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# Preclinical safety profile of sildenafil

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**Sildenafil citrate, marketed as Viagra®, for the treatment of erectile dysfunction, has a proven record of safety in humans as predicted by the results of extensive pharmacological and toxicological testing in animals and *in vitro*, and confirmed by pharmacokinetic exposure data. The aim of this paper is to review succinctly the main findings resulting from these experiments. Daily doses of sildenafil, within and far beyond the human therapeutic range, were given to dogs and rodents for up to 1 and 2 y, respectively. Plasma analyses were conducted to determine the exposure to sildenafil. We found species-specific effects in dogs (Beagle pain syndrome), mice (marked intestinal dilatation) and rats (adaptive reversible hepatocellular hypertrophy associated with secondary thyroid hypertrophy). All these effects in rodents and dogs have no relevance to humans. Morphometric thickness measurements of the retinal layers carried out in response to clinical observations of visual disturbances in humans indicated no difference between treated and control rats and dogs after up to 24 months of treatment. There was no evidence of histopathologic damage to any structures of the visual pathway. Sildenafil had no effects on fertility, no teratogenic potential, was not genotoxic and has no carcinogenic potential. In rats and dogs, safety ratios were 40:1 and 28:1, respectively, in terms of exposure over 24 h ($AUC_{24\,h}$) and 19:1 and 8:1, respectively, in terms of peak plasma concentration ($C_{max}$). These safety ratios illustrate the separation between exposure to sildenafil of animals at large nontoxic doses and the much smaller human therapeutic exposure. This profile highlights the very low risk of human toxicity for sildenafil. The favourable results of the nonclinical safety evaluation of sildenafil in established animal models have been confirmed by many years of clinical experience during the development and marketing of sildenafil.**
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## Introduction

Sildenafil (short for sildenafil citrate) is the citrate salt of 1-(4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo(4,3-d)pyrimidine-5-yl)phenyl-sulphonyl)-4-methylpiperazine. The drug has been developed for the treatment of erectile dysfunction. Overall, more than 130 *in vivo* animal and *in vitro* toxicology, safety pharmacology and toxicokinetic studies have been conducted using rigorous study protocols developed and validated by nationally and internationally recognized scientific toxicology associations (Toxicology Working Groups of ICH, American Society of Toxicology, EUROTOX, etc). The studies were also performed according to international Good Laboratory Practice guidelines issued by the FDA and other Health authorities around the World. These studies, for the most part, have up to now not been widely published. In order to assist physicians in their search for data and to avoid unnecessary additional animal experimentation, we offer for publication a concise review of our preclinical safety evaluation of sildenafil. The assessment reports issued by the Health Authorities are in the public domain.[1,2]

## Methodology

About 70 pharmacology investigations in rodents and dogs were carried out to characterize the mode

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of action (MOA) of sildenafil and its pharmacological profile related or unrelated (safety pharmacology) to the MOA.

The preclinical pharmacokinetic programme (about 30 studies) for sildenafil comprised single dose studies in all animal species used in the toxicology programme. Routine pharmacokinetic parameters (ADME) were supplemented by plasma drug levels in specific pharmacology studies and during most toxicology studies under the actual conditions of safety evaluation. Monitoring for metabolites of sildenafil was included. Methodology included specific HPLC, radiolabelled sildenafil and *in vitro* metabolism studies.

Sildenafil citrate has been subjected to a standard toxicology testing programme (about 30 studies) that was essentially identical to what has later been agreed by the ICH (International Conference on Harmonization) in their testing guidelines for standard drugs.[3] CD-1 mice and Sprague–Dawley rats were supplied by Charles River, France or USA. Pure-bred Beagle dogs were supplied by Marshall Farms, USA and New Zealand White rabbits by La Garenne or Lago, France. Studies, with single and repeated daily administration, comprise general toxicity, genotoxicity (*in vivo* and *in vitro* studies), carcinogenicity and reproduction toxicity (Table 1).

In addition, studies have been carried out to further clarify findings observed in the course of the toxicology programme (Table 2).

As may be evident from the sheer number of studies, the numbers of animals in each study, the exhaustive number of clinical, laboratory and histopathology parameters, and the multiplicity of observations and sampling over frequently extensive periods, an incredible number of individual data has been generated. These covered all aspects of life such as development from neonate to old age, body weight, food consumption, general health status, cardiovascular, pulmonary, renal, gastrointestinal, CNS, autonomous nervous system, reproductive function, tumour development, spontaneous and drug-induced morbidity and mortality. The vast majority of such data were in the normal range for the respective animal population and age bracket. The data were scrutinized by classical statistical analyses used in toxicology studies (eg Dunnett's test for continuous data, Peto's analysis for tumour data).

**Table 1** Standard nonclinical toxicology programme with sildenafil

| *Species or test system, study type* | *Doses (mg/kg bodyweight) or concentrations* |
|---|---|
| Single dose studies | |
| Rats | 300, 500 and 1000 |
| Mice | 500 and 1000 |
| Repeated dose studies | |
| Rat | |
| 10 days | 50, 150, 500 |
| 1 month | 10, 45, 200 |
| 6 months | 3, 12, 60 |
| Mouse | |
| 3 months (2 studies) | 10, 50, 100, 200 and 20, 40, 100 |
| Dog | |
| 10 days | 10, 30, 100 |
| 1 month | 5, 20, 80 |
| 6 months | 3, 25, 80 reduced to 50 |
| 12 months | 3, 10, 50 |
| Carcinogenicity studies | |
| Rat 24 months | 1.5, 5, 60 |
| Mouse 24 months (2 studies) | 3, 10, 30 and 1, 3, 5 |
| Reproduction toxicity | |
| Rat fertility | 3, 12, 60 |
| Rat embryo/foetal toxicity | 10, 50, 200 |
| Rat peri/postnatal | 10, 30, 60 |
| Rabbit embryo/foetal toxicity | 10, 50, 200 |
| Genotoxicity | |
| Ames microbial assay *in vitro* | 0.002 to 1 mg/plate[a] |
| CHO/HGPRT assay *in vitro* | 65 to 240 μg/ml[a] |
| Human lymphocyte assay *in vitro* | 10–25 μg/ml and 10–250 μg/ml[a] |
| Mouse bone marrow assay *in vivo* | 2000 mg/kg (single dose) |

[a]With and without exogenous metabolic activation.

**Table 2** Additional studies exploring specific findings in the toxicology programme

| *Species* | *Details of study* | *Study end points* |
|---|---|---|
| Rat | *In vivo* exploratory study on the relationship between liver enzyme induction and thyroid hypertrophy | Exogenous thyroxin clearance; plasma TSH, thyroid hormones, histopathology of liver and thyroid, liver UDPGT activity |
| Mouse and rat | Five exploratory studies of intestinal transit (charcoal meal test) | Gut motility, intestinal transit time, total length of small intestine |
| Dog | Isolated retina; light challenge | Kinetics of response to light |
| Dog | Electroretinography (ERG) | Response to flashes of blue light |
| Dog and rat | Morphometry of the retina in treated and control animals | Counting of the numbers of nuclear layers of the retina after 6, 12 and 24 months |

## Results

### *Pharmacology relevant to the understanding of the safety of sildenafil*

Sildenafil is a selective and potent inhibitor of the cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) enzyme, with an inhibitory concentration—$IC_{50}$ between 2 and 7 nM. During sexual stimulation, nitric oxide (NO) is released from penile nerve endings. This acts to increase levels of cGMP in the *corpus cavernosum* smooth muscle, which results in erection. PDE5, abundantly present in the *corpus cavernosum*, breaks down cGMP levels. Sildenafil prevents this breakdown and thus enhances the induced erectile response. PDE5 is also present in visceral smooth muscle. Sildenafil also inhibits, albeit with a 10-fold lower potency ($IC_{50}$ between 27 and 68 nM), another PDE isoenzyme, PDE6. This is found in the human, dog and rat retina in cones and rods. Special exploratory studies were carried out to investigate the main potential safety issues for sildenafil. These relate to the pharmacological effects arising from the elevation of cGMP levels in tissues other than the *corpus cavernosum*, in particular, the retina and smooth muscle (Table 2).[4] Safety pharmacology studies were unremarkable.

### *Synopsis of pharmaco/toxicokinetic data*

As in humans, in all toxicology animal species, sildenafil, after oral administration, is rapidly and nearly completely absorbed from the gastrointestinal (GI) tract. Presystemic metabolism results in similarly high oral bioavailability in man (41%) and dog (54%), less in male rat (15%) and in mouse (17%). Sildenafil is metabolized primarily in the liver, mediated by two cytochrome *P*450 isoforms, *viz.* CYP2C9 and CYP3A4. Inhibitors of cytochrome *P*450 isoenzymes could, in principle, reduce the clearance of sildenafil, and thereby increase bioavailability and extend the half-life. However, even potent enzyme inhibitors do not increase peak plasma levels by much more than a factor of 2. Sildenafil is highly bound to plasma proteins in the rat (95%), dog (86%) and in humans (96%). Biotransformation of sildenafil in animal species used in the nonclinical programme overall resulted in similar metabolite profiles to those observed in man. No human-specific metabolites have been identified. Sildenafil is predominantly excreted in the faeces. Toxicokinetic analyses showed dose-dependent increases in plasma exposure ($C_{max}$ and $AUC_{24\,h}$) with some deviation from linearity observed at doses beyond the human therapeutic range.

### *Safety ratios and exposure multiples*

Plasma concentrations measured in rats and dogs at doses that did not cause adverse effects ('no-toxic-effect-levels') and plasma concentrations in humans at the highest therapeutic dose (100 mg) can be used to determine safety ratios (Table 3). These were, for rats and dogs, respectively, 40:1 and 28:1, calculated from total exposure ($AUC_{24\,h}$), and 19:1 and 8:1, in terms of peak plasma concentration ($C_{max}$). The plasma concentrations have been expressed as free sildenafil, that is, sildenafil not bound to plasma proteins, to take into account species differences in plasma protein binding.

Recently, Geelen *et al*[5] reported effects of sildenafil on cardiac repolarization in isolated guinea-pig hearts and results of patch-clamp experiments in HEK293 and in HERG-transfected CHO cells. The multiples, over the human therapeutic exposure, in terms of sildenafil concentrations required for effects in these test systems are given in Table 4.

### *Toxicology*

Sildenafil, with documented impurity profile for each lot used, was given by intubation (oral gavage)

**Table 3** Safety ratios: exposure to sildenafil of animals at 'no-toxic-effect levels' in toxicology studies *vs* free plasma concentrations in humans at the maximum clinical dose (100 mg)

| | *Dog* | | | *Rat* | | |
|---|---|---|---|---|---|---|
| | | *Plasma concentration* | | | *Plasma concentration* | |
| | *Dose (mg/kg)* | *Free* $C_{max}$ *(ng/ml)* | *Free* $AUC_{24\,h}$ *(ng/h/ml)* | *Dose (mg/kg)* | *Free* $C_{max}$ *(ng/ml)* | *Free* $_{AUC24\,h}$ *(ng/h/ml)* |
| Dog | 15[a] | 174 | 1890 | | | |
| Rat | | | | 60 | 422 | 2705 |
| Human | 1.43[b] | 22 | 67 | 1.43[b] | 22 | 67 |
| Safety Ratios | | 8:1 | 28:1 | | 19:1 | 40:1 |

[a] = dose level not associated with BPS (see section Toxicology).
[b]This refers to a human of 70 kg body weight having received 100 mg of sildenafil.

**Table 4** Exposure multiples: concentration of sildenafil during perfusion$^{+++}$ of isolated guinea-pig hearts (IGPH) causing 15% prolongation of cardiac repolarization and in patch-clamp experiments (IKr block)[5] *vs* free plasma concentration in humans (70 kg) at the maximum clinical dose (100 mg)[6]

| | *Sildenafil 1 μM/l = 474.6 ng/ml* | | |
|---|---|---|---|
| | *IGPH*[5] | $IC_{50}$ *for IKr block*[5] | *Humans 70 kg dose 100 mg* |
| Molar concentration | 30 μM/l | 100 μM/l | 1.18 μM/l |
| Total concentration | 14 238 ng/ml | 47 460 ng/ml | 560 ng/ml |
| Free concentration | 14 238 ng/ml | 47 460 ng/ml | 22 ng/ml |
| Exposure multiples | 647 | 2157 | |

*Note*: $+++$ in Krebs–Henseleit buffer containing no plasma protein.

to rodents and rabbits, and in gelatine capsules to dogs or was used in a number of recognized vehicles in *in vitro* studies.

*Single dose studies*. The administration of single oral doses of sildenafil indicated a maximum nonlethal dose of 500 mg/kg in mice and 300 mg/kg in rats. The risk for humans of acute poisoning following overdosing with sildenafil is minimal, given the 150-fold multiple over the oral clinical dose.

*Repeated dose studies*. The main effects in *rats*, in the 1- and 6-month studies were dose-related increases in liver weight and hepatic centrilobular hypertrophy associated with hypertrophy of the thyroid follicular epithelium. This is a rat-specific phenomenon. In an exploratory rat study (Table 2), daily treatment at 200 mg/kg over 29 days produced, in addition to the known liver/thyroid changes, an induction of hepatic UDP-glucuronyltransferase (UDPGT), an increase in the clearance of thyroid hormone, and a compensatory increase in plasma thyroid-stimulating hormone (TSH) that stimulated the thyroid gland. Isolated deaths occurred after 1 to 3 days of treatment at doses of 500 and 200 mg/kg. The 'no-toxic-effect' dose level in rats was 60 mg/kg.

Sildenafil was administered to *mice* for 3 months, at daily doses up to 200 mg/kg, to select doses for the carcinogenicity study. Treatment-related mortality occurred at doses from 40 mg/kg. No toxic effects were seen after 10 and 20 mg/kg.

No specific target organs for toxic effects were identified in *Beagle dog* studies up to 1 month at doses up to 80 mg/kg. Consistent increases in the heart rate with only occasional mild and inconsistent decreases in diastolic and systolic blood pressure were seen at doses from 10 mg/kg and are regarded as physiological reflex response to the vasodilatory activity of sildenafil. ECG examinations carried out frequently during the toxicology studies did not indicate any rhythm disturbances or other treatment-related abnormalities. Shortening of the QT interval was associated with increase in the heart rate. At the mid- and high doses (20 and 80 mg/kg), slight dilation of pupils, emesis and transient salivation were observed in a few dogs. In the 6-month study, the clinical signs were similar and the dogs developed resistance to the dosing procedure. Consequently, this dose was reduced to 50 mg/kg, whereupon the signs of GI intolerance disappeared. In this study, one of four male dogs, and later in the 12-month study, all four male dogs, both at the highest dose of 50 mg/kg, developed one or more episodes of the following: hyperthermia, subdued behaviour, stiffness,

equilibrium disturbance, arched back and a stiff neck, which appeared painful when manipulated; neutrophilia, mild anaemia, increased fibrinogen; alkaline phosphatase increase, decrease in albumin, sodium, potassium and chloride; and disseminated polyarteritis. These changes, consistent with idiopathic juvenile arteritis syndrome, also known as Beagle pain syndrome (BPS), presented from week 11 of the studies. Doses of sildenafil in dogs that did not cause BPS were 15 mg/kg.

*Reproduction toxicity studies*. In none of the reproduction toxicity studies were any signs of impaired fertility, foetotoxicity or major adverse effects on peri- and postnatal development seen. No changes in testis weight and no histopathological alterations of the testes were noted in the many repeat-dose studies in rats, mice and dogs.

*Fertility*: Male and female rats received sildenafil at daily doses up to 60 mg/kg. In the preceding repeat-dose studies in the rat and dog (Table 1), there were no treatment-related changes to the testes or ovaries in terms of organ weight and histopathology. In the fertility study, no treatment-related effects on mating behaviour, pregnancy success or on the other reproduction parameters were seen. Thus, there was no evidence of disturbances of fertility.

*Foetotoxicity (teratogenicity)*: Inseminated rats and rabbits received sildenafil at daily doses of up to 200 mg/kg during organogenesis. Slight or minimal maternal toxicity was recorded at 200 mg/kg in both species. No foetotoxicity was observed. Sildenafil is not an animal teratogen.

*Pre- and postnatal development*: Female rats received sildenafil from day 6 postcoitum until the end of lactation. Minimal toxicity to the treated dams at the high dose of 60 mg/kg manifested as a decrease in the number of viable pups and lower pup body weight at birth. These findings are not a safety issue for humans.

*Genotoxic potential*: Sildenafil neither induced mutations in bacterial or mammalian cells *in vitro* nor did it cause clastogenic activity *in vivo* or *in vitro*, and, therefore, shows no genotoxic potential.

*Carcinogenic potential*: *Mice* received sildenafil for 18 or 24 months at daily doses of up to 30 mg/kg in the first study and up to 5 mg/kg in the second study. The main cause of deaths was, as already observed in the 3-month study, marked abdominal swelling, due to GI dilation. This led to abrupt loss of body weight before death.

*Rats* received sildenafil for 24 months at daily doses of up to 60 mg/kg. No differences in mortality were observed between treated and control rats. Body weight was decreased in high-dose rats compared to the controls. Increased incidence of thyroid follicular cell hyperplasia was seen as expected from earlier studies. Sildenafil is not a carcinogen to mice and rats.

*Morphometric analysis of the retina*. The high-dose animals in the 6-month and carcinogenicity studies in rats (60 mg/kg) and in the 6- and 12-month dog (50 mg/kg) studies were subjected to morphometric analysis of the retina. This involved counting the number of cell layers in central and peripheral parts of the retina. In none of these studies were there differences in the retinal thickness between control and treated groups.

## Discussion

Pharmacokinetic studies established the rat and dog to be appropriate animal models for investigating the toxicity of sildenafil. In rats and dogs, safety ratios were 40:1 and 28:1, respectively, in terms of total exposure ($AUC_{24\,h}$) and 19:1 and 8:1, respectively, in terms of peak plasma concentration ($C_{max}$). The highest exposure levels of the animals to sildenafil that did not cause toxicity are useful comparators to the maximum therapeutic exposure of humans. The derived safety ratios illustrate the separation between exposure to sildenafil of animals and the much smaller human therapeutic exposure. They highlight the very low risk of human toxicity for sildenafil.

### *Liver and thyroid changes in rats*

An interdependence of thyroid and liver changes was seen in the subchronic and chronic studies in rats. This is explained by an activation of hepatic UDPGT, which brings about an increased T4 clearance, resulting in a reduction in circulating concentrations of this hormone. This enzyme is responsible for conjugation of thyroxine prior to excretion into the bile.[7–9] Low thyroxine plasma concentrations stimulate the pituitary, through a well-characterized negative feedback mechanism, to release TSH, resulting in a hypertrophic response on the thyroid epithelium and eventually to proliferative changes of thyroid follicular cells.[10–12] The thyroid of rats is particularly sensitive to disturbances in thyroid hormone metabolism because rats lack thyroxine binding globulin, resulting in a shorter half-life of T4 than in humans. Thus, treatment-related increase in follicular cell proliferative changes in the thyroid of rats has no relevance to humans.[13]

### *BPS (idiopathic juvenile arteritis syndrome)*

BPS is a well-documented, dog-specific condition, reported mainly in Beagle dogs.[14,15] In the literature, an immune-complex mechanism and genetic pre-

disposition are discussed as possible pathogenesis.[16–18] Known to occur spontaneously, BPS appears with greater frequency and severity in treated dogs of toxicological studies.[19,20]

In the studies with sildenafil, the manifestations were incomplete. At the lower doses, in these studies up to 15 mg/kg sildenafil, no BPS was seen. The safety margin over the human dose calculated for the latter dose in dogs is 28 in terms of AUC and eight in terms of $C_{max}$. This and the species specificity of the syndrome indicate that treatment with sildenafil does not constitute a hazard to humans.

*Intestinal dilation in mice*

Unexpected mortality of mice, especially in the carcinogenicity studies was associated with marked dilation of large gas-filled intestinal segments. Exploratory studies (Table 2) revealed a marked slowing of intestinal transit in mice already after single administration of sildenafil from 10 mg/kg. Identical experiments in rats showed similar but less prominent effects, and only at doses 10 times higher than in mice. The effects seen in rodents could not be produced in dogs. In dogs, minor digestive disturbances were seen only at 80 mg/kg. Changes in the contractile patterns of the small intestinal musculature leading to a relaxation of the intestinal smooth muscle, and consequently to a prolongation of the intestinal transit time are considered as pathogenetic factors.[21] Mice appear especially sensitive to these effects. No intestinal disturbances of any kind have been associated with sildenafil in humans after extensive clinical use of sildenafil.

*Ocular safety in humans*

The findings of minor visual adverse effects (blue tinge to vision, increased perception of brightness of light), reported in clinical studies, have been investigated extensively in laboratory animals. No deleterious effects on visual function have been detected in the pharmacology and toxicology investigations in this programme. The ocular effects in humans are likely to have a pharmacological basis, that is, resulting from PDE inhibition, rather than manifestations of toxicity. ERG recordings in the dog (Table 2) demonstrated reversible effects, which were proportional to plasma sildenafil concentrations. In rat and dog toxicology studies, plasma concentrations of sildenafil that are pharmacologically active on the retina did not cause structural changes to the retina. Ophthalmologic examinations in rats, mice or dogs were unremarkable after treatment for 24, 18 or 12 months, respectively. Histopathological examination of all major structures along the visual pathway did not reveal any evidence of toxicity. Counting the nuclear layers in the retina of the high-dose rats in the 6 months and carcinogenicity studies and of the high-dose dogs in the 6- and 12-month studies did not reveal differences compared to the respective controls groups. In conclusion, the reversible effects on dog ERG recordings are related to the pharmacology of sildenafil and do not represent a safety risk to humans.

## Conclusion

A comprehensive drug safety evaluation programme has been carried out, following rigorous scientific study protocols validated by professional scientific associations and in compliance with GLP regulations. Daily doses of sildenafil, within and far beyond the human therapeutic range, were given to dogs and rodents for up to 1 and 2 y, respectively. During most studies, plasma analyses were conducted to determine the exposure to sildenafil. We found species-specific effects in dogs (BPS), mice (marked intestinal dilatation) and rats (adaptive reversible hepatocellular hypertrophy associated with secondary thyroid hypertrophy). All these effects in rodents and dogs have no relevance to humans. Morphometric thickness measurements of the retinal layers, carried out in response to clinical observations of visual disturbances in humans, indicated no difference between treated and control rats and dogs after up to 24 months of treatment, and no evidence of histopathologic damage to all structures of the visual pathway were found. Sildenafil had no effects on fertility (no teratogenic potential), was not genotoxic, and has no carcinogenic potential. In rats and dogs, safety ratios were 40:1 and 28:1, respectively, in terms of total exposure ($AUC_{24\,h}$) and 19:1 and 8:1, respectively, in terms of peak plasma concentration ($C_{max}$). These safety ratios illustrate the separation between exposure to sildenafil of animals at large doses and the much smaller human therapeutic exposure. They highlight the very low risk of human toxicity for sildenafil. The favourable results of the nonclinical safety evaluation of sildenafil in established animal models have been confirmed by many years of clinical experience during the development and marketing of sildenafil.

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