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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DOXAPRAM

SUMMARY REPORT

1. Doxapram hydrochloride (1-ethyl-4-[2-(4-morpholinyl)ethyl]-3,3-diphenyl-2-pyrrolidinone hydrochloride, CAS 113-07-5, anhydrous) is a respiratory stimulant in humans and animals. It stimulates chemoreceptors of the carotid and aortic regions and has secondary analeptic properties by direct action on the central nervous system.

   Doxapram is used in horses and in newborn cattle and sheep. For adult horses an intravenous dose of 0.5 to 1 mg/kg bw is recommended, for neonates intravenous, subcutaneous or sublingual administration is used. In initiating or stimulating neonatal respiration, doses of 40 to 100 mg/calf (intravenous, intramuscular, subcutaneous, sublingual administration), and 5 to 10 mg/lamb (intravenous, intramuscular, subcutaneous or sublingual administration) are recommended depending on size of neonate and degree of respiratory depression.

2. In a pharmacological study doxapram was given intravenously (2 mg/kg bw or 4 mg/kg bw at 10 hour intervals) to 14 dogs (bodyweight 8.2 to 17.2 kg). At dose of 2 mg/kg bw, the respiratory effects and pressor effects were manifested without the transient hypotension. The transient hypotension seen after intravenously administration of doxapram (4 mg/kg bw). No changes in electrocardiogram pattern which could be related to the direct action of doxapram on the central nervous system were seen.

   Three dosages of doxapram (0.275, 0.55 and 1.1 mg/kg bw) were administered intravenously to six horses (bodyweight 473 to 623 kg) on 3 separate occasions at least 10 days apart. Doxapram produced cardiovascular stimulation, as well as respiratory stimulation, at all dosages given. Increasing dosages of doxapram increased intensity, but not duration, of respiratory stimulation and increased both intensity and duration of cardiovascular stimulation.

   Two groups of five newborn lambs (2 to 6 days old) received, respectively, intravenous infusions (2.5 mg/kg bw) of doxapram or keto-doxapram (an oxidative metabolite of doxapram) over a period of 1 minute. Both drugs stimulated ventilation, but the effect of doxapram was significantly higher. Also doxapram but not keto-doxapram, caused an increase in systolic blood pressure, as well as a change in neuro-behaviour. Because the ventilatory effects of keto-doxapram occurred during the first minutes of injection (5 to 10 minutes), it is probable that plasma concentrations of keto-doxapram between 5.3 mg/l (at 1 minute) and 1.3 mg/l (at 10 minutes) are necessary to maintain respiratory stimulation. In contrast, in as much as the ventilatory effects of doxapram lasted 20 minutes, a concentration in plasma of 0.9 mg/ml (range 0.48 to 1.3 mg/ml) to 3.1 mg/ml (range 2.7 to 3.6 mg/ml) appears to be necessary for maintenance of the ventilatory responses of doxapram.

3. Pharmacokinetic and biotransformation data have been provided from studies on rats and dogs. In female albino Wistar rats, the distribution of radioactivity in the tissues followed a similar pattern after oral and after intravenous administration of ^{14}C-doxapram (5 mg/kg bw; 0.69 µCi/mg). Radioactivity was excreted in the urine (23.7% and 21.4% in 24 hours after oral and intravenous treatment, respectively), faeces (63.8% and 59.9% in 24 hours, respectively) and bile (23.4% and 47.0% in 2 hours, respectively), but not in the expired air.

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After intravenous administration, the half-life in plasma was short (8.2 minutes). In 72 hours, 96.8% and 91.2% of radioactivity were excreted after oral administration and intravenous administration, respectively. After intraperitoneal injection (^{14}C-doxapram, 20 mg/kg bw), 28% of the radioactivity recovered from urine by 24 hours originated from unchanged drug. The level of radioactivity in the plasma and tissues declined with time and was undetectable after 12 hours. Information on the pharmacological activities of the metabolites was not available.

In dogs (n=8), doxapram (20 mg/kg bw intravenously) was rapidly and completely metabolised. In 24 hours about 33% and 29% of dose was excreted in the urine and faeces, respectively. Only traces of unchanged drug were found in urine. The results were similar in labelled and unlabelled studies. Doxapram concentration in bile was over 10 times higher than that in blood. The highest levels of metabolites were found in fat, liver, pancreas and adrenal glands. The levels in blood seem to decrease rapidly, followed by a more gradual decline.

One male dog received, over a 5 hour period, three intravenous doses of doxapram totalling 1.5 grams and urine was collected for 48 hours. Seven urinary metabolites were detected. It is suggested based on this data that there are two possible pathways of doxapram metabolism in dog, namely, de-ethylation to its analogue de-ethyldoxapram (over 50% of radioactivity), oxidation to keto-doxapram, and further opening of the morpholine ring of keto-doxapram producing a third metabolite.

Doxapram was administered intravenously to six horses (bodyweight 473 to 628 kg) at three doses (0.275, 0.55 and 1.1 mg/kg bw) given at least 10 days apart. Median values of total body clearence were 10.9, 10.6 and 10.9 ml/kg bw/min for the three doses and were thus independent of the dose. The steady-state volume of distribution was approximately 1.2 l/kg bw and the median biological half-life ranged from 121 to 178 minutes. Renal clearence of doxapram was a minor route of elimination. Metabolic clearance of doxapram appeared to be major route of elimination. Four metabolites of doxapram were isolated from urine.

Two groups of five newborn lambs (2 to 6 days old) received, respectively, intravenously infusions (2.5 mg/kg bw) of doxapram or keto-doxapram over a period of 1 minute. The elimination half-life for doxapram in lambs was 5.2 hours (range 1.2 to 11.6) and for keto-doxapram 2.3 hours (range 0.7 to 3.4). The apparent volume of distribution for doxapram was 1.2 l/kg (range 0.5 to 2.0) and for keto-doxapram 1.1 l/kg (range 0.3 to 2.1).

4. Doxapram showed a low acute oral and parenteral toxicity. The LD_{50} values in mice were 85 mg/kg bw intravenously, 153 to 175 mg/kg bw intraperitoneally and 270 to 326 mg/kg bw orally. In rats, the LD_{50} values were of the same magnitude. In neonatal rats, oral LD_{50} value was 83 mg/kg bw. In dogs and cats the intravenous LD_{50} was 40 to 100 mg/kg and the oral value was 150 to 300 mg/kg bw. The predominant adverse effect was impairment of the central nervous system. It was seen as hyperactivity, ataxia, and mild convulsions at higher doses.

5. Toxicity studies with repeated doses were conducted in rats and dogs using oral and intravenous routes. Doxapram was given to rats at 20 mg/kg bw intravenously for 10 to 20 weeks or 40, 80 or 160 mg/kg bw/day (5 days/week) orally for 4.5 weeks followed by a recovery period of 10 weeks. In all the doses studied there were signs of hypoxic effects (petechial haemorrhages in brains, loss of Purkinje cells in cerebellum). Consequently no NOEL could be determined from these experiments.

   In the dog study, doxapram was given orally to groups of 2 to 3 dogs each, at daily doses of 2.5, 5, 10, 20 (2 groups), 50, or 125 mg/kg bw for 6 days per week for 4 to 5 weeks. At the two highest dose levels the following effects were seen: respiratory stimulation, hypertension, tremors. Three dogs receiving 125 mg/kg bw/day and one of the two given 50 mg/kg bw/day died. Dogs given 20 mg/kg bw/day were not affected except for mild tremors and occasional vomiting and diarrhoea. No changes were observed in the clinical chemistry, haematological parameters or the histopathology in the dogs after the doses of 10 mg/kg bw or lower. However, due to limited number of animals used no toxicological NOEL could be established from this study.

6. Doxapram was studied for embryotoxic/teratogenic effects in the rat (15 or 25 mg/kg bw orally, vehicle not reported, 25 females in both groups) and the rabbit (18 mg/kg bw intravenously or 30 mg/kg bw intraperitoneally, 25 females in both groups) 15 rats and rabbits were used as controls. The drug was given from day 5 to day 11 after impregnation. The percentage of abnormal foetuses and newborn, resorption sites and maceration showed no differences between the studies on the treated animal and those on the control animal. The study did not comply with GLP-standards.

   In another study, albino rats (20 male and 20 female) were given doxapram in the diet equivalent to 100 mg/kg bw for about 70 days at which time the rats were mated and the compound administered until weaning after which point the rats were remated with different pairing. After remating the parent rats were treated intramuscularly with 25 mg/kg bw/day until termination of the study. The only effects were reduced survival and lowered body weight in pubs at weaning in the first litter of the animals treated orally. No abnormalities that could be attributed to compound administration were seen in pups.

   Based on the available information doxapram is not considered to have reproductive toxicity including teratogenicity.

7. Doxapram was tested in the *Salmonella*-microsomal assay for induction of reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538. Doses of 100, 333, 1000, 6670 and 10 000 µg per plate were employed in the presence and absence of an exogenous metabolic activation system of Aroclor induced rat liver microsomes. In addition there was a negative solvent control and ten known mutagens acted as positive controls. The results indicate that under the conditions of the experiment doxapram hydrochloride did not cause a positive response on any of the tested strains with or without the activator. It can be concluded that doxapram does not possess a mutagenic activity in this test.

8. No data on carcinogenicity were presented.

9. No specific studies were performed on the potential of doxapram for immunotoxicity. No alterations of haematological parameters were detected in the repeated dose toxicity studies. Doxapram has been used also in man and as far as is known, no allergic reactions have been reported. Based on the available information doxapram is not considered to have immutoxicological properties.

10. Doxapram is used in humans as a central and respiratory stimulant in a dose of 0.5 to 1 mg/kg bw intravenously at 5 minute intervals or by intravenous infusion (dose 2 to 4 mg/kg bw), the total daily dose not exceeding 3 grams per person.

    Typical adverse effects involve central and autonomic nervous systems (headache, dizziness, hyperactivity, convulsions), respiratory systems (cough, dyspnea) and cardiovascular systems.

    Biotransformation of doxapram was studied in explants from 15 human foetal livers. The fastest rate of doxapram metabolism occurred during the first 3 hours of incubation and the amount of doxapram metabolized was significantly higher at concentrations of 10 and 5 µg/ml than at 2.5 µg/ml. The oxidative pathway producing keto-doxapram and a second metabolite, is more active than the de-ethylation producing the analog of doxapram. Explant histopathology and alkaline phosphatase activity showed no direct toxic effects of the drug on liver tissue.

    In healthy men, after intravenous bolus injection (1.5 mg/kg bw, seven men) or infusion (3.5 mg/kg bw/hour, five men) the mean elimination half-life in plasma was 3.4 to 3.9 hours (a half-life of 7.4 hours was seen from 12 hours onwards after the intravenous infusion), the mean whole body clearance was 367 to 371 ml per minute, and the mean apparent volume of distribution was 110 to 125 l/person. After intravenous bolus injection, 0.4 to 4.0% of the parent compound was excreted in the urine within the first 24 hours. The metabolite keto-doxapram appeared rapidly (after 5 minutes) in plasma. After oral administration of the dose of 300 mg/person to seven volunteers, maximum concentration was achieved after 1 to 2 hours and the half-lives were 7.5 hours for doxapram and 9.2 hours for the metabolite keto-doxapram. Oral bioavailability was about 60%.

In a clinical study, five unanaesthetized male patients were given doxapram at dose rates of 0.20 to 0.23, 0.45 to 0.65 or 0.97 to 1.14 mg/kg bw intravenously. At the lowest dose level, two normal men showed no response, while slight stimulation of ventilation was detected in one normal and two patients suffering from respiratory disease. This stimulation of ventilation caused no changes in blood pH, pCO_2 or in electroencephalogram. At this level no side effects were seen.

Doxapram has also been used in infants (n=12) with a very low birthweight (less than 1250 g) with apnoea by continuous infusion of 0.1 mg/kg bw/hour, which was increased every 3 days up to 0.5 mg/kg bw/hour or until an adequate response was obtained for 2 to 16 days (mean 9.5 days). The only side effect was irritability in one patient, which disappeared immediately after discontinuing the doxapram medication. There were no signs of renal failure or liver toxicity, neither clinically nor by laboratory criteria.

Based on the available information on the use of doxapram in humans including neonates, doxapram is considered as being rather safe with limited side-effects. It is not possible to set a pharmacological NOEL based on these studies in man.

11. Due to the lack of toxicological or pharmacological NOEL values no final conclusion can be reached on an ADI for doxapram.

12. Depletion of doxapram was studied in sheep and horses using GLC with nitrogen sensitive flame ionisation detector (limit of detection 50 µg/kg). The methods used in these studies were not validated according to Volume VI of the Rules Governing Medicinal Products in the European Community.

    Three sheep (bodyweight 25 to 75 kg) were slaughtered one and 48 hours, respectively, after a single intravenous dose of 5 mg doxapram/kg bw. Doxapram concentrations in sheep tissues one hour after treatment (5 mg/kg bw intravenously) were as follows: in muscle 0.11 to 2.38 mg/kg, in liver less than 0.05 to 1.2 mg/kg, in kidney 1.64 to 2.84 mg/kg and in fat less than 0.05 to 7.47 mg/kg. After 48 hours all concentrations except in one liver (0.10 mg/kg) and one kidney (0.084 mg/kg) were below limit of detection.

    Three ponies (bodyweight 165 to 190 kg) were slaughtered 48 hours after a single dose of 5 mg doxapram/kg bw intravenously. Doxapram concentrations in horse tissues were following: in muscle and liver less than 0.05 mg/kg, in kidney less than 0.05 to 0.36 mg/kg and in fat less than 0.02 to 0.305 mg/kg.

    No information was provided on residue depletion in cattle.

Conclusions and recommendation

In spite of the fact that an ADI for doxapram could not be established, nevertheless, 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:

- doxapram is used only for neonates or individual adult horses,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- doxapram is rapidly absorbed and excreted;

the Committee for Veterinary Medicinal Products concluded that there is no need to establish an MRL for doxapram 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
Doxapram	All mammalian food producing species	