kg/day. During 14 consecutive days monkeys were dosed daily with either vehicle or with Aviptadil at a concentration of 162 or 3857 µg/kg/day.

The following parameters were measured during the study:

Mortality, signs of illness, respiratory minute volume, ECG, ophthalmology, laboratory investigations (hematology, clinical biochemistry, coagulation, urinalysis), gross pathology and histopathology of distinct organs. In addition toxicokinetic investigation was performed.

All animals survived during the course of the study. No clinical symptoms occurred during the escalation and the repeated dosing period that could be attributed to the treatment with Aviptadil.

No drug related changes in body weight or food consumption were measured. Respiratory minute volume was considered not to be changed by the treatment. Aviptadil did not appear to have any clinical significant effects on ECG recording that were attributed to the treatment with Aviptadil during the escalation and repeated dose administration period. No ocular changes could be observed. There were no treatment related changes in the laboratory parameters (hematology, clinical biochemistry, coagulation, and urinalysis).

Using a RIA test, toxicokinetic investigations during the repeated dosing phase showed that the administration of Aviptadil may lead to a systemic exposure of the drug.

Macroscopic and organ weight findings were interpreted as incidental or agonal of origin.

Histopathology findings: Aviptadil at a dose of 3857 µg/kg/day administered for 14 consecutive days produced findings in the nasal cavity/sinuses, which included moderate erosion of the squamous epithelium in 1/2 males (Animal 401) and squamous metaplasia of the respiratory epithelium in 1/2 males (Animal 401) (graded slight) and 1/2 females (Animal 452) (graded minimal). In the larynx, minimal epithelial hyperplasia was observed for 1/2 males (Animal 401) and minimal squamous metaplasia was present in 1/2 females (Animal 452). Other parameters evaluated did not produce any treatment related changes.

Conclusion: Oronasal inhalation exposure of Aviptadil of monkeys at escalating doses of 338, 959, 2139 and 3102 µg/kg/day did not produce any treatment related changes in any of the

parameters evaluated. Aviptadil in a concentration of 5.5 mg/ mL is the maximal feasible dose due to the limitation of aerosolization of the drug. Toxicokinetic investigations showed that repeated dosing of Aviptadil via inhalation may lead to a systemic exposure of the drug. Aviptadil at a dose of 3857 µg/kg/day administered for 14 consecutive days produced minimal to slight findings in the nasal cavity/sinuses and the larynx of male and female monkeys.

Based on these observations, the no observable adverse effect level (NOAEL) for Aviptadil was considered to be at 162 µg/kg/day when administered over 14 consecutive days to cynomolgus monkeys (Study Report No. 79350).

28-day DRF inhalation toxicity study in cynomolgus monkeys

The objective of this study was to investigate the potential toxicity of a nebulized aerosol formulation of Aviptadil during a 28-day repeated dose administration to the monkey by head-only inhalation. Furthermore the safety of the vehicle was assessed. The vehicle contains among other excipients sucrose which is not contained in any approved medicinal products for inhalation so far.

Three animals per group and gender were treated either with Aviptadil dissolved in the vehicle containing polysorbate 80, sucrose, and mannitol along with either acetic acid or sodium acetate to adjust the pH, or with the vehicle alone or with saline. Target dose levels and theoretically achieved doses are presented in the table below.

Table 3: Target and achieved concentrations of Aviptadil or placebo applied to five dosing groups (3 males and 3 females/ group) of rats

Group Number Identification	Target Dose [µg/kg/day]	Exposure Time [min]	Theoretical achieved Dose [µg/kg/day]
1 Saline Control	0	60	0
2 Vehicle Control	0	60	0
3 Low Dose	733	15	619
4 Mid Dose	1466	30	722
5 High Dose	2932	60	1855

The target doses could not be reached although the maximum concentration of 6 mg/mL Aviptadil was used which is the maximum soluble concentration of the test item to produce the aerosol in the chamber.

Results

There were no Aviptadil-related changes when compared to controls (saline and vehicle) with respect to mortality, clinical observations, body weights and body weight gains and appetite.

There were no changes compared to saline and vehicle control in clinical pathology (hematology, serum chemistry and in urinalysis).

Organ weights (absolute and relative to body weights) were not affected by the test item.

Macroscopic observation revealed no test item related changes. All changes recorded were considered incidental in origin.

Microscopic observations

Microscopic changes attributable to head-only inhalation of Aviptadil for 28 days in monkeys were limited to the nasal cavities of most monkeys in all dose/duration groups as summarized in the table below:

Table 4: Incidence and Severity of Aviptadil-related Histopathological Changes in the Nasal Cavities of Cynomolgus Monkeys

	Males					Females				
Dose [µg/kg/day]	0	0	619	772	1855	0	0	619	772	1855
Nasal cavities N° of Animals examined	3	3	3	3	3	3	3	3	3	3
Degeneration resp. epithelium N° animals affected	-	-	2	1	3	-	-	3	3	2
Minimal	-	-	1	-	2	-	-	3	3	-
Slight	-	-	1	1	1	-	-	-	-	2

The changes observed in almost all animals receiving Aviptadil but not in the vehicle and saline control groups were rated minimal to slight in severity and were located in general at the ventral aspect of the nasal turbinate in the second level of the nasal cavity. Occasionally the affected area was accompanied by neutrophil infiltration. The affected epithelial cells had no cilia, were basophilic and pavimentous contrasting with the pseudostratified columnar ciliated normal respiratory epithelium. A lower number to total disappearance of goblet cells was noted in the affected areas.

All other microscopic findings were not considered drug-related. The nature of these findings was considered incidental and therefore it was considered that these findings are not of toxicological significance (Study No. 79707).

26-week inhalation toxicity study in cynomolgus monkeys

The objective of this study was to investigate the potential toxicity of a nebulized aerosol formulation of Aviptadil during daily inhalation administration to the monkey for 26 consecutive weeks followed by a 4-week recovery period.

Table 5: Target and achieved concentrations of Aviptadil or placebo applied to five dosing groups (Main study: 4 males and 4 females/ group; Recovery: 3 animals/gender/group) of monkeys

Group Number Identification	Target Dose (µg/kg/day)	Exposure Duration (minutes)	Mean Theoretical Achieved Dose (µg/kg/day)
1/ Saline Control	0	60	0
2/ Vehicle Control	0	60	0
3/ Low Dose	250	60	263
4/ Mid Dose	730	60	867
5/ High Dose	2350	60	2525

Monkeys were examined twice daily for mortality and signs of ill health following arrival. Detailed examinations were performed weekly starting during the last week of pretreatment and throughout the treatment period, and on the day of necropsy (fasted). Individual body weights were measured at least once during the pretreatment period and weekly during the treatment period. In addition, each monkey was weighed (fasted) before scheduled necropsy. Appetence was assessed daily by visual inspection (generally in the a.m.) commencing the last week of the pretreatment period and extended through the treatment period.

Once prior to commencement of treatment, and again during Weeks 13, 26 ophthalmology and electro cardiology assessments were performed on all animals. In addition, once prior to commencement of treatment, and again during Weeks 13, 26 and 30 (recovery animals only), laboratory investigations (hematology, serum chemistry and urinalysis) were performed, with toxicokinetic sampling on Day 1 and again during Weeks 13 and 26 on all animals. Furthermore, twice prior to the commencement of treatment the respiratory minute volume of all animals was assessed.

At study termination, necropsies were performed, macroscopic observations collected, selected organs weighed, and selected tissues were retained for histopathologic examination.

A single female receiving 867 µg/kg/day of Aviptadil was preterminally euthanized on study Day 47 due to a fractured humerus, sustained during manipulation.

There were no test article-related clinical observations or changes in body weights, food consumption, clinical pathology parameters, organ weights or macroscopic observations.

Minimal to slight degeneration/regeneration of the respiratory epithelium of the nasal cavities in both sexes at all dose levels (without a clear dose relationship) was noted histopathologically.

VIP exposure increased after repeated daily inhalation for 13 and 26 weeks, with accumulation ratios reaching 43.6-fold for the AUC(0-t) between Week 26 and Day 1 in females receiving 263 µg/kg/day dose group. Exposure was generally greater in females (up to 7-fold) than in males, in particular for the AUC(0-t) parameter and females exhibited greater accumulation ratios than males, particularly at the 263 and 2525 µg/kg/day doses levels. No general trend could be established regarding dose proportionality due to the variability of the data.

In conclusion, Aviptadil given by head-only inhalation to cynomolgus monkeys for 26 consecutive weeks at dose levels of 0, 0, 263, 867 and 2525 µg/kg/day was well tolerated. Compound-related microscopic findings observed at dose levels of ≥ 263 µg/kg/day included degeneration/regeneration of the respiratory epithelium of the nasal cavities of monkeys at all dose levels, without a clear dose relationship. Following the 4-week recovery period, minimal degeneration/regeneration of the respiratory epithelium was still present in a single male exposed to 2525 µg/kg/day Aviptadil suggesting partial recovery in males.

Due to the extent of the histopathological changes the no-adverse-observable-effect-level (NOAEL) was determined to be 2525 µg/kg/day (Study No.79641).

4.2.1.3.2 Repeated Dose Toxicity of Aviptadil Following Intravenous Application

26-week long-term toxicity study in dogs

To assess the long-term toxicity of Aviptadil supporting the intended use as a chronic treatment, a 26-week repeated-dose toxicity study in Beagle dogs using intravenous application has been performed.

Four Beagle dogs per group and gender were dosed by intravenous infusion into the vena cephalica of the right or left forelimb once daily either with Aviptadil or vehicle for 26-weeks.

Dose levels per group were as follows: Group 1 (control): 0 µg Aviptadil/kg b.w.; Group 2: 20 µg Aviptadil/kg b.w.; Group 3: 60 µg Aviptadil/kg b.w.; Group 4: 200 µg Aviptadil/kg b.w.

No signs of local intolerance reactions were noted at the daily inspections of the injection sites at any of the tested dose levels. Macroscopic inspection at necropsy revealed no signs of local intolerance reactions.

Histopathology did not reveal any test item-related local intolerance reactions.

No mortality occurred. The following clinical signs were observed:

All animals treated with 20, 60 or 200 µg Aviptadil/kg b.w./day revealed an increased heart rate, pale or reddened gingiva, reddened ears, abdomen and/or conjunctiva, salivation, emesis, a prolapse of the nictitating membrane and chewing movements from test day 1 onwards. The severity and duration of the changes were dose-dependent. Animals treated with either 60 or 200 µg Aviptadil/kg b.w./day revealed also pilo-erection. In addition, reduced motility (slight) was observed for the animals treated with 200 µg Aviptadil/kg b.w./day. Defaecation occurred in individual animals on single test days. In general, the symptoms started up to 5 min after the end of the administration and lasted up to 20 min. The increase of the heart rate started during or immediately after the end of the administration and lasted up to 60 min.

The body weight and body weight gain were not influenced. No influence was observed on the food consumption. The visual appraisal of the drinking water consumption revealed no differences between the control and the test item-treated animals.

The visual assessment of the ECG revealed an-increase of the heart rate and a decrease of the PQ and QT interval as well as of the QTc value starting at 20 µ/kg Aviptadil/kg b.w./day. Details are given in Table 6 below.

Table 6: Changes in ECG Parameters of Dogs Treated with Aviptadil

Timepoint	Group 2 20 µg/kg		Group 3 60 µg/kg		Group 4 200 µg/kg	
	males	**females**	**males**	**females**	**males**	**females**
Changes in the <u>heart rate</u> compared to the control in %						
TW 26 (5 min p.a.)	+83**	+108**	+120**	+156**	+124**	+131**
Changes in the <u>PQ interval</u> compared to the control in %						
TD 1 (5 min p.a.)	none	-21**	none	-16	none	-17
TW 26 (5 min p.a.)	-23**	-31**	-23**	-31**	-21**	-17
Changes in the <u>QT interval</u> compared to the control in %						

TW 26 (5 min p.a.)	none	none	-20**	-29**	-23**	-28**
Changes in the QTc value compared to the control in %						
TW 26 (5 min p.a.)	none	none	none	-11**	none	-12**

** statistically significant at $p \leq 0.01$

Treatment with Aviptadil led to a decrease (statistical significant $p< 0.01$ after the mid and the high dose) in the peripheral arterial systolic and diastolic blood pressure as well as the resulting mean blood pressure 10 min after the end of the administration in test week 26. Details are given in the table below:

Table 7: Changes in Blood Pressure of Dogs Caused by Aviptadil

Parameter	**Changes in blood pressurecompared to the control in %**					
	Group 2 20 µg/kg		**Group 3 60 µg/kg**		**Group 4 200 µg/kg**	
	males	**females**	**males**	**females**	**males**	**females**
Test week 26						
Systolic blood pressure (10 min p.a.)	-15	-17	-23**	-9	-15**	-17
Diastolic blood pressure (10 min p.a.)	-16	-21	-25**	-16	-25**	-25**
Mean blood pressure (10 min p.a.)	-16	-19	-24**	-13	-21**	-22**

** statistically significant at $p \leq 0.01$

No test item-related changes were noted in haematological, in clinical biochemistry and in urinalysis parameters. Ophthalmological examination revealed no test item-related changes. There was no indication of any impairment to auditory acuity. No test item-related changes were noted. No test item-related influence was observed on the relative or absolute organ weights. The myeloid/erythroid ratio of the male and female animals was not influenced in the bone marrow. The histopathological examination did not reveal any test item-related morphological organ changes which are considered to be related to the administration of the test item.

The analysis of plasma samples revealed a nearly linear dose-related exposure of the animals to Aviptadil. The mean peak plasma levels of Aviptadil were measured 0.5 or 1 minute after administration on test day 1 and test day 182 as shown in the table below. A nearly dose-related increase was observed both in C_{max} and AUC values. The dose proportion factor for the AUC

revealed a relative increase in the exposure on test day 1 and 182 which was roughly linear but somewhat higher as compared to the dose increase. Test week 26 revealed an accumulation with time in comparison to test day 1. The mean plasma elimination half-life ranged from 1.8 to 4.0 min. There was no difference between the genders.

Table 8: Toxicokinetics of Aviptadil in Dogs

Dosage	Non-compartment analysis							
	Sex	C_{max} (ng/mL)	T_{max}* (min)	$t_{1/2elim}$ (min)	$AUC_{0\text{-}10\ min}$ (ng*h/mL)	AUC/dose (ng*h*kg/mL/µg)	R	DPF
	Test week 1(Test day 1)							
20 µg/kg	m	45.7	0.7	2.6	2.347	0.117	1	1
	f	76.2	1.0	2.7	5.573	0.279	1	1
60 µg/kg	m	179.2	1.0	3.3	12.520	0.209	1	1.8
	f	277.0	0.5	2.9	14.170	0.236	1	0.8
200 µg/kg	m	958.1	0.7	3.1	67.020	0.335	1	2.9
	f	930.8	0.5	4.0	72.693	0.363	1	1.3
	Test week 26 (Test day 182)							
20 µg/kg	m	114.0	1.3	1.8	6.433	0.322	2.7	1
	f	152.1	0.7	2.4	9.723	0.486	1.7	1
60 µg/kg	m	464.1	1.3	3.0	35.947	0.599	2.9	1.9
	f	481.9	0.5	2.6	31.907	0.532	2.3	1.1
200 µg/kg	m	2123.1	0.8	2.9	105.557	0.528	1.6	1.6
	f	2410.1	0.8	3.0	139.010	0.695	1.9	1.4

* Values obtained from plasma analysis, all other values calculated by toxicokinetic analysis; R: accumulation factor ($AUC_{TW26\ 0\text{-}10\ min}/AUC_{TW1\ 0\text{-}10\ min}$); DPF: dose proportion factor [$AUC_{0\text{-}10\ min}$ (x µg/kg)/$AUC_{0\text{-}10\ min}$ (20 µg/kg)]/ [(x µg/kg)/ (20 µg/kg)] for the same day

At the end of the recovery period the peripheral arterial systolic and diastolic blood pressure as well as the resulting mean blood pressure of the previously high dosed female animals was still reduced by up to 20% compared to the control while male animals were within the normal limits.

The signs of systemic toxicity in the form of an increased heart rate, a decrease of the PQ and QT interval or of the QTc value as well as all findings of behaviour, external appearance and faeces had completely subsided at the end of the recovery period in test week 30.

Body weight and body weight gain, food consumption, eyes and ears, haematological, biochemical and urinary parameters were within the normal limits.

Conclusion
The aim of the experiment was to obtain information on the toxicity of Aviptadil, administered daily by intravenous administration to Beagle dogs for 26 weeks at dose levels of 20, 60 or 200

µg/kg b.w./day and to assess the reversibility of any effect after a 4-week recovery period. No test item-related local intolerance reactions were noted.

No mortality occurred. From the low dose of 20 µg Aviptadil/kg b.w./day onwards, an increased heart rate, pale or reddened gingiva, reddened ears, abdomen and/or conjunctiva, salivation, emesis, a prolapse of the nictitating membrane and chewing movements were observed - in a dose-related way - in nearly all animals from test day 1 onwards. Further, starting at 20 µ/kg Aviptadil/kg b.w./day, an increase of the heart rate and slightly decreased values of the PQ and QT interval and of the QTc value of males and/or females were revealed 5 min p.a. in test week 1 and/or 26. Correlating, a dose-related decrease in the peripheral arterial systolic and diastolic blood pressure as well as the resulting mean blood pressure was observed 10 min after the administration. The severity of the findings was dose-related. In addition, pilo-erection was observed at 60 and 200 µg Aviptadil/kg b.w./day and a reduced motility at 200 µg Aviptadil/kg b.w./day. All effects noted are considered to be due to marked pharmacodynamic effects, as Aviptadil is a potent vasodilator and an inhibitor of smooth muscle cell proliferation.

No test item-related changes were observed for haematological or biochemical parameters.

Macroscopic inspection at necropsy did not reveal any test item-related changes.

The histopathological examination did not reveal any test item-related morphological organ changes which are considered to be related to the administration of the test item.

The analysis of plasma samples revealed a nearly linear dose-related exposure of the animals to Aviptadil. Pharmacokinetic evaluation revealed nearly a dose-related increase both in C_{max} and AUC values. Test week 26 revealed an accumulation with time in comparison to test day 1. The mean plasma elimination half-life ranged from 1.8 to 4.0 min.

At the end of the 4-week recovery period the peripheral arterial systolic and diastolic blood pressure as well as the resulting mean blood pressure of the female animals treated with 200 µg Aviptadil/kg b.w./day were still 20% below the control values. All other symptoms had subsided.

Under the present test conditions of this study, the no-observed-adverse-effect-level (NOAEL) could be consider 200 µg Aviptadil/kg b.w./day, i.v. (Study Report No.19219/05).

Sumary of results of acute and repeated dose toxicity studies

The results of the acute and repeated dose toxicity studies are summarized in the table below.

Table 9: Summary of Results of Acute and Repeated Dose Toxicity Studies

Study	Route of Admin. and Dose	Clinical Signs at Applied Dose	Histopathological Findings at Applied Dose	NOAEL
Acute Toxicity in mice LPT No. 19275/05	IV Single Dose 1, 3, 10 mg/kg	3 mg/kg Slightly reduced motility, slight ataxia, slight dyspnoea 5/5 ♀; 5/5 ♂; 10 mg/kg Slightly reduced motility, slight ataxia, slight dyspnoea, slightly reduced muscle tone 5/5 ♀; 5/5 ♂;	No findings attributable to the treatment with Aviptadil	1 mg/kg
Acute and 10-day in mice CRM No. 79354	Inhalation Single dose 224, 1391, 3082, 3920 µg/kg/day	No findings attributable to the treatment with Aviptadil	No findings attributable to the treatment with Aviptadil	3.92 mg/kg
	Rep. Dose Phase 1546 µg/kg/day for 10 days	No findings attributable to the treatment with Aviptadil	Eosinophilic granules in nasal epithelium 3/5 ♀; 1/5 ♂ Epithelial hyperplasia 1♀3.92 mg/kg	1.55 mg/kg for 10 days
Acute Toxicity in rats LPT No. 19274/05	IV Single Dose 1, 3, 10 mg/kg	3 mg/kg Slightly reduced motility, slight ataxia, slight dyspnoea 5/5 ♀; 5/5 ♂ 10 mg/kg Slightly- moderate reduced motility, ataxia, dyspnoea, muscle tone , slight ptosis 5/5 ♀; 5/5 ♂	No findings attributable to the treatment with Aviptadil	1 mg/kg
Acute and 10-day in rats CRM No. 79349	Inhalation Single dose 162, 1170, 1885, 2630 µg/kg/day	No findings attributable to the treatment with Aviptadil	No findings attributable to the treatment with Aviptadil	2.63 mg/kg
	Rep. Dose Phase 1017 µg/kg/day for 10 days	No findings attributable to the treatment with Aviptadil	Eosinophilic granules in nasal olfactory epithelium 2/5 ♀; In laryngeal ventrolateral epithelium 3/5 ♀and 3/5 ♂; in tracheal epithelium 1/5 ♀	1.017 mg/kg for 10 days

Study	Route of Admin. and Dose	Clinical Signs at Applied Dose	Histopathological Findings at Applied Dose	NOAEL
14-day repeated dose in rats CRM no. 79426	Inhalation 0, 72,2, 549, 3228 µg/kg/day	No findings attributable to the treatment with Aviptadil	No findings attributable to the treatment with Aviptadil	3.228 mg/kg for 14 days
DRF and 14-day repeated dose in cynomolgus monkeys CRM No. 79350	Inhalation 0, 338, 959, 2139, 3102 µg/kg/day	No findings attributable to the treatment with Aviptadil	No findings attributable to the treatment with Aviptadil	
	Repeated dose phase 0, 162, 3857 µg/kg/day	No findings attributable to the treatment with Aviptadil	At 3857 µg/kg/ 14-days In the nasal cavity/sinuses moderate erosion of squamous epithelium 1/2♂; slight squamous metaplasia of respiratory epithelium 1/2♂ and minimal squamous metaplasia 1/2 ♀ In the larynx minimal epithelial hyperplasia 1/2♂ and minimal squamous metaplasia in 1/2 ♀	0.162 mg/kg for 14 days
DRF 28-day repeated dose in cynomolus monkeys. CRM No. 79707	Inhalation Repeated dose 0, 0, 679, 790, 1885 µg/kg/day	No findings attributable to the treatment with Aviptadil or vehicle control	In all Aviptadil dose groups a degeneration of the nasal epithelium has been observed with incidence and/or severity being greater in animals receiving 1885 µg/kg/day. No findings attributable to sucrose-containing vehicle.	1.885 mg/kg/day
26-week repeated dose toxicity in cynomolgus monkeys CRM No. 79641	Inhalation by head-only exposure 0, 0, 263, 867, 2525 µg/kg/day	No findings attributable to the treatment with Aviptadil or to vehicle control	Minimal to slight degeneration of the respiratory epithelium in nasal cavities of most monkeys in all Aviptadil treated animals without clear dose-relation.	2.525 mg/kg/day for 6 months
26-week in beagle dogs LPT No. 19219/05	IV 0, 20, 60, 200 µg/kg/day	All well known side effects were observed after systemic exposure of the test item which were resolved after the recovery period. Details are given above in the text.	No findings attributable to the treatment with Aviptadil	0.2 mg/kg/day for 6 months

4.2.1.4 Genotoxicity

Aviptadil is an endogenous peptide and the various biological activities are well documented. There are generally no concerns about carcinogenicity and genotoxicity of endogenous peptides such as Aviptadil.

4.2.1.4.1 In vitro

No evidence of mutagenic potential has been detected following several tests on INVICORP™ (combination of Aviptadil and phentolamine mesylate); INVICORP™ Data sheet, Douglas Pharmaceuticals LTD, December 5, 2003).

4.2.1.4.2 In vivo

Aviptadil is an endogenous peptide which has an identical amino acid sequence in most mammalian species. Consequently, it is not expected that Aviptadil exerts any genotoxic potential. Therefore, no specific *in vivo* gentoxic studies have been performed.

4.2.1.5 Carcinogenicity

Due to the fact that Aviptadil is an endogenous peptide, carcinogenicity studies are not considered necessary. This is in line with the ICH guideline for the safety of medicinal products (S1A, point 4.7).

4.2.1.6 Reproductive and Developmental Toxicity

4.2.1.6.1 Effect of Aviptadil on Fertility and Early Embryonal Development

The effects of infused synthetic porcine VIP (sequence similarity to human VIP = Aviptadil) on fertility and early embryonic development were investigated by Fredericks et al. This group investigated the effects of infused Aviptadil upon reproductive function in the female rabbit. Intravenous infusions of Aviptadil (37.5, 75, and 150 pmol/kg per min) induced acute dose-dependent increases in plasma progesterone (P) but not estradiol (E2) or testosterone (T) in estrous rabbits. This P effect was not associated with an increase in plasma prolactin (Prl) and was not altered by pretreatment with a Prl-inhibiting regimen of bromocriptine. In rabbits stimulated to ovulate with 75 IU human chorionic gonadotropin (hCG) and coitus, plasma P and E2 increased, reaching a peak at 180 min following stimulation. VIP (75 pmol/kg per min) infused from 120 to 180 min following the ovulatory stimuli increased this P peak but did not effect E2 levels. This Aviptadil infusion had no effect upon fertility or upon the number of corpora lutea, uterine implants, or viable conceptuses. Infusions of Aviptadil for 60 min at the P peak, and for 240 min at the time of ovulation had no significant effect upon ovum pickup or the rate of ovum transport. These observations suggest that:

a) Aviptadil infusions in rabbits can increase plasma progesterone (P) from both the basal levels of estrus and from the peak levels preceding ovulation.

b) Infusions of Aviptadil at the time of the preovulatory steroid surge or during ovulation have little effect upon fertility or gamete transport in the rabbit.

c) Endogenous VIP may play a role in the regulation of progesterone (P) secretion in the rabbit. [Fredericks et al., 83]

Although Fredericks et al. suggested that intravenous infusion of Aviptadil can increase plasma progesterone levels, it is not expected that such an effect could occur following inhalation of Aviptadil. For the treatment of pulmonary arterial hypertension, Aviptadil will be administered to patients by inhalation in relatively low doses. Even the foreseen maximum clinical dose will not lead to significant systemic exposure. Experience from single-dose and repeated dose toxicity studies in rodents and non-rodents showed different pictures of activity of Aviptadil dependent on the route of administration. Following intravenous application, the well-known pharmacological effects like increase in heart rate and in cardiac output could be observed. These effects could not be detected after administration by inhalation [Study No.79350, 07;Study No.79426, 07].

4.2.1.6.2 Effect of Aviptadil on Embryogenesis

DRF for a study of embryo-fetal development in rabbits with Aviptadil by intravenous administration [Study No.22514, 08]

Himalayan rabbits were dosed either with vehicle (0.9% NaCl) or with Aviptadil 4 times per day with an interval of 2.5 hours between intravenous administrations to mimic the intended clinical dosing frequency. The administration was performed by slow bolus injections of Aviptadil (0.5 mL/kg body weight) into a medial ear vein in the following concentrations: 4 x 5 µg/kg, 4 x 15 µg/kg, 4 x 50 µg/kg, 4 x 150 µg/kg and 5 x 500 µg/kg) during days 6 to 20 of gestation. Although the intended route of administration is inhalation, the intravenous route has been applied to prove the influence of the test item on embryo-fetal development because results from toxicity studies have shown that Aviptadil administered by inhalation did not reach the circulation as a pharmacologically active substance [Study No.79350,07; Study No.79426,07]. No noteworthy findings were noted in the dams but reddening of the ears was reported starting at 4 x 150 µg Aviptadil/kg corresponding to 46 x the maximum clinical dose. There were no findings in the embryos with respect to corpora lutea, implantation sites, weight and number of live fetuses, placental weights, the values calculated for the pre- and post-implantation loss and the sex disturbance in the high dose group (4 x 500 µg/kg/day corresponding to 154 times the maximum clinical dose). No dead fetuses were observed; the viability of the fetuses was within the normal range during 6- to 24-hour stay in an incubator at al tested dose levels. External/internal macroscopic examination of the fetuses revealed no test-item related malformations. No variations were reported in the fetuses during external/internal macroscopic examination.

From these data the following doses were chosen for the main study:

Group 1: Control (vehicle)
Group 2: 200 µg/kg/day (4 x 50 µg/kg/administration)
Group 3: 600 µg/kg/day (4 x 150 µg/kg/administration)
Group 4: 2000 µg/kg/day (4 x 500 µg/kg/administration)

Study of embryo-fetal development in rabbits with Aviptadil by intravenous administration [Study No.21326, 08]

Following the same procedure of drug administration as described in the DRF study above, the following results were obtained in the main study:

Influence of Aviptadil administration on the dam

No test item-related local intolerance reactions were noted. No Aviptadil-related mortality was noted. No test item-related systemic intolerance was noted with regards to clinical signs. The body weights were in the normal range of the control animals. There were no impairments in food and water consumptions reported. Necropsy revealed no test item-related changes. There were slight and not statistically significant reductions (by 20% or 30%) for the gravid uterus weight of the intermediate- and high-dose dams (treated with 4 x 150 µg or 4 x 500 µg Aviptadil/day) caused by the reduced number of fetuses.

Influence of Aviptadil on the embryo/fetus

Significantly increased incidences (at $p \leq 0.01$) for total, early and/or late resorptions (including total post-implantation loss) were noted in the intermediate- and high-dosed dams (treated with 4 x 150 µg or 4 x 500 µg Aviptadil/day). The post-implantation loss was increased (30.8% or 42.0%) when compared to the control (8.9%). Hence, the number of fetuses was significantly reduced (at $p \leq 0.01$).

No test item-related influence was noted on the prenatal fetal development with respect to fetal body weights and lengths and placental weights. No dead fetuses were noted at laparatomy. The viability of the fetuses was within the normal range during a 6- or 24-hour stay in an incubator at all tested dose levels.

No test item-related malformations or variations were noted at external/internal examination skeletal examination, skeletal examination (according to DAWSON) and during soft tissue evaluation of the fetal head (according to WILSON). The external inspection of the brain revealed no changes for any of the examined fetuses after opening of the cranium and removal of the brain. No test item-related influence was noted for the incidence of skeletal retardations.

Toxicokinetics

The analysis of plasma samples revealed a dose-related exposure of the animals to Aviptadil by monitoring plasma levels after intravenous administration of 4 x 50 µg, 4 x 150 µg or 4 x 500 µg Aviptadil/kg body weight/day. Peak plasma values were measures 0.50 to 0.67 minutes after the forth administration: mean peak plasma levels of 315.14, 497.81 and 3495.05 ng/mL were measured on gestation day 6, mean plasma levels of 241.15, 773.00 and 2959.42 ng/mL were measured on gestation day 20. The toxicokinetic data revealed no evidence for an accumulation with time. The plasma elimination half-life ranged from 1.77 to 2.75 minutes.

Mean toxicokinetic parameters are presented in the table below:

Table 10: Mean toxicokinetic values obtained during the study on the influence of Aviptadil on Embryo-Fetal Development in Rabbits

Dosage [µg/kg] i.v.	Non-compartmental analysis					
	C_{max} [µg/kg]	T_{max} [min]	$T_{1/2}$ [min]	Kel [1/h]	AUC $_{0-10}$ min [ng*h/mL]	$AUC_{0-\infty}$ [ng*h/mL]
Gestation day 6						
4 x 50	315.24	0.67	2.69	15.99	16.37	17.98
4 x 150	497.81	0.50	2.75	15.36	21.16	22.77
4 x 500	3495.05	0.67	1.78	23.58	135.96	138.88
Gestation day 20						
4 x 50	241.15	0.50	2.29	18.30	11.20	11.82
4 x 150	773.00	0.50	2.09	20.54	22.06	23.22
4 x 500	2959.42	0.67	1.77	23.51	121.58	123.89

Conclusion

Under the present test conditions the non-observed–effect level (NOEL) was 4 x 50 µg Aviptadil/kg/day (corresponding to 15 times the maximum clinical dose) for the dams. From 4 x 150 µg Aviptadil/kg/day (corresponding to 46 times the maximum clinical dose) onwards, significantly increased incidences were noted for resorptions and hence, the uterus weight was decreased. These findings were regarded to be test item-related and caused by the exaggerated pharmacodynamic properties of Aviptadil being a vasodilator.

The non-observed–effect level (NOEL) was about 4 x 500 µg Aviptadil/kg/day (corresponding to 154 times the maximum clinical dose) for the fetal organism. No test item-related malformations or variations were noted during external/ visceral examination of the fetuses or soft tissue examination of the fetal heads (according to WILSON); skeletal examination (according to DAWSON) revealed no test item-related malformations, variations or retardations.

In conclusion, the test item Aviptadil possesses no teratogenic properties. No Aviptadil-related increase was noted in the incidence of malformations, variations and retardations, not even at materno-toxic dose levels when an increase in resorptions was noted.

Other studies from the public domain on the effect of VIP on embryogenesis

Studies were performed in mice and rats to examine the effects of Aviptadil on embryogenesis. The early post-implantation period (mid-gestational period) comprises in mice embryonic days E9-11 and is characterized by neural tube closure, beginning of neurogenesis, organogenesis, and conversion from yolk sac to placental nutrition. Aviptadil accelerates in vitro cultured mouse embryo at day E9.5. Blockage of Aviptadil functions during this period by treatment of pregnant mice with Aviptadil antagonists from E9 to E11 caused growth retardation of embryos. Blockage of Aviptadil after E11 did not retard growth, suggesting effects of Aviptadil limited to a very brief and specific period following implantation.

In rats, Aviptadil binding sites in the embryo were analysed during embryonic days E10-E13. Aviptadil binding was almost exclusively located to the brain and spinal cord. The investigation of expression of Aviptadil mRNA from embryo in this stage (E11) revealed totally negative results, indicating extra-embryonic, maternal Aviptadil supply to the embryo. Indeed, beginning at day E9, there is an enormous increase of Aviptadil concentration in maternal blood, 6-10 times greater than later on during pregnancy. Data indicate that Aviptadil from maternal tissues may be the source of Aviptadil acting on the embryonic tissues, transferred to the embryo undegraded. On day E17, Aviptadil mRNA expression was easily detectable all over the embryo, and maternal Aviptadil concentration in the blood decreased to very low levels. Aviptadil is required for embryonic development (Hill et al., 1996).

4.2.1.7 Local Tolerance

4.2.1.7.1 4-week intratracheal administration of Aviptadil to rats

To assess the local tolerance of Aviptadil and thereby obtaining information on the possible effects in humans following overdosing, a 4-week local tolerance study in rats with repeated intratracheal administration every other day (15 administrations per animal in total, 2 groups) has been performed according to GLP guidelines. By using the intratracheal route of administration, an accurate dose of Aviptadil, which is identical to the dose to be used clinically, can be directly applied to the lung, which is the site of actual exposure in humans. This is a major advantage compared to the common nose-only animal inhalation model since with this model only a small portion of peptide/protein drugs actually reaches the lung as indicated in a recent publication (Nadithe et al., 2003). In addition, the animal nasal system, which is different from the human nasal system, is highly exposed to the drug and a significant amount of the drug is adsorbed when the nose-only animal model is used.

In this local tolerance study the rats in group 2 received 1.65 µg Aviptadil (6.6 µg/kg/day; 33 µg/ml) per animal and per intracheal administration in an application volume of 50 µl for 4 weeks. A third group achieved 100 µg Aviptadil (400 µg/kg/day; 2 mg/ml) administered intratracheally to rats for 4 weeks to obtain information on the possible effects in humans following overdosing with the test item and to establish the toxic symptoms in respect of body weight and food consumption. The applied dose in the third group was 80 fold higher than the planned clinical single dose (300 µg per subject = 5 µg/kg/day). The control group received vehicle. No signs of systemic toxicity were noted during this study in any of the animals examined. No test item-related influence was noted on body weight, food consumption, weight of lungs or during macroscopy. The histomorphological examination of the lung and trachea did not reveal any morphological changes which are considered to be related to the test item. Under the conditions of this study, no local irritation of the lungs or trachea was noted in the animals treated intratracheally with Aviptadil (LPT Study Report No.19219/05).

4.2.1.8 Other Toxicity Studies

No specific other toxicity study has been performed. However, human data showed that Aviptadil inhibits established allergic contact dermatitis.

Eight female patients with patch-test-verified allergic contact dermatitis (erythema, oedema, papules, or vesicles) were examined. After application of allergen substance for 2 days, Aviptadil was applied topically for 1 day on the test reaction areas, at concentrations of 10^{-7} – 10^{-5} mol/l in a volume of 15 µl. After application of 10^{-5} mol/l of Aviptadil, there was a statistically significant reduction of diameters of test reactions induced by allergens (Bondesson et al., 1996). Other clinical studies performed did not reveal any incidence for Aviptadil being an inducer of contact allergies or showing sensitizing activities (see Section 2.3 Clinical Trial and Previous Human Experience Data).

4.2.1.9 Discussion and Conclusions

Intravenous administration of Aviptadil at high doses causes clinical signs like tachycardia, watery diarrhea, and decrease in blood pressure as it is known from the literature and was found in the 26-week dog study above. All these signs resolve shortly upon termination of application. Furthermore, slightly reduced motility, slight ataxia and slight dyspnoea and ptosis were observed in mice and rats.

From acute and short-term toxicology studies can be considered that administration of Aviptadil by inhalation did not lead to a systemic exposure of the drug. Thus, no clinical signs in mice and rats were detected when dosed by inhalation. However, during the 14-day inhalation toxicity study in monkeys in two animals Aviptadil levels were elevated after administration of the test item by inhalation. Aviptadil, which was tested by a RIA, measures the entire molecule of Aviptadil and in addition its natural and not natural metabolites which are known not to be pharmacological active. It was considered that the elevated levels in these animals contain mainly physiological non-active metabolites of Aviptadil due to the fact that no expected clinical signs could be observed. Histopathological findings in the respiratory tract after inhalation could not clearly be attributed to drug treatment in mice and rats. The observed histopathological findings are known to be a common side effect in animals due to the inhalation procedure. The results from the 14-day inhalation toxicity study in rats confirms the statement that histopathological findings (eosinophilic granules) observed in the acute and 10-day study are not attributable to the treatment with Aviptadil.

Aviptadil regulates embryonic growth in rodents during early postimplementation, midgestational period and accelerates the growth of mouse embryos. Blockage of the peptide during this period causes growth retardation. Maternal Aviptadil can be transferred undegraded to the embryo and is required there for development. Therefore, it can be concluded that Aviptadil is neither genotoxic, teratogenic, embryotoxic, fetotoxic, nor mutagenic in rodents (rats and mice). Alternative: In summary, Aviptadil did not exert any genotoxic, teratogenic, embryotoxic, fetotoxic, or mutagenic effects in the conducted studies.

Important information about the toxicity of Aviptadil is also derived from applications of high doses to humans. Following systemic application, the biological activity of Aviptadil is the same in animals and humans. As described in literature, relaxation of smooth muscles inducing bronchodilation, vasodilation (flushing), watery diarrhoea and increase in heart rate are well-known effects of VIP, which were observed after infusion of high doses of Aviptadil (e.g. . 65 µg/h for 10 hours) in humans.

5 EFFECTS IN HUMANS

5.1 PHARMOKINETICS AND METABOLISM

5.1.1 ABSORPTION

Studies with radiolabeled Aviptadil (single intravenous bolus injection of 300 pmol [1 µg]) demonstrate that after the initial rapid clearance from the circulation, the lung is the primary site of peptide binding. Within 30 min., about 45% of the radioactivity is found in the lungs. Only minimal activity was found in the gastrointestinal tract, and almost no activity was seen in normal liver or spleen during the observation period of 24 hours. The radioactivity in the lungs decreased at 4 hours to 25%, and after 24 hours to 10%. Radioactivity was excreted into the urine (35% of the injected dose after 4 hours, and 90% after 24 hours) (Virgolini et al., 1994). There, Aviptadil was tolerated without side effects apart from a short asymptomatic drop (10% maximum) in blood pressure.

Graded doses of 0.6, 1.3, and 3.3 pmol/kg/min of Aviptadil were infused over 30 minutes in healthy volunteers. Half-life of Aviptadil in blood was 1 minute, the metabolic clearance rate was 9 ml/kg/min and the apparent volume of distribution for Aviptadil was about 14 ml/kg.

5.1.2 DISTRIBUTION

Clinical pharmacokinetic data relevant to the distribution of Aviptadil are discussed in Section 2.3.2.2. The preferential and rapid accumulation into the lungs of intravenously administered Aviptadil was confirmed by Petkv et al. (2003).

As described under "Nonclinical Pharmacology and Toxicology Data" (see Section 2.2.2.4), Aviptadil binding to its receptors occurs in discrete locations within the gastrointestinal, respiratory and genital tracts. Aviptadil is localized on respiratory epithelium, smooth muscles of the airways, blood vessels and alveolar walls (Henning and Sawmiller, 2001). The *in vivo* distribution of Aviptadil was studied using a rat model in combination with labeled VIP (^{131}I-VIP) and a gamma-camera. The results which are described in Section 2.2.2.4 suggest that the lungs play an important role extracting Aviptadil from the circulation after intravenous administration.

The approved medicinal product containing a combination of Aviptadil and phentolamine mesylate (INVICORP™) was administered intravenously to **12** healthy subjects. The mean maximum measured plasma Aviptadil concentration was 396.6 pmol/L and occurred in a mean time of 1.4 min. The mean area under the plasma Aviptadil concentration time curve was 10.4 pmol x hr/l. The mean terminal rate constant was 25.2042 hours^{-1} equivalent to a half life of 1.7 min. After intracavernosal administration in healthy subjects the mean maximum measured plasma Aviptadil concentration was 37.2 pmol/L and occurred in a mean time of 4.3 min. The mean area under the curve was 6.1 pmol x hr/L. The mean terminal rate constant was 7.8231 hours^{-1} equivalent to a half life of 5.3 min. After intracavernosal administration in **21** hypertensive patients with erectile dysfunction, the mean maximum base line adjusted measured plasma Aviptadil concentration was 16.3 pmol/L and occurred in a mean time of 11.2 min. The mean area under the curve was 4.3 pmol x hr/L, and the half life 8.5 min. (INVICORP™ Data sheet, Douglas Pharmaceuticals LTD).

5.1.3 ELIMINATION

Only limited human pharmacokinetic data of Aviptadil are available. The physiological plasma concentration of Aviptadil is about 40 pg/ml (Petkov et al., 2003). After injection of 1 µg radioactively labelled Aviptadil as bolus to patients a very rapid tissue distribution was observed. Within 30 minutes about 45% of the radioactivity was found in the lungs. Over an observation period of 24 hours only minimal activity was detected in the gastrointestinal tract and almost no activity was found in the liver or spleen. Radioactivity in the lungs decreased within four hours to 25% and within 24 hours to 10%. The half-life of Aviptadil in plasma is about 1-2 minutes. After injection of radiolabelled Aviptadil radioactivity was almost completely eliminated by the kidneys, 35% within 4 hours, and 90% within 24 hours (Virgolini et al., 1994).

After intravenous infusion a bi-exponential decline of the Aviptadil plasma level was reported, with half-life of 2 and 21 minutes, respectively. The short half-life of 2 minutes reflects the fast tissue distribution and accumulation in the lung; the longer half-life reflects the elimination by metabolic degradation and corresponds to a metabolic clearance of 42 ml/kg min^{-1} and an estimated virtual volume of distribution of 135 ml/kg (Virgolini et al., 1994).

5.1.4 PHARMACOKINETICS OF ACTIVE METABOLITES

There are no known active metabolites.

5.1.5 PLASMA CONCENTRATION-EFFECT RELATIONSHIP

Aviptadil will be applied as inhalation therapy to the patients. From the pharmacological experiments in animals, the half-life of VIP after pulmonary application in the lung has been established at 19 minutes and no active substance was found in the plasma. Therefore, it is quite conceivable that the plasma concentrations will remain low after inhalation. In addition, Aviptadil is accumulating in the lung after intravenous application with half life of 1 min. There, the peptide is metabolically degraded in inactive products.

5.1.6 DOSE AND TIME-DEPENDENCIES

The acute response to Aviptadil and NO was tested on a population of patients with PAH (Petkov et al., 2003). Administration of 100 or 200 µg of inhaled Aviptadil improved pulmonary haemodynamics in all patients tested (MPAP decreased, CO increased and SvO2 increased). Systemic arterial pressure and pulmonary capillary wedge pressure did not change significantly. All patients demonstrated a negative acute vasodilator response to NO.

A following study named AVICUTE (EudraCT Nr. 2004-003757-70) performed by Leuchte et al., (2008) on patients with pulmonary hypertension, confirmed the a§cute effects of a single inhaled dose (100 µg) of Aviptadil on haemodynamics, i.e. pulmonary vasodilation, stroke volume and blood gases, i.e. mixed venous oxygen saturation. These were qualified as modest and short-lived but significant. Acute effects of higher doses than 200 µg per inhalation are unknown as other clinical studies AVISARCO (EudraCT-Nr. 2004-003759-38), AVIFIBRO (EudraCT Nr. 2006-002174-22) and the study by Petkov et al., (2003) used 50 or 100 µg inhalation doses.

The long-lasting responses to VIP were assessed in 28-day and 24-week studies (Prasse et al., 2010; Petkov et al., 2003 and AVIFIBRO study by mondoBIOTECH) and demonstrated, for certain studies, long-lasting benefits such as improved pulmonary haemodynamics in all patients tested (mean pulmonary artery pressure decreased, cardiac output and mixed venous oxygen saturation increased) or modulation of inflammation biomarkers (Prasse et al., 2010).

Dose-dependent acute effects of inhaled Aviptadil were seen in a PAH patient as shown in the figures below

Figure 3: Dose-dependent acute effect of inhaled Aviptadil on mean pulmonary artery pressure in a PAH patient

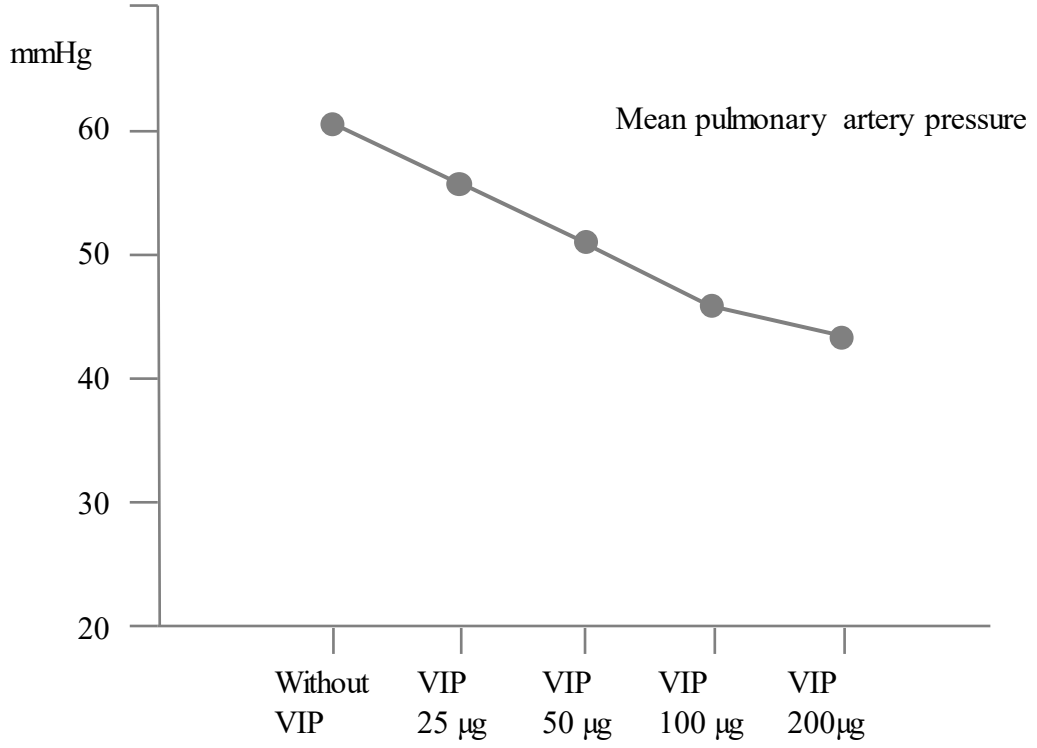


Figure 4: Dose-dependent acute effect of inhaled Aviptadil on cardiac output in a PAH patient

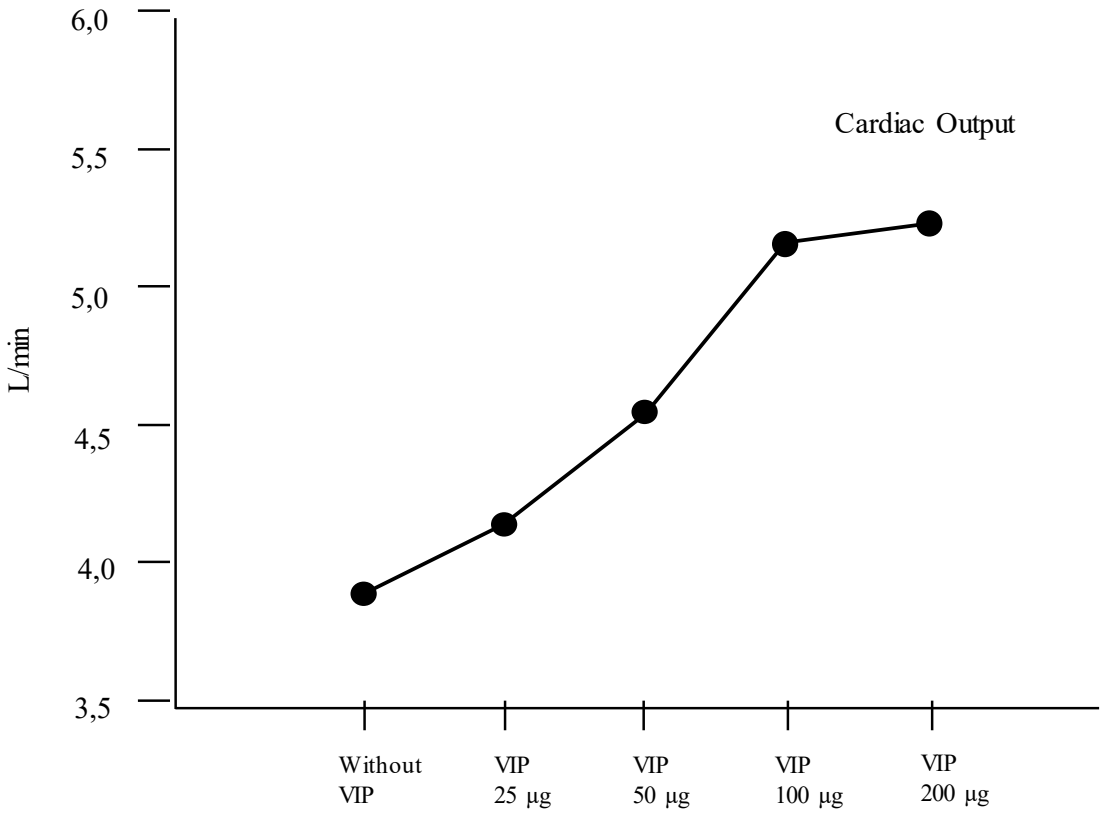
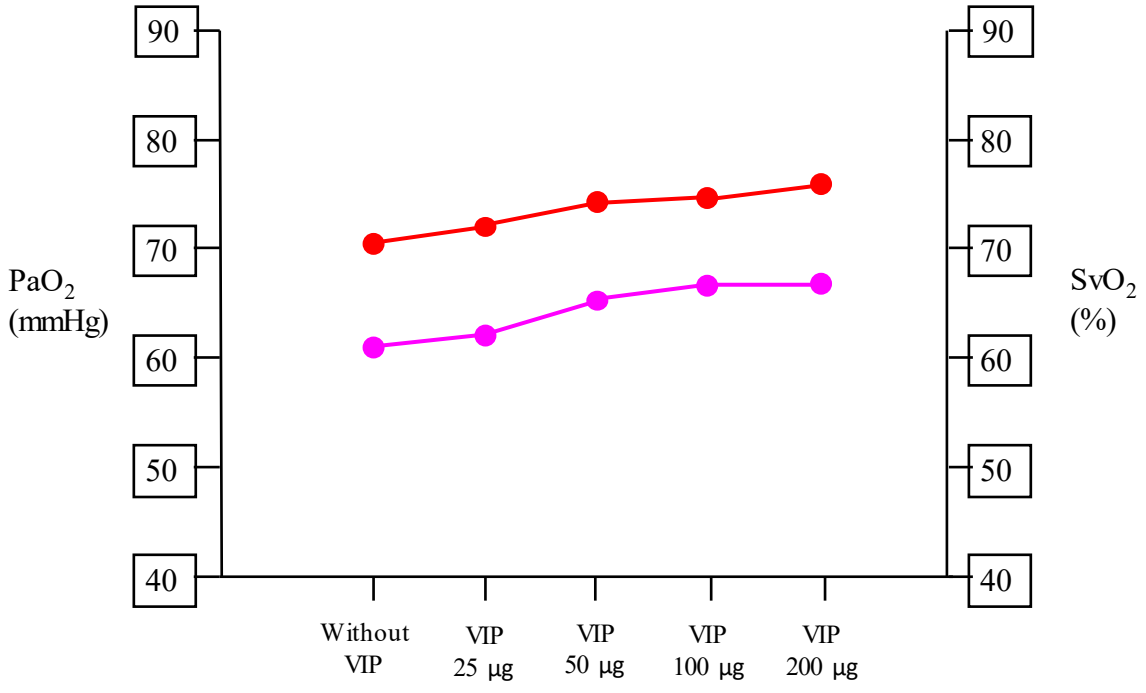


Figure 5: Dose-dependent acute effect of inhaled Aviptadil on blood oxygen content in a PAH patient



Based on these data, it appears that a plateau is reached at a single dose of 50-100 µg. Therefore it seems reasonable to compare the effect of 200 µg/day (4 x 50 µg) and 400 µg/day (4 x 100 µg) of Aviptadil administered for 3 months.

No acute data on the pharmacodynamic half-life of Aviptadil have been generated so far. However, based on the observed clinical effect in the study in PPH patients, the same dosage regimen (4 inhalations a day) has been selected.

5.1.7 SPECIAL PATIENT POPULATIONS

No specific studies in special patient populations have been performed so far.

5.1.8 INTERACTIONS

Clinical studies have shown that Aviptadil administered by inhalation does not reach the systemic circulation and is mainly metabolized in the lung by endopeptidases. Therefore, pharmacokinetic interactions with Aviptadil are not to be expected.

With regard to the approved medicinal product containing Aviptadil and phentolamine mesylate (INVICORP^{TM}), no clinically relevant interaction was observed when INVICORP^{TM} was administered concomitantly to anti-hypertensive drugs or other cardiovascular drugs. (INVICORP^{TM} Datasheet, Douglas Pharmaceuticals LTD).

5.2 CLINICAL PHARMACOLOGY

5.2.1 MECHANISM OF PRIMARY ACTION

There are two mechanisms of primary action, one is a direct effect on vasculomotor tone by acting on vessels smooth muscle cells relaxation, and the second is the induction of an immunomodulatory and immunotolerogenic response reducing inflammation and T cells activation. Both effects are of importance for pulmonary diseases.

5.2.2 PULMONARY CIRCULATION

After intravenous infusion of Aviptadil an increase of heart rate, stroke volume and cardiac output was reported. Right atrial and pulmonary wedge pressure remained unchanged while pulmonary vascular resistance significantly decreased as well as pulmonary arterial systolic pressure (Petkov et al., 2003; Soderman et al., 1993).

Aviptadil vasodilating properties are 50 times more potent than prostacyclin and independent of the endothelium (Barnes et al., 1986, Groneberg et al., 2006). These vasodilating properties result in a primary positive inotropic effect on cardiac muscle that is enhanced by its ability to facilitate ventricular-vascular coupling by reducing mean arterial pressure by 10-15%. When administered by inhalation acute Aviptdil caused a small and temporary but significant selective pulmonary vasodilation, an improved stroke volume and mixed venous oxygen saturation (Leuchte et al., 2008). The pulmonary vasodilating effect of aviptadil

was not accompanied by any side-effects nor systemic blood pressure drop. Similarly, no drop of systemic blood pressure, increase of heart rate or tachycardia events have been observed in the 28-day trial with inhaled Aviptadil in pulmonary sarcoidosis patients, suggesting that not enough VIP passed into the systemic circulation to induce global vasodilating or inotropic effects (Prasse et al., 2010).

5.2.2.1 Immunotolerogenic response

In Pulmonary Sarcoidosis, the presumed primary therapeutic mechanism of action of inhaled Aviptadil is a combination of anti-inflammatory properties and induction of tolerogenic immune response of immune cells localized in the lungs (Prasse et al., 2010, Ran et al., 2015). In contrast to global immune-suppressors that affect the entire body immunity, no systemic effect were observed following VIP inhalation as documented by unchanged production of cytokines from peripheral blood monocytes such as sIL-2R or neopterin level (Prasse et al., 2010).

5.2.3 SECONDARY PHARMACOLOGIC EFFECTS

5.2.3.1 Systemic circulation

In healthy volunteers intravenous Aviptadil reduced systemic vascular resistance due to its potent vasodilatory effects, followed by increase of heart rate and decrease of blood pressure. Cardiac contractility and stroke volume increased. Furthermore cutaneous flushing was often reported. These data document potent vasodilatory and inotropic actions of Aviptadil (Frase et al., 1987; Bennett et al., 2003; Eriksson et al., 1989; Thom et al., 1987; Unwin et al., 1987).

5.2.3.2 Airway Responses

During intravenous infusion (20 ng Aviptadil /kg/min, 30 min), ventilation – perfusion relationships of the lungs (VA/Q) were significantly changed. VA/Q distributions determined by inert gas elimination technique were shifted to lower values but arterial oxygen tension remained unchanged. Therefore Aviptadil alters the ventilation – perfusion distributions but generates no shunt and does not cause hypoxia (Soderman et al., 1993). Aviptadil has no effect on the ventilatory response to carbon dioxide (Morice et al., 1986) and does not cause a change in specific airways conductance(Barnes and Dixon, 1984; Palmer et al., 1986). Infused Aviptadil (6 pmol/kg/min, 15 min) increased FEV_1 in asthmatic volunteers. When bronchoconstriction with histamine was induced in these patients it was ameliorated by Aviptadil when compared with placebo (Morice et al., 1983). This finding could not be supported by Altiere et al. (Altiere et al., 1984).

Inhaled Aviptadil (70 µg) furthermore antagonised the bronchoconstrictory effect of propranolol in asthmatic patients (Crimi et al., 1988). Infusion of Avipatdil (6 pmol/kg/min, 70 min) to patients (n=8) recovering from severe asthma increased peak expiratory flow rate by 26 + 9 (SEM) l/min, but to a lesser degree than seen with salbutamol (5 µg/min; (Morice and Sever, 1986)).

5.2.3.3 Effects on renal function

After intravenous infusion (90 min) of Aviptadil (6 pmol/kg/min) no changes in effective renal plasma flow or glomerular filtration rate could be observed. Urine flow was reduced to about 30% and the fractional

excretion of sodium, potassium, chloride, and calcium decreased to between 50 and 65% of control values. Plasma renin activity increased about 3-fold during the infusion (Calam et al., 1983).

5.2.3.4 Gastrointestinal effects

Aviptadil had no effect on acid secretion when infused (0.5-2.7 µg/kg/h, 1h) in combination with pentagastrin (100 ng/kg/h) in healthy volunteers (n=6). It had also no effect on meal (peptone solution) stimulated gastric acid secretion (Holm-Bentzen et al., 1983). Infused Aviptadil inhibited dose-dependently the response of the lower esophageal sphincter to an intravenous injection of pentagastrin but did not substantially decrease basal lower sphincter pressure (Domschke et al., 1978b).

Aviptadil infusions to healthy volunteers (100, 200 and 400 pmol/kg/h) caused a dose-dependent decrease of water and sodium absorption. Chloride absorption changed to secretion whilst bicarbonate movement remained unchanged (Krejs et al., 1980). When Aviptadil was infused for 10 hours (400 pmol/kg/h) in 5 healthy volunteers, watery diarrhoea developed within 2 to 6 hours. The large faecal bicarbonate loss induced hyperchloremic metabolic acidosis (Kane et al., 1983).

5.2.4 PHARMACODYNAMIC INTERACTIONS

INVICORP™ is an approved medicinal product containing Aviptadil (in combination with phentolamine mesylate for intracavernosal injection for the treatment of erectily dysfunction). In the experience of the use of INVICORP™ with antihypertensives or other cardiovascular drugs, no clinically relevant interactions have been observed. Aviptadil is not known to be incompatible with any medicinal products (INVICORP™ Datasheet, Douglas Pharmaceuticals LTD).

Safety data obtained during AVIFIBRO trial (EudraCT-No: 2006-002174-22), a 24-week trial assessing the benefit of inhaled Aviptadil in patients with pulmonary fibrosis, co-treated with glucocorticoids and/or immunosupresant might suggest that co-administration of Aviptadil with other immunosuppressive agents favours the development of nasopharyngitis and pulmonary infections.

In the planned clinical trials in pulmonary sarcoidosis patients' population, Aviptadil will be used as a stand alone treatment as no medication has formely received EMA or FDA approval for such indication.

In other indications such as pulmonary arterial hypertension (PAH), Aviptadil will be used on top of oral treatments approved for this indication (i.e. bosentan and/or sildenafil). Although Aviptadil has a different mechanism of action compared to bosentan or sildenafil, both Aviptadil and bosentan or sildenafil will finally lead to an increase in cAMP and/or cGMP levels of the smooth muscle cells of the pulmonary vascular system. Combination of prostacyclin which also increases cAMP levels, with an endothelin-1 receptor antagonist has already shown an add-on effect compared to the single agents in a clinical trial. Such an add-on effect has also been clinically demonstrated for the combination of bosentan and sildenafil.

For these reasons, it is not expected that pharmacodynamic interactions would lead to an increased risk for the patients participating in the clinical development programs with Aviptadil.

5.3 OVERVIEW OF EFFICACY [ACUTE RESPIRATORY DISTRESS SYNDROME]

5.3.1 ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

5.3.1.1 Open label Dose Escalation Study

The objective of this Phase I study is to obtain preliminary data, in a open-label study, on the safety and efficacy of IV infused Aviptadil in patients with ARDS complicating sepsis.

The trial was conducted in patients with ARDS complicating the sepsis syndrome. Such patients may or may not have evidence of other organ dysfunction. Although a window of 24-48 h often exists from the time sepsis/septic shock is diagnosed until severe lung and other organ injury occurs, organ injury may develop rapidly and some degree of lung injury may already be present when sepsis is first diagnosed. By limiting the study population to patients with antecedent or associated sepsis/septic shock excluding those with other risk factors for ARDS such as trauma, drug overdose, acid aspiration, and inhaled toxins, the study group was expected to be more homogeneous and well defined.

All patients entered into this trial had the diagnosis of ARDS in the setting of the sepsis syndrome, by recent consensus definitions. Patients were to be observed for a 24-h period, during which time all inclusion criteria had to be met. If all criteria had been met once (not necessarily simultaneously), the patient was to be enrolled, and received the study drug within 12 h of the entry criteria being fulfilled:

Main inclusion criteria were:

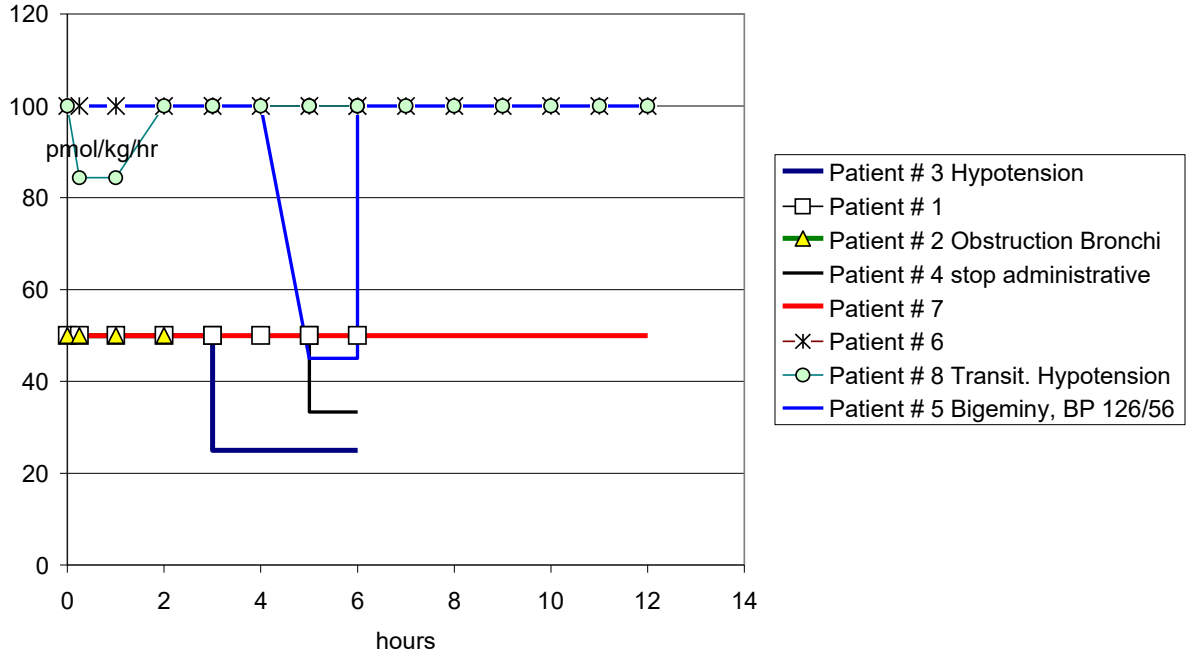
Sepsis / septic shock
ARDS
Hypotension
Inadequate organ perfusion or function

The Aviptadil dosing per patient over time is shown in Figure 6**.**

In the lower dose group with 50 pmol/kg/hr (5 patients) Aviptadil administration was stopped in one (bronchial obstruction due to hypersecretion considered to be unrelated to Aviptadil) and the dose halved in another (Hypotension). In a third case the administration was stopped early in order to keep some of the i.v. solution for analysis.

In the high dose group with 100 pmol/kg/hr (3 patients) Aviptadil administration was stopped in none. However, dose was transitorily reduced in two, due to hypotension (1 case) or to bigeminy (1 case).

Figure 6: VIP Dosing per Patient



The objective of this Phase II study was to obtain preliminary data, in an open-label study, on the safety of Aviptadil, infused iv. (50 or 100 pmol/kg/hr, infusion time 6 to 12 hr) in patients with ARDS induced by sepsis. However, in the majority of the patients the picture was more complex; e.g. in 5 cases there was surgery preceding the infection. Consistent with the polymorbidity of the patients admitted to the trial, they also received a large number of concomitant medications and other supportive measures. This makes any interpretation difficult.

During the administration of Aviptadil, cardiovascular side effects (mainly hypotension; two cases at 100 pmol/kg/hr and 1 at 50 pmol/kg/hr) and watery stools were observed (one case at 100 pmol/kg/hr). These are probably related to Aviptadil but the number of cases treated is insufficient and too heterogeneous to draw any conclusions concerning dose dependence of these adverse events.

Two patients died during the follow-up period. One case was Patient # 5, aged 87 years, male. Three weeks after Aviptadil the patient suffered from massive acute or subacute right-sided middle cerebral artery infarct. Supportive measures were stopped at the request of the family.

The other case was Patient # 2, aged 80 years, male. The patient received the Aviptadil infusion for only 2 of the intended 6 hours because oxygen saturation dropped to 85%. The patient at this point was suctioned from the endotracheal tube and ventilated with an AMBU bag + bronchodilator nebulizer treatment. The endotracheal tube yielded large amount of thick, purulent secretions. The arterial oxygen saturations improved to baseline of 90-92%, but the Aviptadil infusion was not restarted. He died 4 days later; the death was considered an unrelated SAE.

A mortality of 25% is well within the expected mortality in septic ARDS. Until recently, most studies of acute lung injury and the acute respiratory distress syndrome have reported a mortality rate of 40 to 60%, although some reports suggest that mortality from this disease may be decreasing. The majority of deaths are attributable to sepsis or multiorgan dysfunction rather than primary respiratory causes

An overview of the adverse events observed in these severely diseased patients is presented in the following table. It is very likely that the adverse events of hypotension and diarrhoea are attributable to Aviptadil after IV administration.

Table 11: Safety data as recorded in "VIP in ARDS/Sepsis, Open Dose Escalation Study"

Patient No.	AEs	Description of Adverse Event	Severity	Serious	Unexpected	Course	Relationship to Aviptadil
CRF # 1	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
CRF # 2	Yes	Hypoxemia, acute	Severe	Yes	No	N.A.	Unlikely
		Death	extreme	Yes	N.D.	N.A.	No
CRF # 3	Yes	Hypotension	Mild	No	No	disappeared after intervention	Possible
CRF # 4	Yes	Seizures	Severe	Yes	yes	N.D.	No
CRF # 5	Yes	Bigeminy	moderate	No	No	disappeared after intervention	Probable
		Diarrhea	Mild	No	Yes	Spont.disapp.	Probable
		Death	Extreme	Yes	No	N.A.	No
CRF # 6	Yes	Seizures	N.A.	No	Yes	N.D.	No
CRF # 7	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
CRF # 8	Yes	Hypotension	moderate	No	No	disappeared after intervention	Probable
		Pneumothorax	Severe	yes	yes	disappeared after intervention	No

5.4 OVERVIEW OF SAFETY

5.4.1 TABLES OF STUDIES IN HEALTHY VOLUNTEERS AND PATIENTS

Clinical studies investigating the use of Aviptadil in healthy volunteers and in patients are presented in the following tables.

Table 12: Studies Performed with Healthy Volunteers

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Aviptadil in man: pharmacokinetics, metabolic and circulatory effects Domschke S *et al,* 1978	IV infusion 0.6, 1.3, 3.3 pmol/kg/min 30 min	4	T 1/2 : 1 min (in blood) Metabolic clearance rate: 9 ml/kg/min Apparent volume of distribution: 14 ml/kg ↓ of heart rate and amplitude of blood pressure ↑ glucose, free fatty acids, calcium plasma concentration with the highest dose	Cutaneous flushing
Effects of Aviptadil on resting and pentagastrin-stimulated lower esophageal sphincter pressure Domschke W *et al,* 1978	IV infusion 0.8, 1.6, 3.2 µg/kg/h 30 min	4	Dose-dependent ↑ in pulse rate ↓ of the systemic diastolic blood pressure	Cutaneous flushing
Effect of Aviptadil infusion in water and ion transport in the human jejujum Krejs *et al,* 1980	IV infusion after fasting for 12 h 100, 200, 400 pmol/kg/h	22	↑ heart rate at high dose no othe ECG change No blood pressure change Chloride secretion (against the gradient) at the highest dose ↓ absorption sodium, chloride and potassium	Flushing
Effect of Aviptadil on meal stimulated gastric acid secretion in man Holm-Bentzen *et al,* 1983	IV infusion 2 h after fasting for 12 h 1 µg/kg/h	6	↑ heart rate no change in the hematocrit or the blood pressure	None
Effect of Aviptadil on renal function in man Calam *et al,* 1983	IV infusion 90 min after fasting for 12 h 6 pmol/kg/min	6	↑ heart rate ↓ blood pressure no change in the renal plasma flow or glomerular filtration rate ↓ urine flow, osmolal clearance rate, fractional excretion of sodium, potassium, chloride and calcium ↓ urinary pH	Facial flushing

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Production of secretory diarrhea by IV infusion of Aviptadil Kane *et al*, 1983	IV infusion 10 h 400 pmol/kg/h	5	↑ heart rate no change in systolic blood pressure, no cardiac arythmias ↓ diastolic blood pressure ↑ potassium, chloride serum levels ↓ bicarbonate serum level no change in glucose and calcium serum levels	Flushing Watery diarrhea after 4.3 h infusion (no diarrhea anymore after Aviptadil infusion stop)
Aviptadil stimulation of prolactin release and rennin activity in normal man and patients with hyperprolactinemia: effect of pretreatment with bromocriptine and dexamethasone Lightman *et al,* 1984	IV infusion 90 min 65 pmol/min	6	↑ heart rate ↑ plasma rennin activity ↑ circulating prolactin concentrations	None
A comparison of ventilatory, cardiovascular and metabolic effect of salbutamol, aminophylline and Aviptadil Morice *et al,* 1986	IV infusion 30 min 6 pmol/kg/min	6	No increase of the minute ventilation. No change in systolic blood pressure ↓ diastolic blood pressure (74 to 68 mmHg) ↑ mean plasma adrenaline no change in noradrenaline	None
Effect of infused Aviptadil on airway function in normal subjects Palmer *et al*, 1986	IV infusion 15 min 1, 3, 6 pmol/kg/min	6	↑ heart rate ↓ systolic blood pressure no change in specific airway conductance	None

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Cardiovascular effects of Aviptadil in healthy subjects Frase *et al,* 1987	IV infusion 100 min After fasting for 12 h 400pmol/kg/h	6	No arhytmias or ECG changes other than sinus tachycardia. ↓ peripheral vascular resistance no change in systolic blood pressure ↓ diastolic pressure ↓ Cardiac output ↑ left ventricular contractlity	Facial flushing Watery diarrhea in 2 subjects
Action of calcitonin gene related peptide and Aviptadil as vasodilators in man Thom *et al,* 1987	IV infusion 10, 30, or 100 ng/min Dose escalation with 5 min infusion at each dose level into the studied arm	10	Aviptadil is a potent vasodilator in the human forehand vascular bed	None
Effects of indomethacin and propranolol on the cardiovascular and rennin responses to Aviptadil infusion in man Unwin et al, 1987	IV infusion 30 min 6 pmol/kg/min	6	↑ heart rate ↑plasma rennin activity ↓ forearm vascular resistance no change in mean arterial blood pressure, plasma arginine vasopressin concentrations	Cutaneous flushing
Influence of Aviptadil on splanchic and central hemodynamics in healthy subjects Eriksson *et al*, 1989	IV infusion 45 min for each dose 5 and 10 ng/kg/min	6	↑ cardiac output ↑ heart rate ↓ mean systemic arterial blood pressure and vascular resistance	None
Ventilatory effects of substance P, Aviptadil and nitroprusside in humans Maxwell *et al*, 1990	IV infusion at increasing rates at 3 min intervals 1, 3, 6 pmol/kg/min	6	↓ mean arterial blood pressure ↑ heart rate no change in minute ventilation, oxygen consumption, carbon dioxid, and arterial oxygen saturation	None

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Effect of Aviptadil on pulmonary ventilation-perfusion relationship and central hemodynamics in healthy subjects Soderman *et al*, 1993	IV infusion 30 min After overnight fasting 20 ng/kg/min	9	↑ noradrenaline ↑ cardiac output ↑ heart rate ↑ stroke volume ↓ radial arterial blood pressure ↓systemic and pulmonary vascular resistance no change in the right atrial blood pressure, pulmonary arterial blood pressure, pulmonary arterial wedge pressure ↑ mixed venous oxygen tension no shunt, hypoxemia observed during the 30 min infusion	Facial flushing
Evidence for a role for Aviptadil in active vasodilatation in the cutaneous vasculature of humans Bennet *et al*, 1999	Intradermal microdialysis for about 50 min 7.5 µmol to 4 skin areas	8	↑ cutaneous vascular conductance	

Table 13: Studies Performed with Patients

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Idiopathic Pulmonary Arterial Hypertension				
Aviptadil in IPAH Petkov *et al,* 2003	Inhalation Total dose a day: 200µg four times a day Up to 6 months	20	After 3 months: ↑ exercise capacity in the 6-MWT test ↓ dyspnea index ↓ pulmonary artery blood pressure ↑ cardiac output ↓ pulmonary vascular resistance ↑ mixed venous saturation no change in the systemic arterial blood pressure, the heart rate and pulmonary capillary wedge pressure	None
Examination of pulmonal hemodynamics in patients with pulmonary hypertension following inhalation of Aviptadil Leuchte *et al,* 2008 AVICUTE, EudraCT-N°: 2004-003757-70	Inhalation Single dose of 100 µg of Aviptadil	20 Patients 9 IPAH 8 PH in lung disease 3 chronic thromboembolic PH	Aviptadil aerosol caused a small and temporary but significant selective effects: ↑ pulmonary vasodilatation ↑ stroke volume ↑ mixed venous oxygen saturation ↓ Pulmonary vascular resistance No change in the systemic arterial blood pressure	No side effects were observed in 19 of the 20 treated persons. One patient has an AE probably not related to Aviptadil

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Pulmonary Sarcoidosis				
Influence of inhaled Aviptadil on the immunologic activity of alveolar macrophages in Pulmonary Sarcoidosis Prasse *et al,* 2010 AVISARCO EudraCT N°: 2004-003759-38	Inhalation (nebulizer) 200 µg/day, splitted in 4 equal inhalations of 50 µg each for 28 days	20	Biochemical analyses of bronchoalveolar labage (BAL): ↓ MIP-1a and TNF-a cytokines ↑ bronchoalveolar Treg population ↓ CD4+/CD8+ ratio Improvement of cough and dyspnoea spontaneously reported by patients No effect on pulmonary function tests No change in heart rate, blood pressure	No SAEs 12 patients reported 21 mild AEs Most AEs are related to BAL procedure
Idiopathic Pulmonary Fibrosis				
Influence of inhaled Aviptadil on CCL18 serum concentrations in patients with pulmonary fibrosis AVIFIBRO EudraCT-No: 2006-002174-22 Relief Therapeutics Holding SA, unpublished data	Inhaled (nebulizer) 300 µg/day, split into 3 equal inhalations of 100 µg for 24 weeks Placebo controlled, centrally randomized 2:1.	20	Addition of VIP to the standard therapy did not cause a detectable change of CCL-18 levels No change in the 6-MWT No change in the pulmonary function tests, blood gases, diffusing capacity and compliance after 24 weeks No differences between treatments in global assessments of efficacy and of tolerability by investigators or patients No change in haematology and blood chemistry	3 SAEs in the Aviptadil group None in placebo Uncertain relationship between SAEs and Aviptadil 82 AEs reported in Aviptadil group vs. 56 in placebo group. Most common AEs concern respiratory disorders : acute nasopharyngitis and respiratory infection

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Erectile dysfunction				
A clinical trial of intracavernous Aviptadil to induce penile erection Roy et al, 1990	Intracavernosal injection 200 or 400 pmol/injection in three consecutive weekly visits	24	Dose-related increase in penile length, diameter and rigidity. None of the patients achieved penile rigidity adequate for intromission.	No local or systemic side effects
Intracavernous self- A clinical trial of intracavernous Aviptadil to induce penile erection Gerstenberg *et al,* 1992	Intracavernosal injection 30 µg Aviptadil combined with either 0.5 mg, 1 mg or 2 mg phentolamine mesylate Median duration of treatment was 6 months (range 1 to 22)	52	All patients obtained erection sufficient for penetration. Following ejaculation rigidity decreased normally. No change in the blood pressure or heart rate	Facial flushing No priapism 9 patients discontinued treatment. One patient died of a myocardial infarction not associated with this therapy
A 6 month multi-center placebo controlled study of INVICORPTM in the treatment of non-psychogenic erectile dysfunction Data presented by Hackett at 1998 AUA meeting	25 µg Aviptadil combined with either 1 or 2 mg phentolamine mesylate for intracavernosal injection	548	Improved quality of life	No SAEs Mild and transient flushing (37% of injection) 2 cases of priapism

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Treating men with predominantly nonpsychogenic erectile dysfunction with intracavernosal Aviptadil and phentolamine mesylate in a novel auto-injector system: a multi-center double-blind placebo-controlled study Dinsmore *et al,* 1999	Intracavernosal injection 25 µg Aviptadil combined with either 1 or 2 mg phentolamine mesylate	236	Improved quality of life	Facial flushing (40% of injections
Asthma				
Comparative effect of inhaled Isoprotenol and Aviptadil on histamine-induced bronchoconstriction Altiere *et al*, 1990	Inhalation 28µg or 375µg Isoproterol 375 µg Aviptadil	2	Isoproterol produced bronchodilatation in the asthmatic subject and protected against histamine-induced bronchoconstriction while VIP has no effect	None
Effect of inhaled Aviptadil on bronchial reactivity to histamine in humans Barnes *et al*. 1984	Inhalation (nebulizer) 100µg Aviptadil (single dose, acute testing)	6	No change in blood pressure, heart rate No significant effect on baseline airway function Significant protection against histamine-induced bronchoconstriction in subjects with bronchial hyperactivity	None
Effect of inhaled Aviptadil and propranolol-induced bronchoconstriction Crimi *et al,* 1988	Inhalation 70 µg Aviptadil (single dose, acute testing)	6	No significant effect on baseline airway	None

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Effect of inhaled Aviptadil on bronchomotor tone and bronchial responsiveness to inhaled histamine in 6 atopic asthmatic subjects Bundgaard *et al*, 1983	Inhalation 100µg Aviptadil 200µg Salbutamol control solution: 1% human serum albumin, 0.9% saline (single dose, acute testing)	6	Specific airway conductance did not change after control or Aviptadil inhalation but significantly ↑ after salbutamol inhalation The provocation concentration of histamine causing a 35% ↓ in specific airway conductance did not change after control inhalation but significantly ↑ after Aviptadil and salbutamol inhalation. Salbutamol effect is significantly greater than Aviptadil effect. No change in heart rate or blood pressure	None
Aviptadil causes bonchodilatation and protects against histamine-induced bronchoconstriction after IV administration in asthmatic subjects Morice *et al*, 1983	IV infusion 15 min 6 pmol/kg/min Aviptadil	7	↑ heart rate ↓ diastolic pressure Bronchodilatation Aviptadil ameliorated histamine-induced bronchoconstriction	Cutaneous flushing
Aviptadil as bronchodilatator in severe asthma Morice *et al*, 1986	IV infusion 30 min 6 pmol/kg/min Aviptadil	2 groups of 8 patients recovering from severe acute asthma	Aviptadil caused a significant ↑ in peak expiratory flow rate (compared to the bronchodilatation seen with 5 µg/min salbutamol) Aviptadil caused a significant brochodilatation following pretreatment with ipratropium bromide	None

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Aviptadil coronary hemodynamics				
Coronary hemodynamic effects of intravenous Aviptadil in humans Feliciano *et al*, 1998	IV infusion 100 pmol/kg/h for 12 min then 50 pmol/kg/h during the hemodynamic measurements and blood sampling; immediately after, 200 pmol/kg/h for 12 min then 100 pmolkg/h during the next set of hemodynamic measurements and blood sampling	16 patients referred to cardiac catherization and coronary arteriography for chest pain syndrome (5 patients placebo)	↑ heart rate ↓ mean blood pressure ↑ cardiac output right arterial pressure, pulmonary arterial and pulmonary capillary wedge pressure unchanged ↓ systemic, coronary and pulmonary vascular resistance Aviptadil effects seems to be mediated via prostaglandins	Mild facial flushing No SAEs
Liver disease				
Effect of Aviptadil infusion on renal function in patients with liver disease Calam et al, 1983	IV infusion for 90 min 6pmol/kg/min Aviptadil	6 patients with biopsy-proven liver cirrhosis	↑ heart rate ↓ mean blood pressure ↑ plasma rennin activity ↓ urine flow The presence of severe liver diseases may affect renal response to Aviptadil	None

Study title / Reference	Treatment regimen (route/dose of admin, duration)	Subjects	Results	Adverse Events
Venous ulcers				
Venous ulcer: improved healing by iontophoretic administration of calcitonin gene-related peptide and VIP Gherardini *et al*, 1998	Iontophoretic, transdermal administration of calcitonin gene-related peptide (CGRP) and Aviptadil for 12 weeks (3 times per week) Drug reservoir of 3 ml CGRP: 3 X 10^{-9}M/ml Aviptadil: 3 X 10^{-5}M/ml	32 patients with venous stasis ulcers	Significant ↓ of the mean ulcer surface area with the combination: Aviptadil -calcitonin gene-related peptide	

5.5 MARKETING EXPERIENCE

Aviptadil has been approved in Denmark, New Zealand and THE United Kingdom for the treatment of erectile dysfunction. Aviptadil in combination with the adrenergic antagonist phentolamine mesylate (INVICORPTM; an intracavernous injection formulation for the treatment of erectile dysfunction) has been granted marketing approval in Denmark (July 14, 1998, where it was re-introduced on March 16, 2016 under the name Procivni), New Zealand (April 5, 2000) and in the United Kingdom (October 13, 2000, and was re-introduced in the year 2016) and in Finland (August 4, 2015) for the treatment of erectile dysfunction. The approved medicinal product is a combination of 25 µg Aviptadil with either 1 mg or 2 mg of phentolamine mesylate.

6 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

Aviptadil for the treatment of acute and chronic pulmonary diseases seems to be a promising approach to achieve those objectives. At that level of its development path it is possible to state that the benefits which this compound could offer to the patients in their clinical condition and its presumably favourable safety profile exceed any risks which might occur during the Aviptadil development program.

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