The European Agency for the Evaluation of Medicinal Products

EMEA/MRL/011/95

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

JOSAMYCIN (CHICKEN)

SUMMARY REPORT

1. Josamycin (JM), a macrolide antibiotic obtained by fermentation of Streptomyces narbonensis, is effective in vitro against Mycoplasma, Gram positive Cocci (Staphylococcus, Streptococcus, Diplococcus). In veterinary medicine, it is used for the prevention and the treatment of chronic respiratory diseases, sinusitis, synovitis and arthritis caused by mycoplasma and gram positive germs in chicken.

2. The site of action of macrolides is thought to be the ribosomal subunits located in cytoplasm. Josamycin resistance genes may be resulted from alterations in the ribosomal structure and the loss of macrolide affinity.

3. Pharmacology studies, performed in mice, rats, rabbits, guinea pigs, frogs, cats and dogs showed that adverse effects on respiratory rate, on blood pressure and on the electrocardiogram in anaesthetised cats were observed after IV administration for dosage higher than 0.1 mg/kg b.w. No pharmacological studies were carried out with the oral administrations.

4. In rats, 99 % of the radiolabelled JM were excreted within 4 days (23 % in urine vs 76 % in faeces) after oral administration. JM is rapidly absorbed and well distributed. One major urinary metabolite, the deisovalery-JM, representing 96 % of the total urinary metabolites was identified.

   No formal metabolism studies were performed in chicken. It was shown that JM does not accumulate in the blood of chickens receiving repeated doses of 18000 IU/of JM per day during five days via drinking water. JM concentrations were below 0.1 µg/ml at 24 hours after the last administration.

5. The acute toxicity of JM, determined using different routes of administration (IV, SC, IP and oral) in rats and mice is low : the LD50s were above 7000 mg/kg b.w. and 3000 mg/kg after oral and after IP or SC administrations respectively. After IV administration of JM in tartaric acid solution, the LD50 ranged from 355 to 395 mg/kg b.w. in both species.

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6. The short and long term oral toxicity studies performed in rats [a five-week study (0, 300, 1000 and 3000 mg/kg b.w. - JM base) and a 6-month study (0, 300, 100, 3000 mg/kg b.w. - JM base)] revealed that JM induced variations in haematological parameters. A NOEL of 100 mg/kg b.w. could be established from the 5-week study. In the 6-month toxicity study performed in dogs (0, 50, 120, 300, 1800 mg/kg b.w. JM propionate), no toxic effects were reported for animals treated up to 300 mg/kg b.w.

7. Several tolerance studies, carried out in pigs and chicken showed no treatment related abnormalities.

8. A two generation reproduction study was not performed.

9. Two teratogenic studies were performed in rodents : one in rats (0, 300, 3000 mg/kg b.w. from 8 to 14 days of gestation) and the other one in mice (0, 300, 3000 mg/kg b.w. from 7 to 13 days of gestation). In rats, no adverse effect was observed up to 3000 mg/kg b.w.. In the mice group treated at 3000 mg/kg b.w., the resorption rates were significantly increased (15.9 % vs 8.7 % in the control group) and the number of foetuses with growth retardation was increased (8.3 % versus 2.1 % in control group). 300 mg/kg b.w. per day could be considered a no effect level for embryotoxicity in mice.

10. JM was not mutagenic in the following in vitro tests : Ames Test (TA 98, TA 100, TA 1535 or TA 1537), Repair test using Bacillus subtilis, HGPRT- test with cells of Chinese hamster cell line V79 and Micronucleus test in vivo.

11. No carcinogenicity study was performed. However, this was not requested on the basis of the absence of structural alerts and the results of the mutagenicity studies.

12. A toxicological ADI of 0.5 mg/kg b.w., based on the NOEL of 100 mg/kg b.w. observed in the five-week oral toxicity study in rats, could be estimated. Since this study lasted only for 30 days, a safety factor of 200 was chosen instead of the standard safety factor of 100.

13. From the in vitro study performed in order to evaluate the potential effect on the human gut flora, a MIC_{50} of 0.70 µg/ml could be calculated for Bacteroides fragilis, the two other strains tested, Escherichia coli and Bifidobacterium sp. being resistant.

14. Based on the in vitro results obtained on Bacteroides fragilis, a microbiological ADI of 0.002 mg/kg b.w./day was calculated according to the following formula :

$$\text{ADI (µg/kg b.w.)} = \frac{\dfrac{\text{lowest MIC (0.7 µg/ml) x } CF_2\ (1)}{CF_1\ (1)} \text{ x daily faecal bolus (150 ml)}}{\text{fraction available for micro-organism (0.85) x human weight (60 kg)}}$$

with

CF1 = 1 and CF2 = 1, as the MIC of the most sensitive strain was retained to calculate the ADI.
the fraction available for microorganism 0.85 was obtained from human data.

As there are a few data on human flora, this microbiological ADI should be provisional and considered to establish MRL values as it is 250 fold lower than the ADI evaluated from toxicological data (0.5 mg/kg b.w.).

15. After administrations of JM (18 mg/kg b.w./day/5 days) to chicken, no JM residues could be detected in edible tissues at 3 days after the end of the treatment (four animals per sampling time - microbiological method).

16. In the whole egg, no josamycin residues were detected either during the treatment period or any day after cessation of treatment (18 mg/kg b.w./day/5 days). (limit of quantification of the microbiological method 100 µg/kg).

17. As there is no available (physicochemical) analytical method validated according to the recommendations of Volume VI, MRLs based on all active antimicrobial residues should be proposed as provisional for chicken

Chicken

liver	200 µg/kg
kidney	400 µg/kg
fat	200 µg/kg
muscle	200 µg/kg
eggs	200 µg/kg

The residue should be expressed as the sum of josamycin and all its microbiologically active metabolites.

A provisional MRL can be allocated for eggs as this product is also recommended for use in laying hens.

18. These MRL values expire on 01/07/2000.

Before the 01/07/1999, the following information is requested.

1. The applicant should provide the English translations of the Italian and Spanish Marketing authorisations.

2. The applicant should provide information about the name and the structure of the impurities and complete the criteria of solubility of JOSAMYCIN in solvents.

3. The applicant should provide more information on the reproduction toxicity maybe from human data.

4. The applicant should provide information on the adverse effects in humans.

5. The applicant should provide more information about the potential effect of josamycin on the human gut flora.

6. The applicant should propose a residue marker for all edible tissues (muscle, liver, kidney, fat) and evaluate the ratio of the marker residue towards the microbiological active residues in chicken.

7. The applicant should provide suitable analytical physicochemical methods for the determination of residues in edible tissues of chicken species. These methods should be validated according the recommendations of Volume VI and described according a standard layout (Norm ISO 78/2).

Information should be provided on the sensitivity of josamycin in the microbiological residue monitoring assays which are used in the European community.