



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

METHYLPREDNISOLONE

SUMMARY REPORT (1)

1. Methylprednisolone is a synthetic corticosteroid, which is used in veterinary medicine as the free alcohol, and as various esters. It is the 6 α -methyl derivative of prednisolone. The current application refers to the use of methylprednisolone as the free alcohol, in a combination product with antibiotics. The product is intended for intramuscular injection for the treatment of respiratory disease and urogenital infections in cattle. The recommended dose is equivalent to 400 μ g/kg bw/day of methylprednisolone for 4 to 5 days. Methylprednisolone is also used in human medicine, as the free alcohol, as the acetate, the aceponate and as the sodium succinate.
2. The pharmacological activity of methylprednisolone lasts longer than that of hydrocortisone but less than that of the longer-acting glucocorticoids such as dexamethasone. The gluconeogenic potency of methylprednisolone is equivalent to 500% that of hydrocortisone and 125% that of prednisolone, but only around 17% that of dexamethasone. It has hardly any mineralocorticoid activity. A special study was carried out in which groups of Crl:CD BR rats were given oral doses in the range 1 to 16 μ g/kg bw methylprednisolone. The rats were killed 5 hours after dosing and the livers were removed for determination of tyrosine aminotransferase activity. However no significant increase in tyrosine aminotransferase activity was observed in any of the methylprednisolone groups up to 16 μ g/kg bw.
3. Pharmacokinetic studies in humans, laboratory animals, cattle and horses showed that the esters of methylprednisolone such as the acetate, sodium succinate, the hemisuccinate and the phosphate, were rapidly converted *in vivo* to methylprednisolone and that methylprednisolone was responsible for the pharmacological activity. The plasma half-life of the esters was generally less than 10 minutes.
4. In humans, the oral bioavailability was generally high (80 to 99%) but depended on the dosage form. Methylprednisolone was widely distributed to the tissues, crossed the blood-brain barrier and was secreted in breast milk. After oral administration of about 1 mg/kg bw, the half-life of elimination in humans was in the range 1 to 3 hours; the volume of distribution was in the range 1 to 1.5 l/kg. The time to peak plasma concentration was 1 to 2 hours. Over the dose range 10 to 3000 mg, the plasma clearance was around 6.5 ml/min/kg. Plasma protein binding was approximately 77%.
5. In humans and laboratory and farm animals, methylprednisolone is reversibly metabolised to methylprednisone, which is pharmacologically inactive and formed by oxidation of the C11 hydroxyl group to the ketone. In humans the plasma concentration of methylprednisone is around 10% that of the parent methylprednisolone. The metabolism of methylprednisolone followed similar pathways to those of prednisolone, except for the inability of methylprednisolone to undergo hydroxylation at the C6 position. In both humans and dogs, methylprednisolone, like prednisolone was metabolised to plasma and urinary metabolites which lacked corticosteroid activity.

6. The acute oral LD₅₀ values of methylprednisolone aceponate ester in male and female Sprague-Dawley rats was greater than 2000 mg/kg bw. One female (out of 5) administered the top dose of 2000 mg/kg bw died. When methylprednisolone aceponate was administered to rats subcutaneously, the LD₅₀ value was greater than 3000 mg/kg bw, the highest dose administered.
7. A 14-week repeated dose toxicity study was carried out in Sprague-Dawley rats given daily subcutaneous doses of 0, 0.4, 4, 40 or 400 µg/kg bw/day of methylprednisolone aceponate. Body weight gain and food consumption were reduced in males given 40 µg/kg bw and above and females given 400 µg/kg bw and above. Leucocyte counts were reduced in both sexes given 400 µg/kg bw and bone marrow lymphocytes were reduced in males given 400 µg/kg bw and females given 4 µg/kg bw and above. Changes in serum clinical chemistry values at 400 µg/kg were indicative of effects on the liver and kidney. Atrophy of the thymus and adrenal glands was observed in the 400 µg/kg bw group at necropsy. The NOEL was 0.4 µg/kg bw/day.
8. A 52-week repeated-dose toxicity study was carried out in the same strain of rat using subcutaneous doses of 0, 0.16, 0.8, 4, 20 or 100 µg/kg bw methylprednisolone aceponate. At 100 µg/kg bw, the main effects were reduced body weight and food consumption, reduced erythrocyte, haematocrit and lymphocyte counts and atrophy of the spleen and adrenals. At 20 µg/kg bw, body weight gain and food consumption were reduced and there were some minor effects on organ weights and clinical chemistry values. The NOEL was 4 µg/kg bw/day.
9. Repeated-dose toxicity studies were carried out with methylprednisolone in dogs using daily oral doses of 0, 2.5 and 5 mg/kg bw/day for 42 days and daily intramuscular administration of 1.1 to 1.5 mg/kg bw/day methylprednisolone for 5 weeks. In a special study to investigate adrenal gland function, groups of dogs were dosed orally with 2 or 4 mg/day or intramuscularly with 2.5 mg/kg bw once a week. The main effects in these studies were reduced body weight, skeletal muscle atrophy, increased glycogen deposition in the liver and adrenal atrophy. No NOELs were established in these studies.
10. A fertility study was carried out in which groups of 22 rats per sex and dose were given daily subcutaneous doses of 0, 0.004, 0.02 or 0.1 mg/kg bw/day of methylprednisolone aceponate. Treatment of the males commenced 60 days prior to mating. Treatment of females commenced 14 days prior to mating and continued up to day 7 of gestation. The dams were killed on day 21 of gestation and the foetuses examined. There was no effect on fertility. Parental body weight gain and food consumption were reduced at 0.02 mg/kg bw and above and there were significant increases in the numbers of dead foetuses at these dose levels. The NOEL was 0.004 mg/kg bw/day.
11. In a peri/post-natal study, groups of 24 female Sprague-Dawley rats were given daily subcutaneous doses of 0, 0.04, 0.2 or 1 mg/kg bw/day of methylprednisolone aceponate from day 17 of gestation up to day 21 *post partum*. The dams were allowed to litter naturally and the offspring were monitored for growth and functional development. At 1 mg/kg bw, maternal body weight gain and pup weights were reduced but there were no effects on behaviour or development of the pups. The NOEL was 0.2 mg/kg bw/day.
12. Groups of 36 to 40 mated female Sprague-Dawley derived rats were given daily subcutaneous doses of 0, 0.1, 0.3 or 1 mg/kg bw/day of methylprednisolone aceponate from days 7 to 17 of gestation. Two thirds of the dams were killed on day 21 of gestation and the uterine contents examined. The remaining dams were allowed to deliver naturally and rear the offspring. The top dose of 1 mg/kg bw caused reduced maternal body weight gain and food consumption, reduced foetal weight and an increase in delayed ossification. Foetal weights were also reduced at 0.3 mg/kg bw. At 1 mg/kg bw, there was a small but significantly increased incidence of foetuses with ventricular septal defect. In the group allowed to litter naturally, pup body weight was reduced and opening of the eyelids was delayed in the group administered 1 mg/kg bw/day. The NOELs were 0.1 mg/kg bw/day for foetotoxicity and 0.3 mg/kg bw/day for maternal toxicity and teratogenicity.

13. A very brief report of a teratogenicity study in rabbits was provided. Pregnant female rabbits were given intramuscular injections of 0, 0.004, 0.02, 0.1, 0.15 or 0.25 mg/kg bw/day of methylprednisolone acetate from days 7 to 18 of gestation. The dams were killed on day 29 of gestation and the uterine contents examined. No details were given of group sizes or effects on the dams. Resorption rates were significantly increased and foetal viability was significantly reduced in the 0.15 and 0.25 mg/kg bw groups. The incidence of malformations was significantly increased at 0.1 mg/kg bw and above. The malformations included hydrocephaly, limb defects and spina bifida. Due to the limited information provided, it was not possible to draw any conclusion regarding a NOEL.
14. Groups of mated female mice were given a single intramuscular injection of 330 mg/kg bw methylprednisolone sodium succinate or the same dose the acetate, or the solvent vehicle on day 10 of gestation. Treatment with the acetate produced a decrease in the number of viable foetuses and significant increases in the numbers of foetuses born with their eyes open and foetuses with cleft palate. No cases of cleft palate were observed following treatment with the sodium succinate; instead, there was a significant increase in the incidence of foetal exencephaly. No NOEL was established in this study.
15. Negative results were obtained in an *in vitro* assay for gene mutation in *Salmonella typhimurium* TA98, TA100, TA1535 and TA1538 with concentrations of methylprednisolone sulfonate in the range 250 to 2000 µg/plate. An *in vitro* assay for gene mutation at the hprt locus in Chinese hamster ovary cells and concentrations in the range 2000 to 10 000 µg/ml of methylprednisolone sulfonate. Both these studies were carried out in the absence and presence of metabolic activation. There was no increase in unscheduled DNA synthesis in primary rat hepatocytes with concentrations in the range 5 to 1000 µg/ml methylprednisolone sultepanate. Methylprednisolone was not mutagenic in a DNA-cell-binding assay. Although none of the studies satisfactorily investigated clastogenicity, no evidence of clastogenicity had been obtained in published studies with prednisolone, the 6α-methyl derivative of prednisolone. It was therefore agreed that no further mutagenicity data were required.
16. No carcinogenicity studies were carried out with methylprednisolone. Taking into account the absence of alerting features in the chemical structure of methylprednisolone, the negative results in the mutagenicity assays, and the negative results obtained in the carcinogenicity study with prednisolone, it was agreed that such data were not necessary.
17. Methylprednisolone showed no evidence of skin sensitisation in male Hartley guinea pigs.
18. No data were provided concerning potential microbiological effects of methylprednisolone. It was agreed that such data were not needed for this class of substance.
19. In humans methylprednisolone is administered as the free alcohol, the sodium succinate, the acetate, the hemisuccinate and the aceponate. The usual daily oral dose is 4 to 96 mg methylprednisolone/person. The usual daily intramuscular or intravenous dose ranges from 10 to 500 mg/person. The adverse effects are similar to those of the other corticosteroids and include acute adrenal insufficiency and indications of glucocorticoid overactivity such as round face and wasted limbs. Growth retardation may occur in children. Resistance to infection is decreased.
20. Since the toxicological effects were mainly attributable to the pharmacological activity of methylprednisolone, it was considered appropriate to establish an ADI based on the pharmacological NOEL. An Acceptable Daily Intake of 0.16 µg/kg bw (i.e. 9.6 µg/person) was established by applying a safety factor of 100 to the NOEL of 16 µg/kg bw/day which was established in the study investigating tyrosine aminotransferase activity in rats.
21. There were no radiometric residues depletion studies in the target species.

22. Data for humans, laboratory animals, horses and cattle showed that there was an interconversion of methylprednisolone and the biologically-inert metabolite methylprednisone. In humans and dogs, both substances were further metabolised to a number of other metabolites; on structural grounds these were not expected to possess corticosteroid activity. The evidence suggested that like dexamethasone and prednisolone, methylprednisolone was metabolised in both humans and animals to substances with no pharmacological activity. It was therefore agreed that radiometric studies were not required. Methylprednisolone was considered to be an appropriate marker residue.
23. Cattle were given daily intramuscular injections of a proprietary product containing methylprednisolone together with the antibiotics neomycin and benzylpenicillin, for 5 consecutive days. The dose of methylprednisolone corresponded to 400 µg/kg bw/day. Groups of 2 male and 2 female cattle were slaughtered 7, 14, 21, 30, 45 and 60 days after the last dose. Residues of methylprednisolone in tissues were determined using the proposed routine analytical method based on HPLC with UV detection. The limit of quantification was 10 µg/kg. At 7 days after dosing, residues of methylprednisolone in injection site muscle ranged from below 10 µg/kg to 8393 µg/kg. At 14 days after dosing, residues of methylprednisolone in injection site muscle ranged from below 10 µg/kg to 90 µg/kg and were below the limit of quantification in all later samples. Residues of methylprednisolone in all samples of muscle, liver, kidney and fat were below the limit of quantification.
24. The proposed routine analytical method was based on HPLC with UV detection. The limit of quantification was 10 µg/kg for bovine muscle, liver, kidney and fat. The method was not satisfactorily validated concerning accuracy and precision, the raw data relating to the limit of detection were not provided and there was no information concerning the susceptibility to interference from residues of other corticosteroids.

Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 0.16 µg/kg bw (i.e. 9.6 µg/person) was established,
- methylprednisolone was retained as the marker residue,
- residues in bovine tissues were rapidly depleted and MRLs could be set at values equivalent to the limit of quantification,
- there were no satisfactory residues depletion data for milk and so no MRLs could be elaborated for milk,
- an analytical method for the determination of residues of methylprednisolone in bovine tissues was available but the sensitivity of the method needed to be improved and the method was not satisfactorily validated;

the Committee for Veterinary Medicinal Products recommends the inclusion of methylprednisolone in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Methylprednisolone	Methylprednisolone	Bovine	10 µg/kg 10 µg/kg 10 µg/kg 10 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption. Provisional MRLs expire on 1.7.2001

Based on these MRLs, the daily intake will represent about 52% of the ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of methylprednisolone in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The proposed routine analytical method should be fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community and re-presented in a standard international format (e.g. ISO 78/2). The sensitivity of the method should be improved and a lower limit of quantification should be established in accordance with the CVMP Position Paper on Requirements for LOQ/MRL ratio (EMEA/CVMP/274/96-FINAL). Information should be provided concerning the susceptibility to interference from residues of other corticosteroids.