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METABOLISM OF CHLORPROMAZINE IN GOATS

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The investigations on the metabolism of chlorpromazine (Cpz) in goats revealed that soon after, and until 24 hours, intravenous administration, Cpz was excreted in the urine as unchanged drug, Cpz-sulfoxide, monodesmethyl-Cpz, monodesmethyl-Cpz-sulfoxide, 7 hydroxy-Cpz, 7 hydroxy-Cpz-sulfoxide and at least 4 more unidentified compounds. The non-phenolic compounds constitute about 10 per cent and the phenolic compounds constitute about 60 per cent of the urinary excretion as non-conjugated metabolites while remaining 30 per cent are excreted in the conjugated form.

INTRODUCTION

The investigation on the drug metabolism are important for the determination of their fate in any organism. After metabolism the drugs are usually transformed into inactive and rarely to more active derivatives. Chlorpromazine (Cpz) is one of the extensively used drug in human and veterinary medicine and several studies have been instituted to determine its metabolism in man and various species of animals (Carr 1962, Domino 1962, Emerson and Miya 1963, Domino 1965, Gordon 1967, Goldenberg and Fishman 1970, Turano *et al.* 1973 and Breyer *et al.* 1974). However, such a work has not been undertaken in goats. therefore, the present study deals with the metabolism of Cpz in goats.

MATERIALS AND METHODS

For the investigation of Cpz metabolism, the urinary excretion of Cpz metabolites was investigated on 6 healthy goats maintained under similar managemental conditions. Two types of experiments were performed to evaluate the metabolism soon after and 5 to 24 hours after the administration of drug.

(a) Cpz (Klorpromazin, injectabile 2.5 per cent, Novo, Copenhagen) was diluted with 0.9 per cent sodium chloride and given intravenously as a priming dose 2.5 mg/kg.b.wt. Subsequently, in order to achieve a constant

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plasma level, maintenance dose ranging between 0.64 to 2.88 mg/kg/hour diluted with 0.9 per cent sodium chloride was infused (Sigmamotor (R) infusion pump) through a catheter (Intracath(R)) placed in the left jugular vein. At 30 minutes after priming dose urinary bladder was completely emptied and washed with distilled water through a baloon catheter (Rush nr. 14, 30 ml). The urine samples collected at 1 to 2 hours after priming were used for the assay of Cpz-metabolites. For the extraction of metabolites from urine, n-heptane containing 1.5 per cent isoamyl alcohol was used as a solvent.

(b) The urinary excretion of Cpz-metabolites between 5 to 24 hours after the intravenous administration of radioactive ^{35}S-chlorpromazine (12μ Ci/kg. b.wt.) was investigated in 3 goats. The goats were maintained in the metabolic cages and total volume of urine voided between 5 to 24 hours post drug administration was collected completely. A part of this urine was taken for the extraction of metabolites by dichloromethane (DCM) containing 15 per cent propanol-2.

The samples were extracted thrice at pH 12-13. After extraction at pH 12-13, the pH of samples was lowered at 8.5 and the extraction was done thrice. Subsequently pH was brought down at 2 and the samples were extracted. The solvent containing the extracted material at each pH value was pooled and evaporated under reduced pressure at 38°C. The residue was dissolved in methanol and a suitable volume of the methanol was plotted on TLC-plates. The plates were run in 2 solvent systems. After developing the TLC plates were sprayed with the colour reagent and heated at about 100°C for 3 minutes. The colour and the Rf values of the spots from the samples were compared with those of the standards for the identification of metabolites (Chan and Gershon, 1973).

The urine samples containing ^{35}S-Cpz were extracted with DCM. The DCM extracts prepared at each pH value of the urine were used for radio-quantitation. A maximum of 0.5 ml extract was taken in the scintillation liquid containing

200 mg dimethyl-POPOP (Merck art. 7248)
8 g PPO (Merk art. 2946)
100 ml Bio-solv. BBS-3 (Beckman)
1900 ml toluene (Merck art. 8317).

Samples were counted in plastic vials into which 10 ml scintillation liquid and

2 drops of 15 per cent ascorbic acid aqueous were added. The counting was done in liquid scintillation counter (Model LS-133, Beckman).

RESULTS

The heptane extract obtained at the urine pH 12-13 was used for TLC. After spraying the developed TLC-plates with the colour reagent, 4 spots were seen on the plates. The TLC characteristics of these spots are given in the Table 1.

Table 1. *Thin-layer chromatographic characteristics of chlorpromazine metabolites in the urine of goats following intravenous administration of chlorpromazine.*

		Urine samples			*Standards*			
Extraction pH	com-pound	colour after spray	Rf value in system I	II	colour after spray	Rf value in system I	II	identified as
Before hydrolysis								
pH 12-13	1	Pink	0.62	0.41	Pink	0.61	0.36	Cpz-unchanged
	2	Pink	0.44	0.25	Pink	0.46	0.21	Cpz-SO
	3	Pink	0.48	0.13	Pink	0.46	0.14	Cpz-Norl
	4	Pink	0.28	0.06	Pink	0.29	0.06	Cpz-Norl-SO
pH 8.5	as for pH 12-13							
After alkaline hydrolysis								
pH 12-13	5	Purple	0.60	0.36	Purple	0.59	0.37	7 OH-Cpz
	6	Purple	0.49	0.21	Purple	0.48	0.23	7 OH-Cpz-SO
	7	yellow	0.58	0.63				not identified

pH 8.5 and pH 2 did not show any compound.

Comparison of the colours and Rf values of the spots from the samples with those of the standards showed that these spots were of unchanged-Cpz, Cpz-sulfoxide (cpz-SO), monodesmethyl-Cpz (Norl) and monodesmethyl-Cpz-SO (Norl-SO). The heptane extract prepared at pH 8.5 showed only incomplete extraction while the extracts prepared at urine pH 2 did not contain any compound.

The conjugated metabolites were assayed after alkaline hydrolysis of the urine samples. The heptane extract prepared at urine pH 12-13, was subjected

to TLC-procedure and revealed the presence of 3 spots (Table 1). Two spots were of purple colour. The colour and the Rf values of these spots were compared with the colour and Rf values of the standards and were identified as 7 hydroxy-Cpz (7 OH-Cpz) and 7 hydroxy-Cpz-sulfoxide (7 OH-Cpz-SO). The third spot of yellow colour had Rf value 0.58 in system I and 0.63 in system II, could not be identified because of lack of proper standards. The extracts prepared at pH 8.5 and 2 did not contain any compound.

(b) The radioactivity that was extracted with DCM from the urine samples of goats collected at 5 to 24 hours after the administration of ^{35}S-Cpz and the radioactivity that could not be extracted from the residual urine sample are shown in the Table 2. From the table it is seen that 69 to 80 per cent of the radioactivity was extractable from the urine samples at different pH while the residual urine still contained 22 to 27 per cent of the radioactivity. In 3 experiments, the average of radioactivity that was extractable with DCM was 73 per cent in both the unhydrolysed and hydrolysed urine samples.

Table 2. *Percentage of the radioactivity extractable with dichloromethane and remaining in the urine of goats administered ^{35}S-chlorpromazine.*

Extracted at	Without hydrolysis			After hydrolysis		
Goat No.	1	2	3	1	2	3
pH 12-13	32	28	20	34	23	26
pH 8.5	36	35	41	41	42	40
pH 2.0	6	6	6	5	4	3
Residual urine	22	36	28	37	31	29

TLC revealed that essentially similar metabolites were present in both the unhydrolysed urine samples and the samples subjected to the enzyme hydrolysis with β-glucoronidase-aryl-sulfatase. The chromatographic behaviour of these metabolites is shown in Table 3. From the table it is seen that the identified compounds were 7 OH-Cpz and 7 OH-Cpz-SO. The compounds 3, 6, 7 and 8 were not identified because of lack of proper standards.

The urine samples of the goats administered ^{35}S-Cpz were extracted with heptane at pH 12-13 and on radioquantitation showed 9 per cent of the radioactivity. The heptane extract after TLC, demonstrated the presence of unchanged-Cpz, Cpz-sulfoxide, monodesmethyl-Cpz and monodesmethyl-Cpz-sulfoxide.

Table 3. *Thin-layer chromatographic behaviour of chlorpromazine metabolites extracted from the urine of goats administered ^{35}S-chlorpromazine.*

Extraction pH	Compound	Colour after spray	Rf value in system II	identified as
pH 12-13	1	Purple	0.36	7 OH-Cpz
	2	Purple	0.21	7 OH-Cpz-SO
	3 (trace)	Purple (light)	0.82	Not identified
pH 8.5	5	Purple	0.22	7 OH-Cpz-SO
	6	Green on heating purple	0.16	Not identified
	7 (trace)	Purple (light)	0.86	Not identified
pH 1 2	8 (trace)	Purple (light)	0.79	Not identified

DISCUSSION

The results given in Table 1 show that the compounds extractable with heptane at the urine pH 12-13 and 8.5 were unchanged-Cpz, Cpz-sulfoxide, monodesmethyl-Cpz and monodesmethyl-Cpz-sulfoxide. The same compounds were also seen in the heptane extracts prepared from the urine samples containing ^{35}S-Cpz and constituted 9 per cent of the radioactivity in the urine samples.

The observations recorded in Table 2 clearly demonstrate that the β-glucuronidase-aryl-sulfatase used 2.5 per cent in the urine did not cause hydrolysis of the conjugates and therefore, one third to one fourth of the ^{35}S could not be extracted and remained in the residual urine. This may be due to the reason that the enzyme concentration used for hydrolysis was inadequate because Kaul *et al.* (1971) recommended 15 folds higher enzyme concentrations than normally used for obtaining a satisfactory hydrolysis. In view of these observations, the results given in Table 2 reveal that the conjugated fraction constitute 27 per cent while non-conjugated fraction constitute 73 per cent of the urinary excretion of Cpz-metabolites in the goats.

The heptane extract prepared after alkaline hydrolysis showed 3 metabolites, 2 of which were identified as 7 OH-Cpz and 7 OH-Cpz-SO. The third unidentified metabolite was of yellow colour on TLC plates. This colour of the spot might have originated during heating for alkaline hydrolysis because such a colour of Cpz metabolite(s) has not been found in the biological samples (Turano *et al.*, 1973). The same phenolic metabolites were identified in the DCM extracts prepared from the urine containing ^{35}S. Besides, DCM extracts contained 4 spots of purple colour which could not be identified. The colour reaction of these unidentified spots indicated that these metabolites were of

phenolic nature exhibiting purple colour with the colour reagent (Turano *et al.*, 1973). It was confirmed by comparison with the standards that these were not 7, 8 dihydroxy or 8 hydroxy metabolites.

From the results of the present investigation it is concluded that following the administration of Cpz, besides unchanged-Cpz the metabolites of Cpz excreted in the urine of the goats include Cpz-sulfoxide, monodesmethyl-Cpz, monodesmethyl-Cpz-sulfoxide, 7 hydroxy-Cpz, 7 hydroxy-Cpz-sulfoxide and at least 4 more phenolic compounds which were not identified. The non-phenolic compounds constitute about 10 per cent and the phenolic compounds constitute about 60 per cent of the urinary excretion as non-conjugated metabolites while remaining 30 per cent are excreted in the conjugated form.

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