



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

VINCAMINE

SUMMARY REPORT

1. Vincamine (14,15-dihydro-14-hydroxy-eburnamenine-14-carboxylic acid methyl ester) is an alkaloid obtained from the leaves of *Vinca minor* (*Apocynaceae*) or periwinkle. Other alkaloids of this plant are vincine, vincaminine and vicinine. They are structurally totally different from the alkaloids obtained from *Vinca rosea* leaves (vincristine and vinblastine) which have anti-neoplastic effects. Vincamine is mostly used in its hydrochloride and tartrate salt forms. Its use is recommended as a cerebral vasodilator in neonatal calves for cerebral anoxia. It is mostly used in combination with heptaminol (a central nervous system stimulant) and papaverine (a vasodilator). The dose used is 5 ml of an injectable solution containing 7.5 mg vincamine/ml or 35 to 40 mg per animal by intravenous or intramuscular route.

Vincamine is widely used in human medicine to increase global and regional blood flow in patients suffering from acute or subchronic cerebral ischaemia.

2. Vincamine's function and therapeutic use as a vasodilating agent, especially at the level of the central nervous system, has been proven after an intravenous administration of 30 mg over a period of 20 minutes in Mongolian gerbils by an increase of cerebral blood flow of approximately 10% and of regional cerebral blood flow in areas with insufficient blood supply by approximately 15%. The mechanism of this vascular action, whilst not fully clear, seems to be partly due to a reserpine-like noradrenaline depleting effect. Hence, its sedative effects are similar to reserpine. However a complete depletion of the cerebral noradrenaline and the intestinal adrenaline contents occurred only at doses ten times larger than those of reserpine (50 mg vincamine base/kg bw intraperitoneally for 3 days).

Other observed effects of vincamine include cardiovascular effects (e.g. hypotension, pro-arrhythmic effect) and effects on central nervous system (sedation).

Cardiovascular effects consisting of a dose-dependent reduction of heart rate, cardiac output, systolic and arterial mean blood pressure in anaesthetised cats after intravenous bolus injection of doses between 0.1 and 10 mg/kg bw with a waiting time of 5 minutes between the lowest doses and 10 minutes between the highest doses. The same effects were seen in cats after oral application of 20 mg vincamine/kg bw, but lower doses were not administered. Intravenous doses of 0.1 to 30 mg/kg bw provoked the same effects in rats as well as in guinea pigs.

Arrhythmia was observed in rabbits and dogs after intravenous administration of doses of 0.5 to 2.5 mg/kg bw. However, this effect was not only a function of the dose but also of the speed of the infusion. When the speed of the infusion was higher than the rate of metabolic transformation, the effect on the heart increased even up to the death of the animal. The effect was linear with the dose and was present at all speeds of infusion used. In rabbits with an infusion rate of 17 mg/kg bw/minute extrasystoles started at 0.677 mg/kg bw. In dogs the corresponding data were 17 mg/kg bw/minute and 1404 mg/kg bw. Ventricular extrasystoles occurred also in rabbits after oral administration of 20 mg/kg bw. No arrhythmia was seen in rats and guinea-pigs.

Sedative effects were established in mice after subcutaneous administration of doses of 2.5 to 10 mg/kg bw. These doses are far in excess of therapeutic doses in humans (40 to 80 mg/day, approximately 0.7 to 1.3 mg/kg bw). In dogs, cats and rabbits the sedative effect only appeared at sublethal doses (100 times the one of reserpine).

In a GLP study in 5 dogs, vincamine was administered by the intraduodenal route. The animals were first treated with the vehicle (0.5% carboxymethylcellulose hydrogel) of the test substance, and subsequently, 1 hour later with vincamine base at dose of 0.3 mg/kg bw, 1.5 hour later at a dose of 0.9 mg/kg bw and finally 1 hour later at a dose of 5 mg/kg bw. At 5 mg/kg bw, vincamine induced a statistically significant increase in left ventricular output, stroke volume, telediastolic left ventricular pressure, cardiac output, and Q-T interval, associated with a fall in total peripheral resistance. At 0.9 mg/kg bw, vincamine induced no statistically significant haemodynamic changes. The dose of 0.9 mg/kg bw can be taken as a pharmacological NOEL in the dog.

No data were provided on the side effects in the target species.

3. Pharmacokinetic parameters of vincamine in rats were measured after oral administration of 20 mg base/kg bw and intravenous injection of 10 mg vincamine hydrochloride/kg bw. After oral administration, a bioavailability of 58% was found and the concentration/time curve showed a two-compartment open model. The following parameters were observed: an elimination half-life of 1.71 hours, a t_{max} of 1.27 hours, a C_{max} of 0.87 µg/ml, a total clearance of 0.818 l/h (higher than the plasma perfusion volume, which indicates a very quick metabolism in other organs in addition to the liver), and a volume of distribution of 2.018 litres. The amount of unchanged vincamine excreted was very low with 3 to 11% in urine and 2 to 5% in bile. Vincamine is taken up in high concentrations into the different organs resulting in the following ratios: lung/plasma 21, brain/plasma 14.6, kidneys/plasma 14.3, liver/plasma 8.9, heart/plasma 7.6. However, elimination from these organs was significantly more rapid than from plasma. After intravenous injection the pharmacokinetic parameters observed were an elimination half-life of 1.68 hours, a C_{max} of 5.46 µg/ml, a total clearance of 0.866 l/hour, and a volume of distribution of 2.104 litres. The values for elimination half-life, volume of distribution and total clearance did not differ significantly between oral and intravenous administration.

Pharmacokinetics of vincamine in dogs also followed a two-compartment open model. Doses of 10, 20 and 40 mg intravenously showed dose-dependent half-life and clearance rate. After oral administration of 20 mg vincamine hydrochloride, bioavailability ranged between 23 and 58%. Vincamine could be detected in the urine at up to 9.5% depending on urinary pH. After oral administration of 4 mg vincamine hydrochloride/kg bw an elimination half-life of 4.5 hours (longer than for rats) and a total clearance of 0.52 l/hour were observed.

The pharmacokinetics of vincamine were measured in 6 calves aged 2 weeks after an intravenous injection of 1 mg/kg bw of vincamine followed 5 days later by an intramuscular injection of 1 mg/kg bw of vincamine. This GLP study revealed a rapid and high intramuscular bioavailability, a high distribution volume and a very rapid elimination. The following parameters were found: bioavailability 75%; C_{max} 496 µg/l; t_{max} 0.47 hours, area under the curve (AUC) 757 µg.h/l; half life of elimination 2.39 hours; apparent volume of distribution 4.6 l/kg.

Vincamine is very extensively metabolised with only a small percentage of unchanged compound detectable in the urine. Radiolabel studies in rats after an oral dose of 10 mg/kg bw demonstrate the metabolic pathway of vincamine. On the one hand it is hydrolysed by the plasma esterases to the unstable vincaminic acid. The latter is quickly decarboxylised and oxidised to eburnamenine. On the other hand vincamine is hydroxylated to the major metabolite 6-beta-hydroxy-vincamine, which accounts for 40% of total urinary and biliary radioactivity, followed by 6-alpha-hydroxy-vincamine (8%) and 6-keto-vincamine, the oxidised metabolite of both previous metabolites (about 10% of the administered dose). 6-Keto-vincamine is eliminated by conjugation. The same metabolites (hydroxy-keto) could also be detected in the urine of rabbits, dogs and man. Within 72 hours 40% of the total radioactivity are excreted in urine and 23% in the faeces.

4. Oral acute toxicity, expressed as LD₅₀ values, was in the order of 825 mg/kg bw in mice and 810 mg/kg bw in rats. LD₅₀ values for intravenous and intraperitoneal routes of administration were 85 and 215 mg/kg bw, respectively.

Rapid intravenous bolus injection of lower doses (cat: 0.1 mg/kg bw, rat: 0.1 mg/kg bw, rabbit: 0.6 mg/kg bw and dogs: 1.4 mg/kg bw) resulted in cardiac disturbances such as bradycardia and arrhythmia. In the heart vincamine may slow sodium and potassium transport in muscle cells.

5. Repeated dose toxicity studies in the rat with oral doses of 30 and 100 mg/kg bw/day for 6 weeks and of 6.6, 20 and 62 mg/kg bw/day for 3 months showed no adverse effects.

In dogs oral administration of vincamine for 3 months at doses of 1, 7 and 20 mg/kg bw/day did not reveal any toxicity at 7 mg/kg bw/day. At the dose of 20 mg/kg bw/day dogs showed slightly changed behaviour without other effects, including histological anatomo-pathological examination of a series of organs such as the gonads. However it is not mentioned if the dogs were male or female. In second study in dogs, vincamine was administered to beagles by the oral route every day for 91 days at doses of 0, 3, 11 and 45 mg/kg bw/day (3 males and 3 females per dose). Administration of 45 mg vincamine/kg bw/day for 91 days (as 60 mg/kg bw/day of its alpha-ketoglutarate salt) induced bilateral testicular atrophy and azoospermia. No such effect was observed at 11 mg/kg bw. No modification in the gonads of the females dogs was observed. No explanation for the testicular atrophy with azoospermia is available.

In the guinea-pig oral doses of 2.5 mg/kg bw for 3 months did not show any effects.

From these repeated oral dose studies, the NOEL is 7 mg/kg bw/day for 3 months in the dog.

6. No long term repeated dose toxicity studies are available.
7. No data on tolerance in neonate calves are provided.
8. Reproduction toxicity studies, including teratogenicity, were carried out in different species.

In an oral 91-day repeated dose toxicity study in beagle dogs, testicular atrophy and azoospermia was observed in males at the highest dose, 45 mg/kg bw/day.

Vincamine base administered by the intravenous route at dose of 5 mg/kg bw/day to female rats (30 animals per group) from 8 days before mating until two-thirds of gestation or until the end of gestation did not modify the fertility and was not embryotoxic or teratogenic. Female rats (20 animals per group) were treated by oral route from day 6 to day 16 of gestation with doses of 0, 2.25, 3.7, 7.5, 22.5 and 37.5 mg/kg bw/day of vincamine (i.e. 0, 3, 5, 10, 30 and 50 mg/kg bw/day of vincamine alphaketoglutarate). The dose of 7.5 mg/kg bw caused an increase in the number of placental haemorrhages. A dose of 22.5 mg/kg bw/day induced a reduction of fertility, a loss of body weight in females, fewer foetuses, smaller and lighter foetuses and marked delay in ossification of foetuses. High foetotoxicity (only one foetus of 6 gravid females) was recorded for the high dose (37.5 mg/kg bw/day). Administration of 225 mg/kg bw/day by oral route had no effect on the reproductive function in male rats.

In mice (30 animals per group) administered 50 mg vincamine/kg bw/day by stomach tube from 1 week before mating until sacrifice or birth, foetal resorption was increased. No effect was observed at 10 mg/kg bw/day. An oral dose of 22.5 mg/kg bw/day from mating to end of lactation was well tolerated in mice (80 animals per group).

In the rabbit (15 animals per group) intravenous administration of 2.3 mg vincamine/kg bw/day from day 8 before gestation until sacrifice or end of gestation had no effect on reproductive function. At 5 mg/kg bw, it caused a high mortality among the treated females. Vincamine was administered at 0, 2, 4 and 8 mg/kg bw/day by stomach tube to rabbits from day 7 to 18 of gestation (13 animals per group). Up to 4 mg/kg bw/day, no significant effect on reproductive function was observed. At 8 mg/kg bw/day, death occurred in some treated animals. Fertility was significantly diminished. Three foetuses in the same litter displayed oligodactyly, though this could not be definitely attributed to the treatment. Vincamine was considered as not teratogenic.

From these studies the oral NOEL for reproduction toxicity is 4 mg/kg bw/day in the rabbit and was considered as the overall toxicological NOEL.

9. Vincamine was not mutagenic *in vitro* in an *Salmonella typhimurium* assay for gene mutation with and without metabolic activation at concentrations of 16 to 1500 µg/plate using the standard plate incorporation and at concentrations of 147 to 1500 µg/plate using the preincubation method and in an assay for gene mutation in mammalian cells (mouse lymphoma cells at the tk locus) with and without metabolic activation, at doses of 9.3 to 150 µg/ml. In both experiments, higher concentrations were not tested because of the limit of solubility. In an *in vivo* assay (micronucleus test), vincamine did not increase the number of micronucleated polychromatic erythrocytes in bone marrow. Male and female Swiss OF1 mice received two oral treatments of vincamine at 175, 350 and 700 mg/kg bw /day at a 24 hours interval. Overall, it was concluded that vincamine was not mutagenic.
10. Carcinogenicity studies were only performed on the short term effects on the skin of mice, without any tumour-promoting potency as measured by the sebaceous gland hyperplasia tests. Vincamin, being not mutagenic and belonging to a chemical family considered as presenting no carcinogenicity hazard, can be considered as not carcinogenic.
11. No data on effects on the immune system are available.
12. Information on microbiological properties of vincamine was not available, however, such effects are not expected for this group of compound.
13. In humans, the recommended therapeutic dose is between 40 and 80 mg/day (i.e. 0.7 to 1.3 mg/kg bw/day) by oral route for at least 20 to 40 days. In a review concerning 1481 patients with cerebrovascular insufficiencies, the following daily doses were used: intravenously 10 to 120 mg (for 90 days), intramuscularly 15 to 120 mg (45 mg up to 180 days and 90 mg up to 150 days) and orally 40 to 80 mg up to 1 year.

To achieve a pharmacological effect in humans after oral or parenteral administration, the doses of vincamine must be sufficiently high to reach the effective plasma concentration, i.e. about 0.2 µg/ml. By the oral route, 30 mg (0.5 mg/kg bw) are not sufficient to do this. Repeated administration of 20 mg (0.33 mg/kg bw) 3 times a day for 6 days still fails to achieve the effective plasma level, and causes no build-up of the drug.

Pharmacokinetics were studied in humans, by using radio-labelled drug by oral route either in an aqueous solution (169 mg vincamine hydrochloride) or as control-released tablets (33.81 mg vincamine hydrochloride). Half-life of elimination for the solution was 0.57 to 1.07 hours, C_{max} was reached between 60 and 90 minutes after administration proving a rapid oral absorption. In another study, the amount of vincamine eliminated in the unchanged form in urine was 5.85% and 7.23% after 30 and 60 minutes respectively. Intravenous infusion of 30 or 40 mg of vincamine hydrochloride resulted in plasma levels of 0.6 to 1.0 µg/ml. Cerebrospinal fluid concentrations were 5 to 24% of the simultaneous plasma concentrations 2 hours after a 30 mg infusion. Due to the very short elimination half-life, sustained release forms are used in human therapy.

14. A toxicological ADI of 0.04 mg/kg bw of vincamine (i.e. 2.4 mg per person) was established based on the NOEL of 4 mg/kg bw/day observed in the reproduction toxicity study in rabbits, applying a safety factor of 100. From studies performed in dogs, a pharmacological ADI of 0.009 mg/kg bw of vincamine (0.540 mg per person) was established, based on the oral pharmacological NOEL of 0.9 mg/kg bw and applying a safety factor of 100. The latter ADI was considered the most relevant for the safety evaluation of vincamine.

15. A residue depletion study with tritiated vincamine was performed in 6 male calves, 2 weeks of age. The animals received 4 injections of 1 mg/kg bw ³H-vincamine at 0 hour, ½ hour, 6 hours and 12 hours. Calves were slaughtered 12 hours (1 animal), 24 hours (2 animals), 36 hours (1 animal) and 72 hours (2 animals) after the last injection. Vincamine and its metabolites were rapidly eliminated: 24 hours after the end of treatment, 79% of the injected radioactivity was eliminated, half by the urinary route and half by the faecal route. At 72 hours after the last injection, 85% of the total radioactivity was eliminated. The percentage of radioactivity found in muscle, injection site, liver, kidney and fat was, in each tissue, less than or equal to 0.001% of the total dose administered 12 hours after the end of treatment and less than or equal to 0.0008% in each tissue at 24 hours after the last injection.

The calculated daily intake of vincamine equivalents (0.418 mg) 12 hours after the last injection according to the scheme of administration was lower than the ADI.

16. A tissue residue depletion study with unlabelled vincamine was carried out on 20 calves aged 2 weeks (10 males and 10 females), which received a total dose of 4 mg/kg bw distributed to 4 intramuscular injections of 1 mg/kg bw at 0 hour, ½ hour, 6 hours and 12 hours. Calves (4 per time point) were slaughtered 3 hours, 6 hours, 12 hours, 24 hours and 36 hours after the last injection. For muscle, liver, kidney and fat, the limit of quantification was 5 µg/kg and the limit of detection was 2.5 µg/kg. Tissue elimination was very rapid. Three hours after the last injection, the residue concentrations were 104 µg/kg in muscle, 315 µg/kg in injection site, 385 µg/kg in liver, 565 µg/kg in kidney and 1040 µg/kg in fat. Twenty-fourth hours after the last administration, concentrations of vincamine were close to or lower than the limit of quantification in all tissues. Vincamine was still detected in the fat of one animal 36 hours after the last administration, at a concentration close to the limit of quantification.

Conclusions and recommendations

Having considered the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- an ADI of 0.009 mg/kg bw, i.e. 0.540 mg per person, has been established,
- vincamine is rapidly and extensively metabolised and excreted,
- vincamine is used in a small number of individual animals, for infrequent or non-regular treatments, in the new-born calf only,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- at 12 hours after treatment the amount of residues likely to be ingested by consumers is below the ADI;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for vincamine and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Vincamine	Bovine	For use in new-born animals only