XYLAZINE

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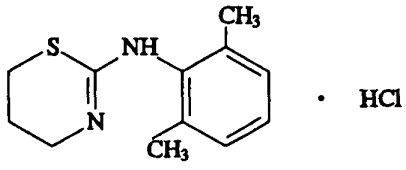
IDENTITY

Chemical name: 2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine hydrochloride (IUPAC name)

N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazine-2-amine hydrochloride (C.A.S. name)

Synonyms: BAY Va 1470, Xylazine hydrochloride, Rompun hydrochloride

Structural formula:



Molecular formula: $C_{12}H_{17}ClN_2S$

Molecular weight: 256.79

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Assay min. 99%

Appearance: White or almost white crystalline substance

Melting point: 165-168°C

Solubility: Freely soluble in water, very soluble in methanol and chloroform, practically insoluble in hexane and ether

UV_{max}: Not indicated

Stability: Not indicated

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITION OF USE

General

Xylazine is a clonidine analoque. It acts on presynaptic and postsynaptic receptors of the central and peripheral nervous systems as an α_2-adrenergic agonist. It is used primarily for sedation, anesthesia, analgesia and muscle relaxation but it has numerous other pharmacological effects. Most of these effects consist of of bradycardia and hypotension. Xylazine inhibits the effects of postganglionic nerve stimulation.

Dosage

Xylazine can be administered intravenously, intramuscularly, subcutaneously or orally. The commercial product contains 23.32 mg/ml xylazine hydrochloride in water based injectable solution. Xylazine can be obtained also as pure crystalline powder. There is a significant species dependent response to xylazine administration. Intramuscular dose of up to 0.3 mg/kg for cattle has been suggested by the manufacturer. The recommended doses for horses were 0.6 mg/kg and for sheep 1.0 mg/kg (Garcia-Villar et al., 1981). For dogs the dose was even higher.

METABOLISM

General

Investigations of rat urine and bile after administration of radiolabelled xylazine (^{35}S and ^{14}C, when both markers were on the thiazine ring) by paper electrophoresis and paper chromatography, approximately 20 metabolites were detected but not identified (Duhm et al., 1969). Only 8% of the labelled parent compound was recovered in the urine. The "principal" metabolite in urine represented 35% of the total radioactivity. The ratio between renal and biliary excretion of the radiolabelled compound was 7:3 but the report did not explicitly indicate if all of the radioactivity was recovered.

Putter and Sagner (1973) showed that less than 1% of the parent radiolabelled compound administered as xylazine hydrochloride could be recovered in cattle urine. Therefore, xylazine in cattle appears to undergo metabolic clearance only. The major metabolite excreted in cattle urine in free and conjugated form was identified as 1-amino-2,6-dimethylbenzene also known as 2,6-xylidine.

In a study utilizing LC/MS/MS and GC/MS techniques xylazine metabolites were characterized in horses *in vivo* and in rat liver *in vitro* (Mutlib et al., 1992). The major metabolites were identified as 2-(4'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, 2-(3'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, N-(2,6-dimethylphenyl)thiourea, and 2-(2',6'-dimethylphenylamino)-4-oxo-5,6-dihydro-1,3-thiazine. There were no data on xylazine metabolism for other species than rats and horses.

Pharmacokinetics

Comparative pharmacokinetics of xylazine in several species was reported by Garcia-Villar et al. (1981). The drug was administered intravenously and intramuscularly at recommended doses. The data was generated by analyzing serum drug concentration in samples obtained at 1, 2, 4, 8, 16, 30 and 120 min after xylazine administration. Compartmental analysis of the data was performed and the data best fitted a two-compartment open model. The major pharmacokinetic parameters are given in Table 1.

Table 1. Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intravenous administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively

Parameter	Horse (n=4)	Cattle (n=4)	Sheep (n=6)	Dog (n=4)
Weight (kg)	415-550	240-440	42-65	14-24
$t_{1/2}$ (min)	50	36	25	30
CL_b (ml/min/kg)	21	42	83	81
$V_{d(area)}$ (l/kg)	2.4	1.9	2.7	2.5

The terminal half-life of xylazine in all species was short indicating that xylazine concentration would decrease to undetectable level within a few hours. The total body clearance varied significantly and was fastest in sheep and dog and slowest in horse. Xylazine clearance has been attributed mainly to metabolic clearance. Therefore, there seems to be species variations in the metabolic rate of the drug. The volume of distribution was large in all species apparently because of the lipophilic nature of the compound.

The pharmacokinetic parameters after IM administration are given in Table 2. There were no differences in the half-lives after IM administration when compared to those after IV administration. The T_{max} values were reached within 15 minutes from drug administration and the peak concentrations were very low. Because of the low concentrations of the drug in bovine plasma, pharmacokinetic parameters after IM administration could not be determined in cattle.

Table 2. Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intramuscular administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively

Parameter	Horse (n=4)	Cattle (n=4)	Sheep (n=6)	Dog (n=4)
Weight (kg)	415-550	240-440	42-65	14-24
$t_{1/2}$ (min)	58	N.D.	22	35
T_{max} (min)	13	N.D.	15	13
$C_{(max)}$ (μg/ml)	0.2	N.D.	0.1	0.4

TISSUE AND MILK RESIDUE DEPLETION STUDIES

Tissues

Two studies using radiolabelled xylazine were performed (Murphy and Jacobs, 1975 and Murphy et al., 1978). One study in which 4 animals, two steer calves, one bull calf and a dairy cow, were given xylazine intramuscularly utilized ^{14}C-label in the 4'-position of the thiazine ring of the compound. The other study was conducted on 5 animals, two steer calves, one bull calf and two dairy cows, and were administered xylazine intramuscularly that carried a ^{14}C-label in 4-position of the aniline ring of the molecule. In both studies a dose of 0.33 mg/kg was used.

In both studies recovery of radioactivity from urine and faeces increased as a function of time (Tables 3 and 4). At 10 hours after administration of the two differently labelled compounds 51-68% of the radioactivity was recovered. Between 24-72 hours post administration 83-100% of the radiolabel was recovered except for one bull calf where a recovery of only 38% was recorded at 72 hours following administration.

Table 3. **Rocovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying ^{14}C-label in the 4'-position of the thiazine ring of the compound**

Animal	Steer calf	Steer calf	Bull calf	Dairy cow
Time after administration (h)	10	24	48	74
	% radioactivity recovered			
Urine	65	71	63	77
Faeces	3	15	20	23
Total	**68**	**86**	**83**	**100**

Table 4. **Rocovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying ^{14}C-label in the 4-position of the aniline ring of the compound**

Animal	Steer calf	Steer calf	Bull calf	Dairy cow	Dairy cow
Time after administration (h)	10	48	72	72	72
	% radioactivity recovered				
Urine	48	82	35	73	85
Faeces	3	15	3	10	14
Total	**51**	**97**	**38**	**83**	**99**

After administration of xylazine ^{14}C-labelled in the 4'-position of the thiazine ring at 0.33 mg/kg, radioactivity equivalent to 0.004 mg xylazine/kg or higher was found in all the 12 different analyzed tissues collected from the treated animals. Highest concentrations were measured in the injection site, kidney and liver (0.022-0.406 mg/kg). When xylazine was administered as above but with a ^{14}C-label in the 4-position of the aniline ring, radioactivity exceeding the detection limit was found in all injection site, kidney and liver samples (0.009-1.152 mg/kg), and in all samples collected from the steer calf sacrificed 10 hours after drug administration (0.009-0.761 mg/kg). The characteristics of these residues were not studied and due to the difference in sensitivity of the radiolabel detection in the two studies it was difficult to predict whether the residues consist of double or single ring structures.

Several other tissue residue depletion studies were conducted (Putter and Sagner, 1969, Dorn and Maasfeld, 1990, Redgrave and Cameron, 1991a, Heukamp, 1991a). The first of these studies showed that the injection site residues declined to less 1/1000 in 20 hours after xylazine administration at 1.0 mg/kg to sheep. In the same study peripheral muscle concentrations were between 0.09 and 0.21 mg/kg during the same period. None of

the other studies were able to detect xylazine residues in tissues when detection level was 0.01 mg/kg an muscle and 0.05 mg/kg in liver and kidney tissues. These studies were conducted in bovine after single IM dose of 0.3 mg/kg. It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

Milk

Detectable radioactivity in milk was found up to 72 hours after administration of the ^{14}C-xylazine labeled in the 4'-position of the thiazine ring and up to 24 hours after administration of the ^{14}C-xylazine labeled in the 4-position of the aniline ring. The chemical nature of these residues was not investigated.

Xylazine residues in bovine milk were investigated (Dorn and Maasfeld, 1990b, Redgrave and Cameron, 1991b and Heukamp, 1991b). A single IM dose of 0.3 mg/kg was used. In the first study xylazine concentrations exceeding the 0.01 ppm detection level were not observed when milk samples were collected after each milking for 7 days. In the second study, in 3 samples out of 6, concentrations ranging from 0.012 to 0.019 were detected 5-8 hours after xylazine administration at 0.3 mg/kg IM to lactating cows.

In an earlier study xylazine milk concentrations in 2 cows after IM administration at 0.2 mg/kg were determined (Putter and Sagner, 1973). In this study concentrations ranging from 0.03 to 0.08 μg/ml were found at 5 and 21 hour after administration.

It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The early reports concerning xylazine residues in tissues were analyzed with a method based liquid-liquid extraction from alkaline solution with hexane, cleaned by passage through basic aluminium oxide column and filtered (Putter and Sagner, 1969; Putter and Sagner, 1973). The hexane fraction was then concentrated and xylazine was extracted to phosphate buffer pH 5.0. A spectrophotometer adjusted at 240 nm was then used for detection. Muscle, milk and urine samples could be analyzed by practically similar procedures. These papers describe also a thin layer chromatography method based on silica gel stationary phase and ethanol:water or ethanol:benzene:chloroform mobile phases. The spots were made visible by $AgNO_3$ and fluorescein.

Xylazine analysis based on paper, liquid and gas chromatography procedures have been described (Duhm et al., 1969, Maasfeld, 1991, Mutlib et al., 1992). Two multiresidue methods for tissue based on reversed phase liquid chromatography using either phenyl or C18 columns and UV and/or fluorescence detection were published (Etter et al., 1984 and Keukens and Aerts, 1989). The first method used a mixture of dichloromethane and petroleum ether for extraction of the compound from alkaline muscle or kidney tissue homogenate. In the second method swine kidneys were homogenized with acetonitrile and sodium chloride was added before solid phase extraction by use oc C18 cartridge. After elution with acidic acetonitrile and hexane extraction of the eluate the aqueous phase was used for chromatography. Both methods claim claim a 5-10 μg/kg detection limit but the reported recovery for xylazine was low (45-70% depending on the tissue and concentration) by use of either method. The performance characteristics of the method of Maasfeld (1991), which was used in the subsequent tissue residue depletion studies, were insufficiently described.

APPRAISAL

Depletion studies with thiazine ring radiolabeled ^{14}C-xylazine administered orally indicated that in rats 2% of radioactivity was still present 48 hours after administration. The ratio for recovery of the radiolabeled compound in urine and faeces was 7:3.

Pharmacokinetic data concerning the parent compound were reported in studies including cattle, horses, sheep, dogs and laboratory animals. Xylazine had a very short plasma half-life which in most species was

approximately 0.5 hours and in horses 0.9 hours. The compound underwent a rapid clearance. Species differences in clearance indicate different metabolic activity and/or different metabolic pathways. The apparent volume of distribution was large 1.9-2.5 l/kg due to the lipophilic nature of the drug. Plasma depletion of unlabeled compound in cattle was more rapid than depletion of total radioactivity in a similar study using ^{14}C-xylazine. Therefore, clarification of xylazine metabolism is required in order to better understand its pharmacokinetics.

The excretion of thiazine ring radiolabeled ^{14}C-xylazine administered intramuscularly to cattle (3 calves and one milking cow) and slaughtered at different time intervals was complete at 74 hours. The ratio of the radioactivity between urine and faeces was 3:1. In a related study using intramuscular administration of ^{14}C-xylazine labelled in the aniline ring, the excretion of the radioactivity was variable, ranging from 38-99%. In a second study, the respective ratios of the radioactivity for urine and faeces ranged from 12:1 to 6:1.

Studies on xylazine in rat and horse urine indicated extensive metabolism. However, no data concerning xylazine metabolism in other animals were available. Due to the lack of these data the possibility that metabolism causes the discrepancy between the depletion studies using radiolabeled compound and the unlabeled compound cannot be evaluated.

Two ^{14}C-radiolabel depletion studies using intramuscular administration of xylazine in cattle were submitted. The first study with three calves and one lactating cow used xylazine, labelled in the thiazine ring, and the second study used four calves and two lactating cows administered xylazine, labelled in the aniline ring. The radiolabeled tissue residue depletion studies showed that total residues in mg/kg xylazine equivalents in kidney, liver, and injection site were 0.009-0.020, 0.022-0.050 and 0.030-1.152, respectively, at 72 hours after administration. Results were similar in the study using a thiazine ring labeled ^{14}C-xylazine. In milk the radioactivity as xylazine equivalents had declined to 0.01 mg/l after treatment with the drug labelled in the thiazine ring or in the aniline ring by 60 and 12 hours, respectively.

The data generated in tissues of cattle and milk residue depletion studies in which only the concentration of the parent compound, xylazine, was determined were in clear contrast with the radiolabel studies. The studies with unlabeled compound failed to detect xylazine at 0.01 mg/kg in muscle, kidney, liver and fat. Similarly, xylazine concentrations in milk exceeded the 0.01 mg/l detection level only occasionally. Thus the majority of the residues were not parent drug, but were unidentified metabolites.

A number of analytical methods, mainly for parent compound, such as photometry, liquid chromatography, gas chromatography, and mass spectrometry, were described. Performance chraracteristics were poorly determined but a limit of detection of 0.01 mg/kg was claimed. No method validation data were available for evaluation.

Maximum Residue Limits

The following factors were considered by the Committee with respect to the assignment of MRLs:

No ADI was established;
Lack of adequate data on metabolism of the compound;
No marker residue could be assigned; and
There were insufficient residue depletion studies available.

The Committee did not recommend MRLs.

The following information would be required before a further review:

Data on xylazine metabolism in target species sufficient to identify a suitable marker residue and target tissues;

Additional data on residue depletion of xylazine and its metabolites in target species. These data should include evidence to show, in particular, whether 2,6-xylidine is present at the recommended withdrawal period; and

A suitable analytical method for determining the marker residue in target tissues.

REFERENCES

Etter, R., Battaglia, R., Noser, J. and Schuppischer, F., (1984). Nachweis von Tranquilizern in Nieren und Muskelfleisch. Mitt. Gebiete Lebensm. Hyg., **75**, 447-458.

Garcia-Villar, R., Toutain, P-L., Alvinerie, M. and Ruckebusch, Y., (1981). The pharmacokinetics of xylazine hydrochloride: An interspecific study. J. Vet. Pharmacol. Therap. **4**, 87-92.

Dorn, H. and Maasfeld, M., (1990). Residue invesgations of tissue samples from cattle following treatment with Rompun. Bayer Study Report Dr.Mf./RA-77

Dorn, H. and Maasfeld, M., (1990b). Residue invesgations in milk samples from cows following treatment with Rompun. Bayer Study Report Dr.Mf./RA-78

Duhm, B., Maul, W., Medenwald, M., Patzschke, K. and Wagner, L., (1969). Untersuchungen mit radioactiv markiertem BAY Va 1470 an Ratten. Berliner und Munchener Tierarztliche Wochenschrift. **82**, 104-109.

Heukamp, U., (1991a). Tissue concentrations of xylazine in calves following intramuscular administration of Rompun - analytical phase. Bayer Study Report BAY 160.

Heukamp, U., (1991b). Milk concentrations of xylazine in dairy cows following intramuscular administration of Rompun - analytical phase. Bayer Study Report BAY 161.

Keukens, H. and Aerts, M., (1989). Determination of carazolol and a number of tranquilizers in swine kidney by high performance liquid chromatography with ultraviolet and fluorescence detection. J. Chromatogr., **464**, 149-161.

Maasfeld, W., (1991). Analytical method for the determination of xylazine in bovine tissues and milk. Bayer Study Report 76/1991.

Mutlib, A., Chui, Y., Young, L. and Abbott, F., (1992). Characterization of metabolites of xylazine produced in vivo and in vitro by LC/MS/MS and GC/MS. Drug Metab. Dispos. **20**, 840-848.

Murphy, J. and Jacobs, K., (1975). Residues of Rompun and its metabolites in cattle. Bayer Study Report No. 43814, Bayer AG. Leverkusen, Germany.

Putter, J. and Sagner, G., (1969). BAY Va 1470. Residue examinations in muscles of sheep and cattle. Bayer Study Report 28018.

Putter, J. and Sagner, G., (1973). Chemical studies to detect residues of xylazine hydrochloride. Vet. Med. Rev. **2**, 145-159.

Redgrave, V. and Cameron, D., (1991a). Tissue concentrations of xylazine in calves following intramuscular administration of Rompun - animal phase. Huntingdon Research Center Study Report BAY 160/91194.

Redgrave, V. and Cameron, D., (1991b). Milk concentrations of xylazine in dairy cows following intramuscular administration of Rompun - animal phase. Huntingdon Research Center Study Report BAY 160/91119.

Walker, D., (1995). Expert report on the residue file for xylazine (Rompun TS and 2%). Report ID 15570, Bayer AG. Leverkusen, Germany.