



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ALFAPROSTOL

SUMMARY REPORT (1)

1. Alfaprostol is a synthetic analogue of prostaglandin F2 α . Its activity is similar to that of the endogenous PGF2 α , causing luteolysis.

Alfaprostol is recommended for use in the cow, sow and mare. It is given as an intramuscular injection and the recommended dosage rate is :

- 1.5 mg per 100 kg bodyweight cow;
- 2.0 mg per sow;
- 3.0 mg per mare.

2. Alfaprostol has a similar pharmacokinetic profile in the three target species, after intramuscular administration it is rapidly absorbed and peak blood levels are achieved in approximately 2 hours. Elimination is equally rapid, mainly through the kidneys and faeces and approximately 75% of the dose is excreted within 24 hours. Only low levels are found in milk.

3. LD₅₀ values were determined in mice and rats following intravenous or subcutaneous administration.

In male mice the mean LD₅₀ following intravenous administration was 53.5 mg/kg, in females the mean value was 56.5 mg/kg. In rats, values obtained were similar, 55.4 mg/kg in males and 52.8 mg/kg in females.

By the subcutaneous route in mice LD₅₀ values of 2096 mg/kg for males and 1900 mg/kg for females were obtained, in rats following administration by this route the LD₅₀ was 110.4 mg/kg in males and 127.8 mg/kg in females.

4. In female rats, using the subcutaneous route, doses of up to 540 μ g/day for 28 days produced no noteworthy effects, except for an increase (statistically significant) in mean white cell count which was noted at the highest dose. However, this was not regarded as being of any toxic consequence.

In female dogs doses of up to 180 μ g/kg intramuscularly were well tolerated, diarrhoea was observed in several dogs 2 to 6 hours post administration but there was no indication of the frequency at any dosage rate. This is related to the pharmacological effect of the compound on the smooth muscle. Increased diuresis was also observed.

5. A thirteen week oral study was conducted in rats in which the test compound was given by oral intubation at doses of 0 (saline control), 0 (vehicle control), 0.01, 0.1, 1, 10 and 100 mg/kg/day.

A dose of 1.0 mg/kg/day produced changes in alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. Focal hepatic necrosis was seen in approximately 50% of the animals and multiple corpora lutea were observed in 25% of the females in this group.

At 10 mg/kg/day and 100 mg/kg/day changes in bodyweight gain, in blood biochemistry, haematology and organ weight were noted.

A number of animals in each group died but this was associated with intubation errors. One male in the 1.0 mg/kg/day group died from unilateral hydronephrosis and pyelitis, but this was considered not treatment related as there were no animals with similar findings at this or higher dosages.

The NOEL based on this study is 0.1 mg/kg/day.

6. A two generation rat study (150 male and 150 female animals) was conducted using doses of, 0 (vehicle control) 0.01, 0.10, 0.20 mg/kg/day by oral intubation. A slight increase in neonatal mortality was seen in a small number of litters (5) at the highest dosage rate - stillbirths were seen in 2 litters and pup mortality in 3 litters. Administration of up to 0.20 g/kg/day to F1 rats did not influence implantation, litter size or pup sex ratios.
7. A study was conducted in pregnant rats at doses of 20, 32 and 50 µg/kg/day by subcutaneous route from day 6 to 15 of pregnancy (25 animals per group), it was not possible to test higher doses as the pharmacological effects of these would interfere with the maintenance of pregnancy).

In the 20, 32 and 50 µg/kg groups 2/25, 9/25 and 19/25 respectively showed metrorrhagia or abortion. None of the control animals showed these signs. Alfaprostol interferes with pregnancy in a dose related manner; this is to be expected from this type of compound at such very high doses. No teratogenic effects were noted.

A second study was conducted in rats to evaluate possible teratogenic effects, this time by stomach intubation, on days 7-15 of pregnancy. Dosage rates of 0.2, 1.0 and 2.5 mg/kg/day were used. Parameters examined included foetal body weight, resorption rates, external, visceral and skeletal abnormalities and skeletal variations.

Skeletal variations indicative of incomplete or retarded ossification were seen. These occurred at a significantly higher frequency in the higher dosage groups. Pregnancy rates were affected at dosages of 1.0 and 2.5 mg/kg/day. The effect was dose dependant and was attributed to the luteolytic properties of PGF₂α. No increase in overall numbers of foetal abnormalities were observed.

A study in rabbits by the subcutaneous route at dose of 0, 0.22, 0.66 and 2 µg/kg/day on days 6-18 using 20 animals per group was carried out. Abortions were seen in the 0.66 and 2.0 µg/kg/day group. No teratogenic effects were noted.

8. A series of 5 mutagenicity tests were carried out. These included the Ames Test, Gene mutation in *Schizosachcaromyces pombe*, mouse lymphoma forwarded mutation test, DNA damage and repair using *Sacchaaromyces cerevisiae* and unscheduled DNA synthesis in rat hepatocytes. The compound was not considered to be mutagenic.
9. Four reports detailing experiences in humans were presented.

Alfaprostol at high doses has some stimulating activity in the uterus of the gravid female.

Doses of 0.2 and 1.0 mg by the intramuscular route four times at 2/4 hour intervals to 25 patients during the first trimester of pregnancy neither interrupted pregnancy or caused dilatation of the cervix.

A trial involving 96 women, during the first trimester of pregnancy using alfaprostol intravaginally in suppositories containing 5, 10 or 20 mg of alfaprostol given as 1 to 8 doses. A response was noted at 20 mg dose given 5 times (dilatation of the cervix). In a trial where vaginal suppositories containing 10 mg alfaprostol were administered 3 hourly for 2 to 5 times to women at term with normal pregnancies, 100% responses were obtained. However doses of 5 mg alfaprostol were ineffective.

10. An NOEL of 0.1 mg/kg/day based on the 13 week rat study was established. An ADI of 60 µg per person was established.

11. The main metabolic pathway is β -oxidation following hydrolysis of the ester bond.

In all 3 target species administration by the intramuscular route is characterised by rapid absorption, distribution, metabolism and excretion. Peak levels were achieved between 30 minutes and 2 hours following administration.

The highest concentrations are seen at the injection sites but alfaprostol is not orally active at the dosage used.

There is relatively rapid elimination through urine and faeces.

12. Following administration of 1.5 mg of alfaprostol/100 kg to 4 cows, the maximum concentrations of compound at 24 hours were as follows: muscle : 0.06 $\mu\text{g/kg}$; liver : 2.5 $\mu\text{g/kg}$; kidney : 3.4 $\mu\text{g/kg}$ and fat: 0.23 $\mu\text{g/kg}$. Injection site concentrations were 0.23 mg/kg at this time. Concentrations in milk of treated cows was 0.76 $\mu\text{g/kg}$ at 30 minutes post treatment, declining to 0.35 $\mu\text{g/kg}$ at 24 hours and 0.03 $\mu\text{g/kg}$ at 5 days.

Following administration of 3.0 mg alfaprostol to 4 mares, the maximum concentrations of compound at 24 hours were as follows; muscle : 0.05 $\mu\text{g/kg}$; liver: 2.0 μg ; kidney : 0.78 $\mu\text{g/kg}$; fat : 0.14 $\mu\text{g/kg}$ and injection site : 26.3 $\mu\text{g/kg}$.

Following administration of 3.0 mg alfaprostol to 4 pigs, the maximum concentrations of compound at 24 hours were as follows: muscle : 0.05 $\mu\text{g/kg}$; liver : 2.8 $\mu\text{g/kg}$; kidney : 2.3 $\mu\text{g/kg}$; fat 0.32 $\mu\text{g/kg}$ and injection site : 41 $\mu\text{g/kg}$.

Using the standard food package, the total residue load from cows slaughtered one day after treatment would be 0.98 μg (including the contribution from milk). This amounts to less than 2% of the ADI. The residue load from horses and pigs is even less than this.

Conclusions and recommendation

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

- alfaprostol is rapidly excreted from treated animals,
- treated animals are unlikely to be sent for slaughter immediately after treatment,
- alfaprostol is not orally active at the doses given to the target animals;

the Committee considers that there is no need to establish an MRL for alfaprostol 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 |
|---------------------------------------|--------------------------|------------------|
| Alfaprostol | Bovine, porcine, equidae | |