THE METABOLITE PATTERNS OF ELTOPRAZINE IN MAN, DOG, RAT AND RABBIT

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CONTENTS

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SUMMARY

To compare the metabolism of eltoprazine of dog, rat and rabbit with that in man, urine samples were collected after dosing with ^{14}C-eltoprazine. The ^{14}C-labelled metabolites were separated by chromatography and detected by their radioactivity. This resulted in so-called metabolite patterns.

The human metabolite pattern contained peaks that were all found in that obtained from the dog's urine. The dog's metabolite pattern had two peaks that were (almost) absent in all other species. The rat's urine gave a pattern which had only two peaks in common with the human pattern.

Unchanged drug was excreted in significant amounts by man, dog, and rat, but not by rabbit. This excretion was even a little more pronounced after intravenous injection of the drug. In man, the ratio between unchanged drug and metabolites was fairly constant with time after dosing, while this ratio decreased in the animal species.

The major part of the metabolites were sulphate- or glucuronide conjugates, but hydrolysis of these required extraordinary amounts of enzyme.

We do not yet know whether the observed species differences reflect differences in conjugating activity or (and) oxidative metabolism.

We could not identify important differences in the metabolite patterns that were due to sex or route of drug administration. Also, the site of the ^{14}C-label in the drug molecule hardly affected the metabolite patterns; the only effect was the excretion by the rat of a very polar but minor component when it was dosed with ^{14}C-piperazine labelled eltoprazine. This component was absent when ^{14}C-phenyl labelled eltoprazine was given.

I. INTRODUCTION

The metabolism of eltoprazine in three animal species has been compared with that in man. This was done by means of so-called metabolite patterns: the chromatograms of the metabolites. In this study, we have made metabolite patterns of ^{14}C-labelled eltoprazine. Urine samples were collected from animals that were dosed with this compound. With high performance chromatography (HPLC), the metabolites were separated and detected by their radioactivity. Of course, the chromatograms do not give the identity of the metabolites (they will be isolated and

identified later) but differences in chemical structures of the metabolites will usually give different metabolite patterns.

Most of the radioactivity was excreted in urine; faeces only contained 1 to at most 15% of the dose /1/. The amount of faecal radioactivity was often too low to produce reliable chromatograms. So, in this paper only urinary metabolite patterns are presented.

II. MATERIALS AND METHODS

2.1 Chemicals

The chemicals were obtained from companies as follows: Ammonium acetate from Baker Chemicals, Deventer, The Netherlands; Zorbax C8 from DuPont Co., Wilmington, DE, USA; Lichroprep RP8 and Lichroprep Si60 from Merck, Darmstadt, FRG; Instagel and Picofluor 30 from Packard, Groningen, The Netherlands; Liquid scintillator NE 267 from Nuclear Enterprises Ltd., Edinburgh, Scotland; Helix pomatia juice from Pharmindustrie, Clichy, France. [Piperazine-U-^{14}C]eltoprazine and [phenyl-U-^{14}C]eltoprazine were synthesized as reported elsewhere /1/.

2.2 Methods

2.2.1 Urine collection

Urine samples were collected from healthy male volunteers who received 10 mg [phenyl-U-^{14}C]eltoprazine. Urine samples were also collected from dogs, rats, and rabbits that were dosed orally (or in some cases intravenously) with 5 mg/kg of [phenyl-U-^{14}C]eltoprazine or [piperazine-U-^{14}C]eltoprazine.

2.2.2 Sample pretreatment

To hydrolyse eventually present glucuronide or sulphate conjugates, urine samples were incubated at pH 5 for at least 16 hours at 37°C, with 1 or 10% v/v Suc d'Helix pomatia. The urine samples were filtered through a 1.2 μm filter before HPLC.

2.2.3 High performance liquid chromatography (HPLC)

Urine samples (0.5 - 2 ml) were injected directly onto the column by a model 7125 loading sample injector equipped with

a 2 ml removable sample loop (Rheodyne, Berkeley, CA, USA). Two HPLC pumps model 510 (Waters Ass., Milford, MA, USA) were used with a gradient controller (Waters). The HPLC column (9 x 500 mm) was packed with Zorbax C8. The two eluents water and methanol both contained ammonium acetate (4 g/l). The flow rate was 3.0 ml/min.

The elution programme is given in Table 1.

2.2.4 Measurement of radioactivity

The HPLC-effluent was counted for radioactivity on line with a Ramona radioactivity monitor (Raytest GmbH, Straubenhardt, FRG) which mixed the effluent with liquid scintillator (Picofluor 30 : NE 267 = 1:1).

Off-line counting was done by mixing one-minute fractions with 3.5 ml Instagel. The radioactivity was measured in a Philips PW4540 scintillation counter (Philips, Eindhoven, The Netherlands).

TABLE 1

HPLC elution programme

Step no.	Percentage methanol in eluent at start of step	Elution time per step (minutes)	Elution gradient
1	0	10	linear*
2	10	10	isocratic**
3	10	5	linear
4	15	5	isocratic
5	15	5	linear
6	20	10	isocratic
7	20	5	linear
8	25	20	isocratic
9	25	30	linear
10	100	20	isocratic

* linear : linear increasing methanol concentration.
** isocratic : constant methanol concentration.

III. RESULTS

3.1 Species differences in metabolite patterns

3.1.1 Some examples of chromatograms and numbering of HPLC peaks

In fig. 1 the metabolite patterns of orally dosed man, dog, rat and rabbit are shown. The dog gave the most differentiated pattern so we used this chromatogram to number the peaks from 1 to 15. The resolution between peaks nos. 3 and 4 and between nos. 7 and 8 was not complete in a number of runs. Therefore, we could not always discriminate between them, e.g. the peaks 7 and 8 in the chromatogram obtained from human urine.

The unchanged drug eluted at the elution time of peak 15. Also, the unchanged drug concentration in the urine, as measured by a different method, corresponded with the levels of radioactivity in this peak. We thus have good evidence that peak 15 is indeed unchanged eltoprazine.

Obviously, man, rat and rabbit produced fewer metabolites than the dog. With their retention time, they could be correlated with the metabolites in the dog's urine as indicated by the numbering in the respective metabolite patterns.

3.1.2 Species differences

Fig. 2 gives the metabolite patterns as histograms with the HPLC-peak numbers. Based on the retention times, the HPLC-peaks obtained with man's urine could all be correlated with HPLC-peaks obtained with dog's urine. However, metabolites nos. 1,2,6,7, and 14 were absent in human urine.

Metabolite nos. 2 and 12 were uniquely present in the dog's urine. Peak no. 1 was observed in very low amounts using non-human urine.

Man excreted much unchanged drug (peak no. 15) ranging between 30 to 65% of the urinary radioactivity, which was more than in the animal species studied. Rabbit did not excrete unchanged eltoprazine at all. In samples obtained later than 24 hours after dosing, the ratio between unchanged drug and metabolites decreased in the urine samples of all species, except in human urine where it remained practically constant.

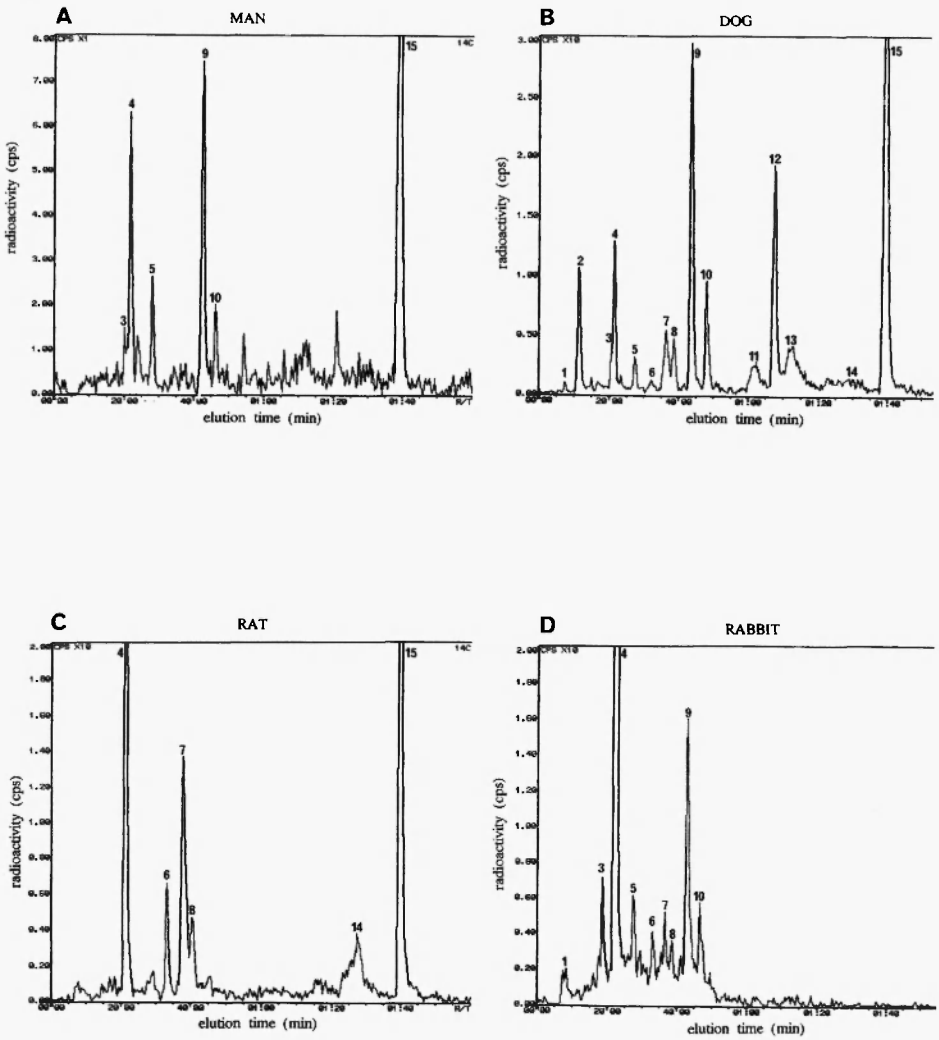

Fig. 1: HPLC-radioactivity chromatograms of urine samples from man (A), dogs (B), rats (C), and rabbits (D). The samples were collected between 0 and 24 hours (man, rabbit) or between 0 and 6 hours (rat and dog) after administration of radiolabelled eltoprazine.

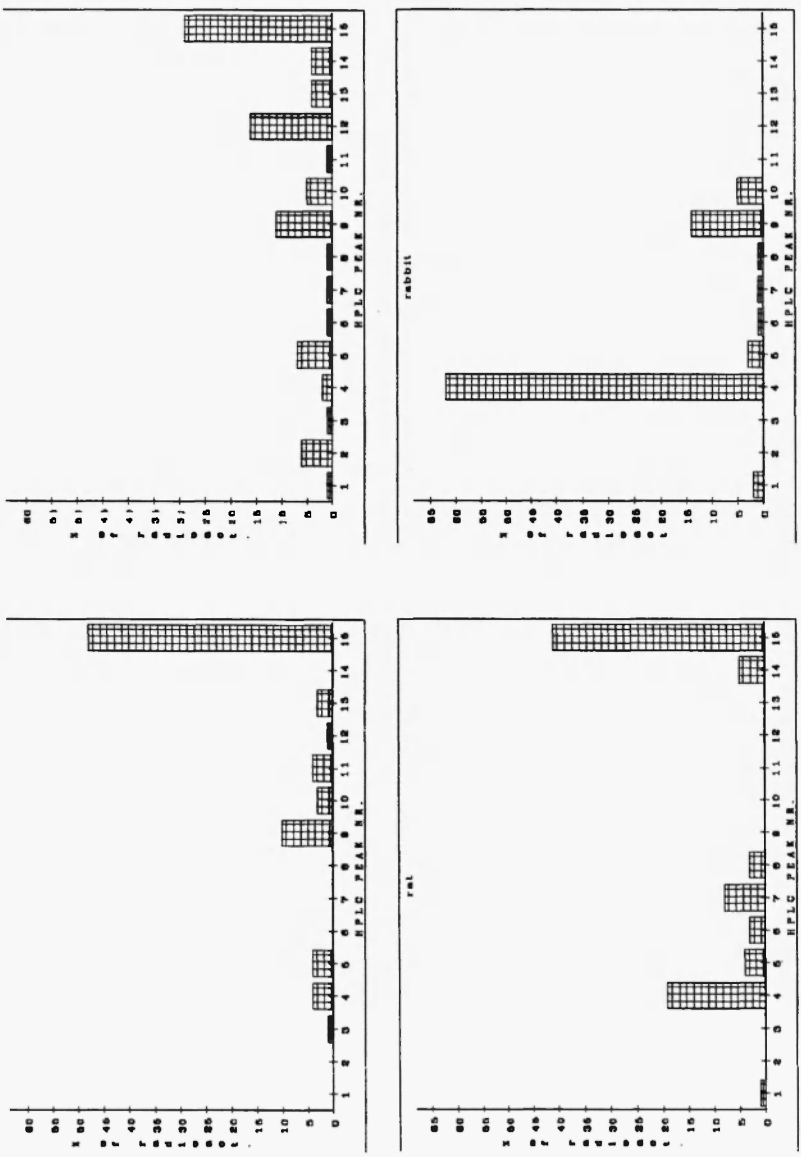

Fig. 2: HPLC-peaks quantified by radioactivity in chromatograms made from urine samples from man, dog, rat and rabbit, collected between 0 and 24 hours after administration of radiolabelled eltoprazine.

3.2 Hydrolysis of sulphate- and glucuronide conjugates of eltoprazine metabolites

Routinely, we treat urine samples from drug dosed animals with 1% (v/v) suc d'Helix pomatia juice to hydrolyse glucuronide and sulphate conjugates. With the urine samples of eltoprazine treated animals, only a few peaks disappeared, while in some cases no effect at all was seen of the enzyme treatment. To provide enough enzyme activity for the conjugates under investigation, we increased the Helix pomatia juice to 10% (v/v). We observed hydrolysis of all major polar metabolite peaks, except peak no. 9 and, perhaps, peak no. 7 (fig. 3). So, the major part of the peaks eluting faster than 70 minutes indeed contained sulphate and/or glucuronide conjugates. The deconjugated metabolites gave quite a few low and quite broad peaks between 60 and 135 minutes retention time.

3.3 Effect of molecular site of radiolabel

To study whether the molecular site of the radiolabel influences the metabolite patterns, we have done identical experiments using the two different ^{14}C-labelled eltoprazine compounds.

3.3.1 Rat

The overall metabolic patterns obtained with the two radiolabelled compounds in rats were very similar. However, the HPLC peak no. 1 was consistently seen using urine from the piperazine-ring labelled eltoprazine treated rats while this component was absent in the urine from the other rats. This could be most clearly visualized using late urine samples (urine obtained between 48 and 72 hours after dosing).

3.3.2 Dog

We could not identify any consistent difference in the metabolite patterns of urine from either ^{14}C-piperazine-eltoprazine or ^{14}C-phenyl-eltoprazine treated dogs.

3.4 Effect of administration route and sex

We analysed samples from intravenously dosed rat, dog and rabbits and compared the results with those obtained after oral dosing. No differences in metabolite patterns were observed after i.v. and oral administration. Only the unchanged drug peak was a little higher after i.v. dosing, as was to be expected. Even the rabbit excreted some unchanged drug after intravenous dosing. We did not observe consistent differences between the sexes.

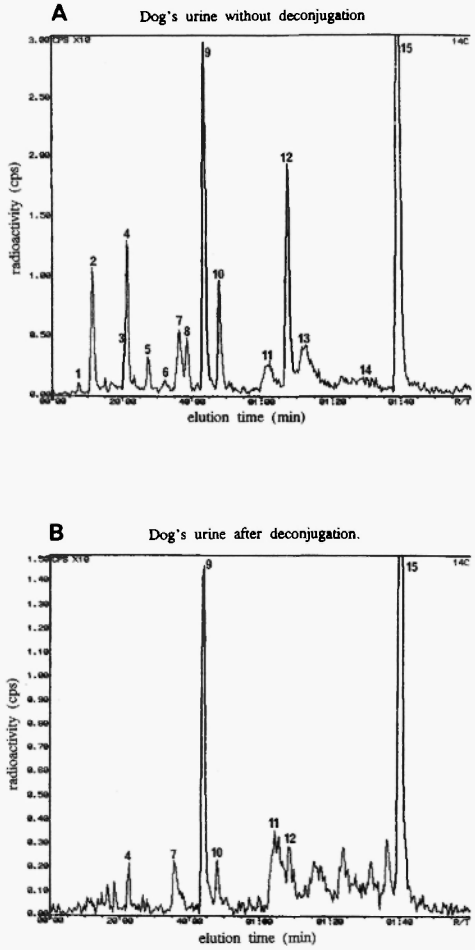

Fig. 3: HPLC-radioactivity chromatograms of dog's urine samples before (A) and after (B) treatment of the sample with aryl sulphatase and aryl glucuronidase. Dogs received ^{14}C-piperazine-labelled eltoprazine orally and urine was collected between 0 and 24 hours after dosing.

IV. DISCUSSION

The main species differences observed were: 1) the extensive metabolism of eltoprazine by the rabbit; 2) the very differentiated metabolism by the dog; 3) the very limited correspondence between the pattern of rat and man; and 4) the constant ratio of unchanged drug and the metabolites in man's urine at later sampling time, while in the other species this ratio decreased considerably.

We could not find significant differences caused by sex, route of administration and molecular site of the radiolabel.

The lack of effect of the molecular radiolabel site on the metabolite patterns shows that almost all metabolites retained (some of) the carbon atoms of the phenyl- and piperazine rings of the parent molecule.

To hydrolyse the conjugates present, a high amount of Helix pomatia juice was required. This may indicate the presence of inhibitors of the hydrolysing enzymes, or may suggest that some of the conjugates are bad substrates for the enzymes. Anyway, the results indicate that the major part of the components eluting faster than 70 min are sulphate- or glucuronide conjugates.

The excretion of unchanged drug in considerable quantities is in accordance with the relatively low log P (1.9 for the basic form) and the high pKa (9.0) of the drug. The rabbit indeed may be a fast metaboliser of eltoprazine as is suggested by the fact that liver microsomes of rabbits metabolised eltoprazine much faster than those from rats and dogs (unpublished observations).

The excretion of unchanged drug by humans closely followed the excretion of total radioactivity with time. In other words, the ratio between unchanged drug and its metabolites was practically constant with time elapsed after dosing. This ratio decreased considerably in the other species. These phenomena could be explained by a human synthesis of the metabolites that would be rate-limiting for their excretion while in the other species metabolism would be faster than the metabolite excretion.

Some characteristic species differences were distinctly revealed even though the excretion rates of total radioactivity were very similar. Our results indicate that the metabolism of eltoprazine in the dog is probably quite similar to that in man. Although the rabbit metabolises eltoprazine far more extensively than man, the metabolites formed by this animal species also seem to be formed by man, but in strikingly different quantities. Rat had only two HPLC-peaks in common with man. It remains to be established

whether the observed differences are due to different conjugating activities or (also) different oxidative metabolism.

V. REFERENCES

1. De Lange, N., and Raghoebar, M. Distribution of radioactively labelled eltoprazine in rat and dog. *Drug Metabolism and Drug Interactions,* 1990; 8: 115-127.