The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CARAZOLOL (extension to cattle)

SUMMARY REPORT (4)

1. Carazolol is a non-specific β-adrenergic receptor blocking agent. It is structurally analogous to the catecholamines (adrenaline and noradrenaline), in that, when administered, it forms reversible bonds with β-receptors; is does not, however, induce adrenergic effects, and it impedes the actions of the catecholamines in times of stress by saturating their sites of operation. In veterinary medicine, carazolol given by intramuscular injection to pigs is indicated in stress-inducing situations. In cattle, the substance is intended to be used at a single dose of 0.01 mg/kg bw for the prevention of shipment stress caused by transportation and formation of new herds (by intramuscular injection), the facilitation of parturition (by intravenous or intramuscular route), the facilitation of expulsion of the placenta (by intramuscular injection), increasing the level of fertility (by intravenous injection), and at 2 to 3 consecutive intramuscular doses of 0.01 mg/kg bw for training to mechanical milking.

   An ADI of 0.1 µg/kg bw based on the NOEL of 20 µg/kg bw for pharmacological effects in rabbits and a safety factor of 200, which provided a sufficient margin of safety to the reduction of vital capacity and forced expiratory volume in humans suffering from chronic bronchitis or asthma by carazolol, was established by the Committee for Veterinary Medicinal Products (CVMP). The same ADI has also been adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

   Currently carazolol is included in Annex I to Council Regulation (EEC) No 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Carazolol	Carazolol	Porcine	5 µg/kg 5 µg/kg 25 µg/kg 25 µg/kg	Muscle Skin + fat Liver Kidney	

   The Committee recently considered an application for the extension of the MRLs for carazolol to bovine and recommended the inclusion for carazolol in Annex III in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Carazolol	Carazolol	Bovine	5 µg/kg 5 µg/kg 15 µg/kg 15 µg/kg 1 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.1.2000

   The information requested for the establishment of final MRLs for carazolol in bovine has now been submitted.

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2. Three male and 3 female Italian Friesian cattle (bodyweight 154 to 203 kg) received a single intramuscular injection of the final medicinal product at a dose volume of 4 ml/100 kg bw, equivalent to 0.02 mg carazolol/kg bw. Blood samples were taken from each animal before dosing and at approximately 5, 15 and 30 minutes and 1, 2, 4, 8, 12 and 24 hours after dosing. Urine samples were taken before dosing and at approximately 6, 12, 24, 48 and 72 hours after dosing. Carazolol and the presence of 5 potential metabolites (carazolol acetate, carazolol lactate, carazolol diol, carazolol glucuronide and 4-hydroxylcarbazole) were determined in plasma and urine samples by means of an HPLC method with fluorimetric detection.

3. In plasma, the parent compound and 4 metabolites (-lactate, -diol, -glucuronide and 4-hydroxylcarbazole) could be detected. Carazolol acetate was not detected in any of the plasma samples. Carazolol was rapidly absorbed in the above cattle. A C_{max} of 5.07 to 6.90 µg/l was reached within 15 to 30 minutes after injection. Thereafter, carazolol concentrations declined rapidly, being detectable in only one animal at 8 hours after dosing.

   Carazolol lactate was detectable in plasma of 4 of 6 animals from 5 minutes to 8 hours post dosing, with maximum concentrations ranging from 0.77 to 2.85 µg/l. Carazolol diol was detectable in plasma of 5 of 6 animals from 5 minutes to 24 hours after injection, with maximum concentrations ranging from 2.47 to 6.26 µg/l. Low concentrations of the glucuronide conjugate and 4-hydroxylcarbazole were found in plasma, the former compound being detected in one animal in the first 8 hours post dosing with a maximum concentration of 0.59 µg/l, and the latter metabolite being identified in 2 animals at only one time point.

4. Analysis of urine samples revealed the presence of the parent compound and 3 metabolites (-lactate, -acetate and the glucuronide conjugate). Carazolol was found in the urine of all animals, and was mainly excreted within the first 24 hours. Carazolol lactate was found from 6 hours on, reaching peak levels at 48 to 72 hours after administration. The acetate metabolite was present in the urine of 4 animals, but only between 48 and 72 hours post dosing. The glucuronide conjugate was detected in the urine of 4 animals from 6 hours after dosing onwards. Urinary recovery expressed as a percentage of the dose was 9.8 to 25.5% in males and 3.9 to 8.9% in females.

   The relative concentrations of the metabolites in urine found were similar to those observed in pigs after intramuscular administration and in dogs after intravenous administration, and the same metabolites were also found in urine from humans after oral adminstration. Hence, it can be concluded that carazolol follows the same metabolic pathways in all species studied.

5. JECFA evaluated a study in which the pharmacological activity of several potential metabolites of carazolol was assessed in rabbits. The compounds examined were carazolol amine, -lactate, -diol, -acetate, -glucuronide, and 4-hydroxylcarbazole. JECFA concluded that carazolol-lactate, -diol, -acetate, -glucuronide, and 4-hydroxylcarbazole were pharmacologically inert. Carazolol amine (not detected in metabolism studies) showed some activity, but was about a 10-fold weaker than carazolol. As the metabolism in cattle was comparable to that of pigs and humans and the metabolites of carazolol were pharmacologically inactive, the parent compound carazolol is considered the most suitable marker residue.

6. Six male and 6 female cattle (Italian Friesian cattle, bodyweight 350 to 452 kg) received a single intramuscular injection of the final medicinal product at a dose volume of 2 ml/100 kg bw, equivalent to 0.01 mg carazolol/kg bw. The animals were slaughtered in groups of 2 males and 2 females at 8, 24 or 48 hours after administration. In tissues from treated cattle carazolol concentrations in tissues, including injection sites, deplete very rapidly to undetectable levels, with levels of carazolol being quantifiable in liver, kidney and injection site at 8 hours after treatment only. In untreated muscle and fat carazolol levels were below the limit of quantification of 1.5 µg/kg at this time point. Within 24 hours, the carazolol levels in liver, kidney and injection site had depleted to below the limit of quantification of 1.5 µg/kg.

7. In milk from treated cattle, levels of carazolol were quantifiable during the first milking after treatment only. Already after 12 hours, the carazolol levels had depleted to below the limit of quantification of 0.5 µg/kg.

8. A fully validated and well described (ISO 78/2 format) routine analytical method for the determination of carazolol residues in bovine tissues and in bovine milk is available. The limit of quantification is 1.5 µg/kg for bovine tissues and 0.5 µg/kg for bovine milk.

9. Final MRLs for bovine milk are confirmed at 1 µg/kg (i.e. twice the limit of quantification). Subsequently, based on the relative tissue distribution at 8 hours after treatment (which is approximately 3:1 for liver and kidney : muscle and fat), 15 µg/kg for bovine liver and kidney, and 5 µg/kg for bovine muscle and fat are retained as final MRLs.

Conclusions and recommendation

Having considered that:

- an ADI of 0.1 µg/kg bw, i.e. 6 µg/person, was set for carazolol on the basis of pharmacological activity, hence for pharmacologically active components only,
- the metabolism of carazolol in cattle is comparable to that in pigs and humans,
- carazolol is the marker residue, as the metabolites of carazolol possess no pharmacological activity,
- the relative tissue distribution at 8 hours after intramuscular treatment is 3 : 1 for liver and kidney : muscle and fat,
- the indication for use of carazolol in porcine and cattle, in combination with the rapid elimination of the compound in both species, allows in this particular case a theoretical excess of the ADI (in the case of pig meat and cow's milk being combined in the total food basket), since the probability for slightly exceeding the ADI in practice would be extremely low,
- a validated routine analytical method for the determination of carazolol in tissues and milk from cattle is available;

the Committee recommends the inclusion of carazolol for bovine in Annex I to 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
Carazolol	Carazolol	Bovine	5 µg/kg 5 µg/kg 15 µg/kg 15 µg/kg 1 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs values, the theoretical maximum daily intake will represent about 116.7% of the ADI.