DISTRIBUTION OF RADIOACTIVELY LABELLED ELTOPRAZINE IN RAT AND DOG

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SUMMARY

Eltoprazine labelled with ^{14}C either in the phenyl or the piperazine ring was administered orally to rats and dogs. While distribution of radioactivity was not affected by the position of label during the first hours after dosing, from six hours onward concentrations of radioactivity in the organs of rats were higher after [^{14}C]piperazine-labelled eltoprazine. In most organs, radioactivity originating from the [^{14}C]phenyl-labelled compound was detectable up to 72 hours, while [^{14}C]piperazine-labelled material could still be detected 384 hours after dosing. These results suggest that the piperazine ring may be broken down to aliphatic amines which are retained in the body. Quantitatively, however, this is of minor importance, representing about 1% of the dose.

With either label, the highest concentrations of the radiolabel were found in liver and kidney. Excretion of radioactivity in rat and dog urine and faeces was not influenced by the position of the radiolabel.

I. INTRODUCTION

Eltoprazine hydrochloride (hereafter eltoprazine) is a representative of the serenics, a class of compounds which specifically inhibit offensive behaviour without sedation, muscle relaxation or inhibition of defensive or other behaviours /1/.

In preliminary studies of the kinetics of this compound, which was ^{14}C-labelled in the piperazine moiety, the excretion and distribution of the radioactivity in rats were investigated. The results indicated that even though more than 95% of the radioactivity left the body within 48 hours, there was for the residual a very long residence time in practically all organs. One possible explanation for this finding was that the piperazine ring of a very small proportion of the dose was cleaved and that small radioactive molecules entered the circulation resulting in a long half-life of the radioactivity in the organs.

The present study was designed to test this hypothesis. Rats and dogs were given either [piperazine-U-^{14}C]eltoprazine or [phenyl-U-^{14}C]eltoprazine orally and the excretion and plasma profiles compared. In addition, the distribution pattern in the rat in a number of organs was compared after dosing with both radiolabelled compounds in various studies.

II. MATERIALS AND METHODS

2.1 Compounds

The site of the labels of the two [^{14}C]eltoprazine preparations are given in Figure 1. [Piperazine-U-^{14}C]eltoprazine had a specific activity of 717.8 MBq/g (19.4 mCi/g), a chemical purity of 98.5% and a radiochemical purity of 96.4%. [Phenyl-U-^{14}C]eltoprazine had a specific activity of 884.3 MBq/g (23.9 mCi/g), its chemical and radiochemical purities were 97.9% and 99.8%, respectively. The purities of the two preparations were determined by a HPLC method. The compounds were dissolved in demineralized water just before dosing.

2.2 Rat experiments

In the studies, male Sprague-Dawley rats supplied by Charles River Farm, England, were used. They received rat-feed "RMH-TM" supplied by Hope Farms, The Netherlands. From 16 hours before until 6 hours after administration, the rats were fasted. Tap-water was available during the whole experiment. The animals were housed individually in stainless steel cages, providing for separate collection of urine and faeces. Eighteen rats received orally 5 mg/kg of [piperazine-U-^{14}C]eltoprazine as a solution. Another eighteen rats received the same amount of [phenyl-U-^{14}C]eltoprazine also as a solution. Rats from both groups were bled under anaesthesia of a

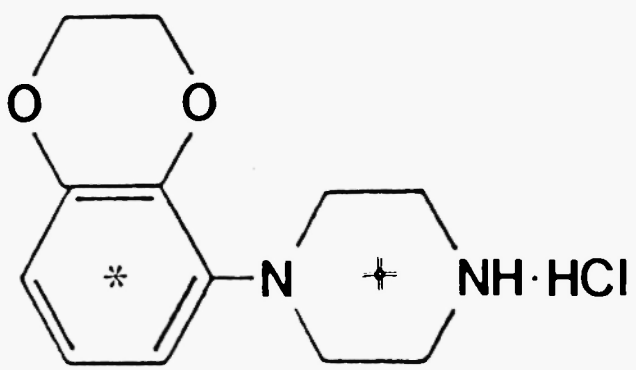

Fig. 1: Structural formula of eltoprazine hydrochloride and site of labelling.
* [U-phenyl-^{14}C]labelling
=O= [U-piperazine-^{14}C]labelling.

mixture of Halothan/O_2/N_2O at 1, 2, 3, 6, 24, 48, 72, 216 and 384 hours after administration. At each time point two rats from each group were sacrificed. Radioactivity was measured in blood, plasma and a number of organs.

Radioactivity in urine and faeces was determined up to 216 hours after administration.

2.3 Dog experiments

Four male (10.8-13.6 kg) and four female (10.8-12.9 kg) Beagle dogs, supplied by CPB-TNO, The Netherlands, were used in the study. The dogs were housed individually in cages providing for separate collection of urine and faeces.

The dogs received daily about 250 g feed (LDB, from Hope Farms, The Netherlands). From 16 hours before until 4 hours after administration the dogs fasted. Tap-water was available *ad libitum* during the whole experiment.

Two male and two female dogs received orally 0.2 mg/kg of [piperazine-U-^{14}C]eltoprazine. The other four dogs received 0.2 mg/kg [phenyl-U-^{14}C]eltoprazine. The drug was given in hard gelatine capsules. In the experiments, urine was collected at 13 and 24 hours after administration and after that in 24-hour portions up to 7 days after administration. Faeces were also collected in 24-hour portions up to 168 hours after each dose.

Blood was drawn from the *vena brachialis* into syringes (Terumo) moistened with heparin solution or into heparin monovettes (Sarstedt). Blood was drawn at several time points up to 7 days after dosing. Blood cells and plasma were separated by centrifugation.

2.4 Measurement of radioactivity

Radioactivity in plasma and urine were determined directly, after mixing with Emulsifier Scintillator 299 (Packard) by scintillation counting in a Philips Liquid Scintillation Counter, type PW 4540 (Philips, Eindhoven, The Netherlands). Radioactivity in faeces and organs was determined after incineration of weighed samples in a Packard Combustion Analyser.

The CO_2 formed was trapped in a mixture of Carbosorb and Perma Fluor (Packard) and radioactivity in the resulting solution was measured again by scintillation counting.

III. RESULTS

3.1 Effect of site of labelling on the excretion of radioactivity

The excretion of radioactivity after administration of [phenyl-U-^{14}C] and [piperazine-U-^{14}C]labelled eltoprazine to rats is shown in Tables 1 and 2, respectively. Although the mean urinary excretion of radioactivity after administration of the [phenyl-U-^{14}C]labelled material was higher than the excretion after dosing with the [piperazine-U-^{14}C]labelled preparation, this difference appeared not to be statistically significant.

TABLE 1

Excretion of radioactivity after administration of 5 mg/kg [phenyl-U-^{14}C]-eltoprazine to four rats, expressed as cumulative percentage of the dose (in parentheses: standard deviation).

Time after Dosing (h)	Urine	Faeces	Total
24	73.1 (4.5)	11.3 (4.7)	84.5 (4.3)
48	75.7 (5.1)	13.1 (4.6)	88.8 (4.2)
72	76.6 (5.1)	13.6 (4.5)	90.2 (3.8)
96	77.0 (5.0)	13.8 (4.5)	90.7 (3.6)
120	77.2 (5.0)	13.9 (4.5)	91.0 (3.5)
144	77.3 (5.0)	14.0 (4.5)	91.3 (3.4)
168	77.5 (4.9)	14.1 (4.5)	91.6 (3.2)
192	77.6 (4.9)	14.2 (4.4)	91.8 (3.2)
216	77.7 (4.9)	14.2 (4.4)	91.9 (3.1)
Cage wash included	78.4 (4.7)		92.7 (2.7)

The urinary excretion of radioactivity after administration of the two preparations to dogs was identical (Tables 3 and 4).

TABLE 2

Excretion of radioactivity after administration of 5 mg/kg [piperazine-U-^{14}C]-eltoprazine to four rats, expressed as cumulative percentage of the dose (in parentheses: standard deviation).

Time after Dosing (h)	Urine	Faeces	Total
24	65.0 (7.0)	15.1 (5.7)	80.1 (2.7)
48	67.8 (7.4)	18.5 (4.7)	86.0 (3.4)
72	68.1 (7.6)	19.1 (4.7)	87.2 (3.5)
96	68.4 (7.6)	19.3 (4.7)	87.7 (3.5)
120	68.6 (7.6)	19.5 (4.7)	88.1 (3.6)
144	68.8 (7.5)	19.6 (4.7)	88.4 (3.5)
168	69.0 (7.5)	19.8 (4.7)	88.7 (3.5)
192	69.1 (7.5)	19.8 (4.7)	88.9 (3.5)
216	69.2 (7.5)	19.9 (4.7)	89.1 (3.5)
Cage wash included	69.9 (7.6)		89.9 (3.8)

TABLE 3

Excretion of radioactivity after oral administration of 0.2 mg/kg [phenyl-U-^{14}C]-eltoprazine to four dogs, expressed as cumulative percentage of the dose (in parentheses: standard deviation).

Time after Dosing (h)	Urine	Faeces	Total
6	26.8 (11.0)		
13	62.0 (9.7)		
24	73.6 (8.7)	4.9 (0.9)	78.5 (8.0)
48	76.5 (8.7)	6.2 (0.8)	82.8 (8.0)
72	77.1 (8.7)	6.5 (0.8)	83.6 (8.1)
96	77.3 (8.7)	6.5 (0.7)	83.8 (8.1)
120	77.4 (8.7)	6.6 (0.8)	84.0 (8.0)
144	77.5 (8.6)	6.6 (0.8)	84.1 (7.9)
168	77.5 (8.6)	6.6 (0.9)	84.2 (7.9)

TABLE 4

Excretion of radioactivity after oral administration of 0.2 mg/kg [piperazine-U-^{14}C]eltoprazine to four dogs, expressed as cumulative percentage of the dose (in parentheses: standard deviation).

Time after Dosing (h)	Urine	Faeces	Total
13	63.5 (8.5)		
24	75.6 (4.9)	6.9 (3.8)	82.4 (6.6)
48	78.4 (4.4)	9.0 (3.6)	87.4 (5.4)
72	79.3 (3.9)	9.3 (3.5)	88.2 (5.3)
96	79.3 (4.2)	9.4 (3.5)	88.7 (5.3)
120	79.4 (4.2)	9.4 (3.5)	88.8 (5.3)
144	79.5 (4.2)	9.4 (3.5)	88.9 (5.3)
168	79.5 (4.2)	9.4 (3.5)	88.9 (5.3)

3.2 Effect of site of labelling on the plasma and brain levels of radioactivity

In Figures 2 and 3, concentration-time profiles of radioactivity in plasma and brain, respectively, are presented after administration of both ^{14}C-labels to rats. It is clear (Figure 2) that after administration of the preparation, [^{14}C]labelled in the piperazine moiety, the plasma disappearance is much slower than after dosing with the [phenyl-U-^{14}C]labelled compound. The same effect can be seen after administration of the two preparations to dogs (Figure 4).

3.3 Effect of the site of labelling on the distribution of radioactivity in organs in rats

The complete results of the distribution of the radioactivity between the organs and tissues at the different times are given in Table 5 for the phenyl-labelled compound and in Table 6 for the piperazine-labelled preparation.

The distribution of the radioactivity among the different organs during the first hours of administration is practically identical for

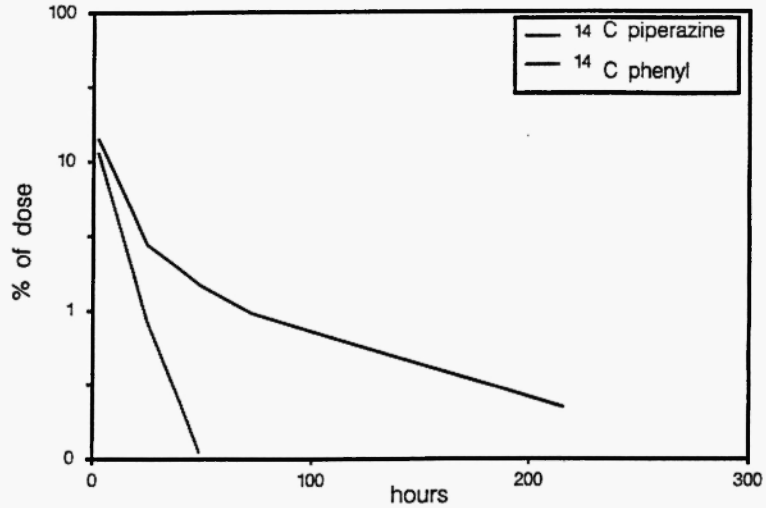

Fig. 2: Concentration of radioactivity in plasma of rats after administration of 5 mg/kg of [piperazine-U-^{14}C]eltoprazine or 5 mg/kg of [phenyl-U-^{14}C]eltoprazine expressed as percentage of the dose.

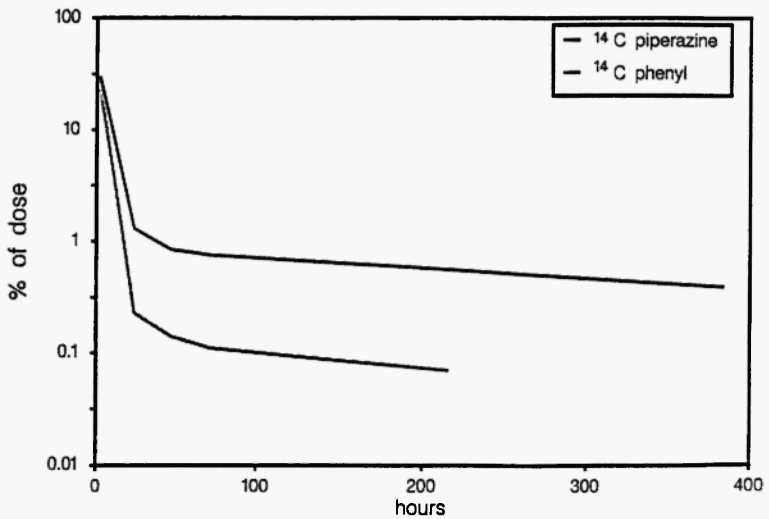

Fig. 3: Concentration of radioactivity in brains of rats after administration of 5 mg/kg of [piperazine-U-^{14}C]eltoprazine or 5 mg/kg of [phenyl-U-^{14}C]eltoprazine expressed as percentage of the dose.

the two preparations. From 6 hours up to 384 hours after dosing, however, the concentration of radioactivity originating from the [piperazine-^{14}C]eltoprazine is strikingly higher.

These data indicate that eltoprazine and/or its metabolites are widely distributed in the rat. Irrespective of the preparations, highest levels of radioactivity were found in the liver and kidneys 1 or 2 hours after dosing. The concentrations in all organs declined rapidly during the first day after administration of both [^{14}C] preparations.

TABLE 5

Concentration of radioactivity in organs and tissues after oral administration of 5 mg/kg [phenyl-U-^{14}C]eltoprazine to rats. Data are expressed as kBq/g. Radioactive dose is 4.4 MBq/kg.

Time	1h	2h	6h	24h	72h	384h
Blood	0.522	0.363	0.073	0.021	0.019	<0.004
	0.733	0.423	0.100	0.016	0.011	<0.004
Plasma	0.667	0.411	0.087	0.042	<0.004	<0.004
	0.821	0.510	0.144	0.034	<0.004	<0.004
Liver	15.774	8.647	2.139	0.597	0.155	0.004
	9.598	8.374	2.712	0.557	0.120	0.004
Kidney	6.614	4.879	0.978	0.215	0.058	0.004
	16.442	5.656	1.169	0.199	0.049	0.005
Lung	2.611	2.519	0.370	0.033	0.011	<0.003
	5.296	3.355	0.495	0.038	0.012	0.002
Brain	0.622	1.208	0.123	0.009	0.004	<0.002
	1.872	0.858	0.137	0.011	0.006	*
Spleen	1.916	2.148	0.260	0.029	0.015	0.003
	4.263	1.832	0.263	0.037	0.013	0.004
Adrenal	3.211	1.465	0.317	0.083	0.036	<0.016
	2.359	1.897	0.298	0.099	0.064	<0. 020
Brown Fat	2.267	1.650	0.182	0.047	0.022	<0.002
	2.758	4.105	0.187	0.104	0.026	<0.006

* No sample

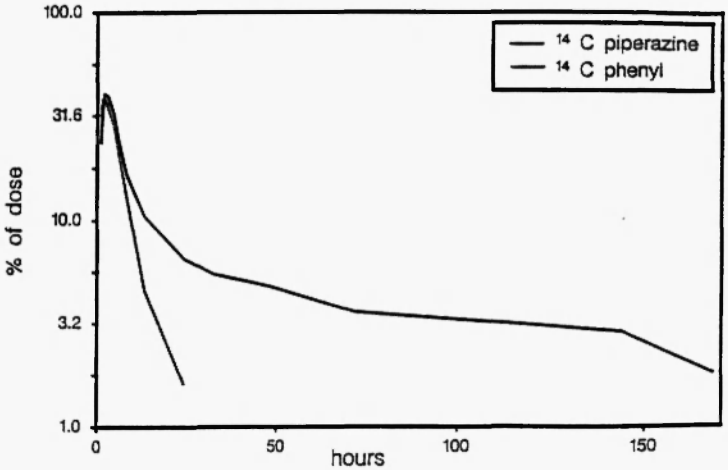

Fig. 4: Concentration of radioactivity in plasma of dogs after administration of 0.2 mg/kg of [piperazine-U-^{14}C]eltoprazine or 5 mg/kg of [phenyl-U-^{14}C]eltoprazine expressed as percentage of the dose.

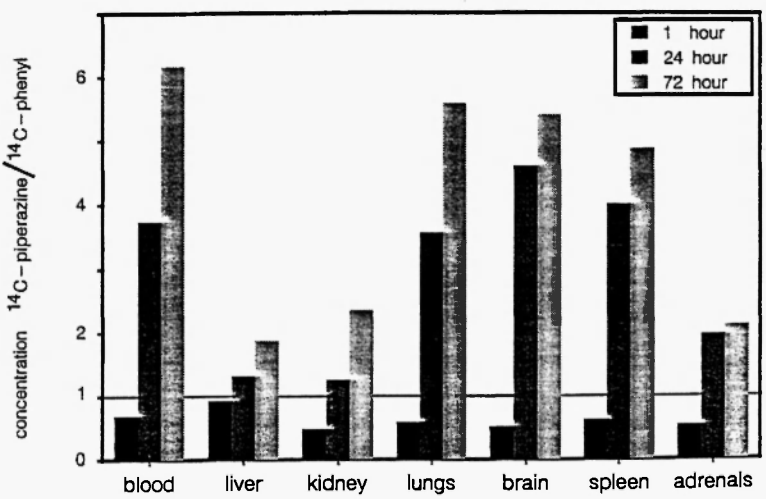

Fig. 5: Ratio concentration of ^{14}C-piperazine/^{14}C-phenyl in some tissues and organs of rats after administration of 5 mg/kg (3.6 MBq/kg) [piperazine-U-^{14}C]eltoprazine and of 5 mg/kg (4.4 MBq/kg) of [phenyl-U-^{14}C]eltoprazine.

TABLE 6

Concentration of radioactivity in organs and tissues after oral administration of 5 mg/kg [piperazine-U-^{14}C]eltoprazine to rats. Data are expressed as kBq/g. Radioactive dose is 3.6 MBq/kg.

Time	1h	2h	6h	24h	72h	384h
Blood	0.350	0.495	0.221	0.070	0.040	0.011
	0.527	0.504	0.271	0.071	0.034	0.012
Plasma	0.434	0.537	0.334	0.107	0.038	<0.004
	0.669	0.488	0.397	0.090	0.032	<0.004
Liver	10.979	11.145	3.425	0.755	0.308	0.037
	12.717	10.306	3.541	0.769	0.208	0.026
Kidney	3.424	5.457	1.839	0.267	0.145	0.017
	7.845	5.849	2.356	0.254	0.109	0.017
Lung	1.494	3.303	0.985	0.115	0.074	0.013
	3.197	3.645	1.087	0.140	0.060	0.016
Brain	0.268	0.911	0.311	0.050	0.032	0.014
	1.025	1.250	0.352	0.041	0.022	0.014
Spleen	1.251	2.792	0.598	0.131	0.095	0.015
	2.622	2.766	0.694	0.133	0.040	0.017
Adrenal	1.815	2.650	0.612	0.169	0.095	0.020
	1.182	2.131	0.592	0.189	0.116	0.024
Brown Fat	1.048	1.939	1.231	0.102	0.077	0.024
	1.453	1.271	0.420	0.083	0.047	0.014

For a number of organs the relative concentration of the radioactivity from the piperazine moiety, as a function of the radioactivity from the phenyl-labelled compound at the same time, is given in Figure 5. At 24 hours after dosing, there is already a substantial difference in [^{14}C] concentrations between the two labelled compounds.

IV. DISCUSSION

The results of the present studies clearly indicate that the residual concentrations detected up to 384 hours after administration of [piperazine-U-^{14}C]eltoprazine are not caused by accumulation of the entire molecule. The piperazine ring is indeed probably cleaved, resulting in small [^{14}C]labelled molecules which might be aliphatic amines.

Evidence for such a cleavage of the piperazine ring already existed for a number of phenothiazines: fluphenazine, perphenazine, perazine, prochlorperazine and trifluorperazine /2-5/ and was recently also described for a phenyl-piperazine /6/.

Quantitatively, the accumulation of radioactivity after administration of the [piperazine-U-^{14}C]labelled material is of minor importance. After 324 hours, the highest concentration was found in the liver, but this concentration was less than 1% of the peak concentration in this organ.

Comparison of the HPLC-patterns of radioactivity of urine after dosing of the two preparations shows only minor differences in the rat: i.e. the presence of a polar metabolite in an amount less than 1% of the total urinary radioactivity /7/.

From these results, we conclude that the amount split from the piperazine ring is so small that for quantitative distribution and metabolic studies, it does not matter which of the two [^{14}C]labelled preparations are used.

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