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Unequal Disposition of Enantiomers of the Organic Cation Oxyphenonium in the Rat Isolated Perfused Liver

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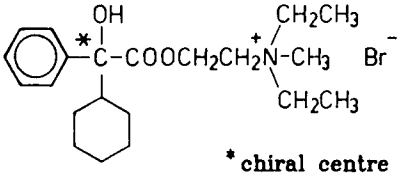
Abstract—This paper describes the results of pharmacokinetic experiments in the rat isolated perfused liver with enantiomers of oxyphenonium. The study was performed with the [^{14}C]methyl labelled compounds. In this preparation both metabolism and biliary excretion were significantly different for the (+)- and the (−)-isomer. Hepatic uptake rate was similar, but total biliary excretion (including metabolites) of the (−)-isomer was only 55% compared with the excretion of the (+)-isomer. In line with these data, after 2 h only 30% of the dose of the (+)-isomer and over 50% of the dose of the (−)-isomer was still found in the liver, predominantly in the form of metabolites. The metabolic profile was investigated using ion pair TLC. At least two metabolites were detected in bile for both enantiomers. However, unchanged (−)-oxyphenonium persisted for longer in bile, indicating either a more rapid canalicular transport of the (+)-isomer and/or a more rapid metabolism of (+)-oxyphenonium to cholephilic metabolites.

Differences in absorption, distribution, metabolism and excretion of enantiomers of drugs and the importance of pharmacokinetic studies of enantiomers has been widely recognized (Ariëns 1984; Drayer 1986; Testa 1986; Walle & Walle 1986). But, so far, no information is available concerning enantio-selective disposition processes of quaternary ammonium compounds. As we are interested in these, as well as in anti-acetylcholine drugs, we have studied the enantiomers of oxyphenonium bromide.



(I). Oxyphenonium bromide.

Oxyphenonium bromide (I) is a quaternary ammonium compound with strong anti-acetylcholine properties. The enantiomers have been made available and their antimuscarinic activity has been studied in radioligand binding experiments. The (+)-isomer is the biologically active compound having a 38 times higher affinity for the muscarinic receptor in-vitro than the (−)-isomer (Feitsma 1987).

In the first instance we examined the disposition of oxyphenonium in the intact rat but it became clear that various enantio-selective factors were playing a role in the drug's disposition, especially at the hepatic level. To disentangle the various processes, we decided to investigate the hepatic uptake and excretion processes in more detail. Therefore, the experiments were performed with rat isolated perfused livers.

Hepatic uptake and biliary excretion of three non-chiral quaternary ammonium compounds have been extensively studied by Neef et al (1984a). It was concluded that quarternary ammonium compounds with intermediate or high lipophilicity are transported by carrier-mediated processes, both at the level of hepatocyte uptake and bile canalicular transport. Ion pair formation may play different roles at the uptake and secretion level in the hepatobiliary transport of organic cations (Neef et al 1984b).

Hepatic transport of racemic oxyphenonium and some other organic cations was studied by Schanker (1965). He investigated the influences of co-administration of these cations on the biliary excretion of procainamide ethobromide (PAEB). The excretion of PAEB was characterized as a saturable process with passive transfer accounting for little of the amount totally excreted. The addition of oxyphenonium lowered the biliary excretion of unchanged PAEB by 67%. Also its uptake by rat liver slices was inhibited by oxyphenonium.

The aim of the present study was to establish whether enantioselectivity is involved in the hepatic transport processes of oxyphenonium bromide in the rat liver and to obtain more insight in the mechanistic aspects of these processes. As well as total hepatic uptake and biliary excretion of the enantiomeric parent compounds, metabolite formation was also studied.

Materials and Methods

Chemicals

^{14}C-Labelled (+)- and (−)-oxyphenonium iodide were synthesized as was described by Feitsma (1987). The enantiomeric purity of the precursor was assessed by chiral HPLC (Feitsma et al 1986), and the enantiomeric purities of oxyphenonium enantiomers were deduced from these figures, being 99·6 and 99·9% for (+)- and (−)-oxyphenonium, respectively. The solutions for injection were prepared by adding adequate amounts of the labelled material to 0·9% NaCl (saline) solutions, containing 0·1 mg mL^{-1} of (+)- or (−)-oxyphenonium bromide, so that the resulting radioactivity concentration amounted 2 μCi mL^{-1}. The specific activities of the solutions thus obtained were about 300 Bq nmol^{-1}.

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Bovine serum albumin (BSA) was obtained from Organon Teknika (Oss, The Netherlands) and sodium taurocholate was purchased from Fluka (Buch, Switzerland). All other chemicals were from Merck (Darmstadt, FRG).

Isolated perfused rat liver preparation

The perfusion technique used, was based upon the method described by Meijer et al (1981), in which the liver was isolated from male Wistar rats (200–260 g) and perfused by a recirculating medium, the perfusate being run into the portal vein and drained via the hepatic vein. The perfusate consisted of a Krebs-bicarbonate solution, supplemented with 1% BSA (Meijer et al 1981), and was circulated by a peristaltic pump in a closed system. A volume of 100 mL perfusion medium was used and the perfusate flow was set at 3·5 mL min^{-1} g^{-1} liver. The medium was oxygenated by a film oxygenator combined with a silastic tube oxygenator with carbogen gas (5% CO_2 and 95% O_2). The temperature of the system was maintained at 38°C. The pH of the medium was maintained at 7·40±0·05 by careful adjustment of the oxygen and carbogen gas flows. During the experiments a constant infusion of sodium taurocholate (15 μmol h^{-1}) was given to the perfusion medium to sustain bile flow.

Before drug injection, the bile flow was monitored for 30 min. If the flow was constant, at about 10 mg min^{-1}, the experiment was started. A solution of (+)- or (−)-[^{14}C]oxyphenonium (55 nmol) was added to the main reservoir. Medium samples (250 or 500 μL) were taken at t = 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80, 100 and 120 min. Bile was collected at 5 min-intervals during the first 30 min and at 10 min-intervals during the rest of the experiment. Bile flow was determined by weight.

The perfusion was stopped after 120 min, the remainder of the perfusate was collected quantitatively and the volume and radioactivity determined. The liver was homogenized with an Ultra Turrax (Ika-Tron DZM 5, Wilten Woltil, De Bilt, The Netherlands) in a 4-fold amount of saline and 250 mg fractions were submitted to liquid scintillation counting in triplicate.

Analysis

Radioactivity was measured by liquid scintillation counting after mixing the samples with 4 mL Rialuma (Lumac, Schaesberg, The Netherlands) in 6 mL polyethylene microvials. The vials were counted in a Packard Minaxi Tri-Carb B4450 liquid scintillation counter for 40 000 counts (2s = 1·00) or for 10 min. The counts min^{-1} were converted to disintegrations s^{-1} (Bq) using a quench curve based upon determination of the spectral index of the external standard.

Data analysis

The content of radioactivity in the liver at various times was calculated by subtracting the amount in the perfusate and the amount excreted into the bile from the administered dose.

Medium disappearance (up to 6 min) and biliary excretion rate versus time curves were fitted using the computer program RUGFIT (Scaf 1988).

All data were based upon the radioactivity (Bq) found in the biofluid or in liver tissue and were expressed in nmol oxyphenonium; they include the presence of metabolites.

Protein binding

The degree of binding to BSA was determined by ultrafiltration. Three mL of medium (1% BSA in Krebs-buffer) containing 0·25 or 1 nmol mL^{-1} ^{14}C-labelled (+)- or (−)-oxyphenonium, was incubated during 15 min at 37°C in a shaking waterbath. 750 μL of the solution was placed in an Amicon filter set, containing YMT-membrane filters (Oosterhout, The Netherlands) and centrifuged at 1000*g* for 5 min at 37°C in a Beckmann J2-21 centrifuge (Irvine, USA). From the ultrafiltrate as well as from the incubation solution 100 μL samples (triplicate) were taken and radioactivity was measured by liquid scintillation counting. Each concentration of both enantiomers was determined twice.

Metabolism

Samples of bile (25 μL) taken at various times were spotted on TLC-plates of 20 × 20 cm (Silicagel G, Merck, Darmstadt, FRG). Blank bile spiked with ^{14}C-labelled oxypheno-

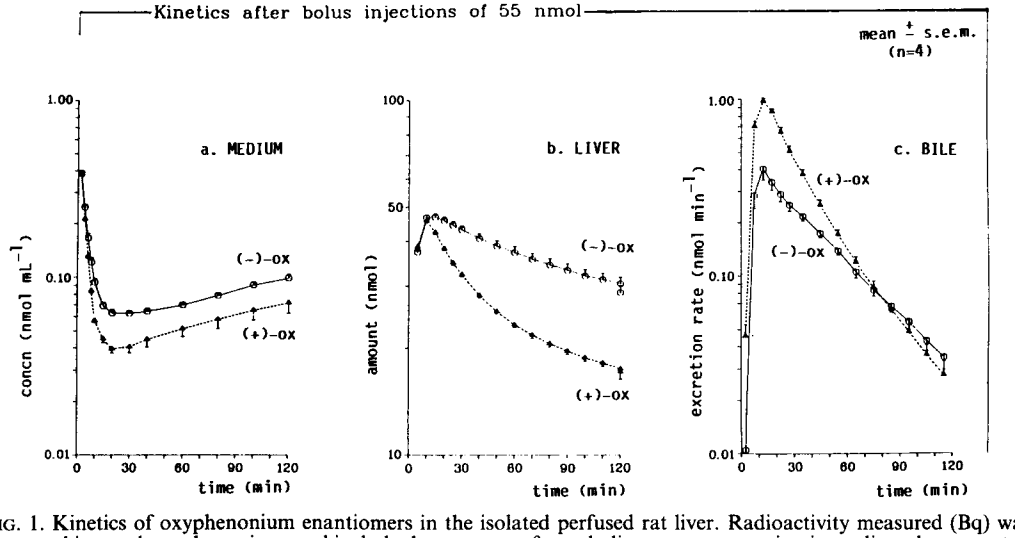


FIG. 1. Kinetics of oxyphenonium enantiomers in the isolated perfused rat liver. Radioactivity measured (Bq) was expressed in nmol oxyphenonium, and include the presence of metabolites. a. concentration in medium. b. amount in liver. c. biliary excretion rate expressed as nmol substrate min^{-1} and plotted at the midpoint of the sampling time interval.

Table 1. Pharmacokinetic parameters of (+)- and (−)-oxyphenonium concentration decay in perfusate during the first 6 min (mean ± s.e.m., n = 4).

Parameter	(+)-Ox	(−)-Ox
C_0 (nmol mL^{-1})	0·640 ± 0·022	0·588 ± 0·013
$t_{\frac{1}{2}}$ (min)	2·61 ± 0·06	3·26 ± 0·21
Cl (mL min^{-1})	22·9 ± 0·8	20·7 ± 0·9
V (mL)	86·1 ± 3·1	97·4 ± 2·4

nium was used as a reference sample on every plate. The plates were developed over about 12 cm in an unsaturated chamber for 75 min using 0·2 M sodium perchlorate in methanol–chloroform (80:20). The labelled compounds were detected using a Berthold TLC scanner II (LB 2723) with a slit width of 10 × 1 mm.

Some bile (50 μL, fraction 5–10 min) was treated with 2 mg β-glucuronidase-sulphatase (Limpet Acetone Powder, type I, Sigma, St. Louis, USA) in 50 μL acetate buffer pH 5·0 for 36 h at 37°C. From the resulting mixture 25 μL was analysed by TLC.

Samples (1·0 and 0·5 mL, respectively) of the perfusate taken after 2 h and of the aqueous liver homogenates were extracted with 2·0 mL dichloromethane after sodium perchlorate had been added to a final concentration of 0·1 M. Samples of the aqueous and the organic layer were submitted to liquid scintillation counting.

Results

Disappearance from the medium

The mean medium curves of (+)- and (−)-oxyphenonium are presented in Fig. 1a. The pharmacokinetic parameters, derived from these curves (for 0–6 min, due to the plasma profile) are listed in Table 1. No significant differences ($P < 0·05$) were observed.

Both enantiomers were rapidly removed from the medium by the liver. Soon after the start of the perfusion labelled material was apparently excreted into the medium resulting in an increase of concentration after about 20 min.

The radioactive compounds in the medium after 120 min could not be extracted with dichloromethane using perchlorate as a counter ion.

Excretion in the bile

No significant differences were observed in bile production between the experiments with (−)- and (+)-oxyphenonium. In Fig. 1c the biliary excretion rates of (+)- and (−)-oxyphenonium are compared. Radioactive compounds rapidly appeared in the bile with both enantiomers. With the RUGFIT-program the data were fitted to a model with monoexponential ascending and descending phases. The resulting pharmacokinetic parameters are listed in Table 2.

Content in liver

The amounts of (+)- and (−)-oxyphenonium and derived radioactive compounds in the liver, calculated from the administered dose, the medium concentration and the amount cumulatively excreted in the bile, are presented in Fig. 1b.

After 8 min, 70% of the dose of both enantiomers was found in the liver. However, their liver content differed significantly at 120 min. The amount of radioactivity found after 120 min is shown by two separate points (Fig. 1b). It can be seen that these agree fairly well with the calculated amounts. The recovery is presented in Table 3.

The radioactive compounds in the liver homogenates could not be extracted with dichloromethane using perchlorate as a counter ion.

Table 2. Pharmacokinetic parameters of biliary excretion rate of ^{14}C-containing material after doses of (+)- or (−)-oxyphenonium (mean ± s.e.m., n = 4).

Parameter	(+)-Ox	(−)-Ox
Lag time (min)	4·00 ± 0·16	4·08 ± 0·14
$t_{\frac{1}{2}},\alpha$ (min)	2·10 ± 0·25	2·03 ± 0·41
$t_{\frac{1}{2}},\beta$ (min)[a]	19·15 ± 0·94	29·02 ± 1·60
AUC (nmol)[a]	31·15 ± 1·28	18·55 ± 1·74

[a] significant ($P < 0·05$).

Table 3. Mean total amounts of (+)- and (−)-oxyphenonium and radioactive metabolites found in medium, bile and liver at the end of the experiment (mean ± s.e.m., n = 4).

% of dose at t = 120 min present in:	(+)-Ox	(−)-Ox
Removed from medium during experiment	0·65 ± 0·04	0·79 ± 0·02
Bile[a]	55·72 ± 2·17	30·42 ± 2·84
Liver[a]	31·22 ± 1·46	50·14 ± 2·31
Medium[a]	12·83 ± 1·58	17·46 ± 0·91
Total recovery	100·4 ± 1·2	98·8 ± 1·0

[a] significant ($P < 0·05$).

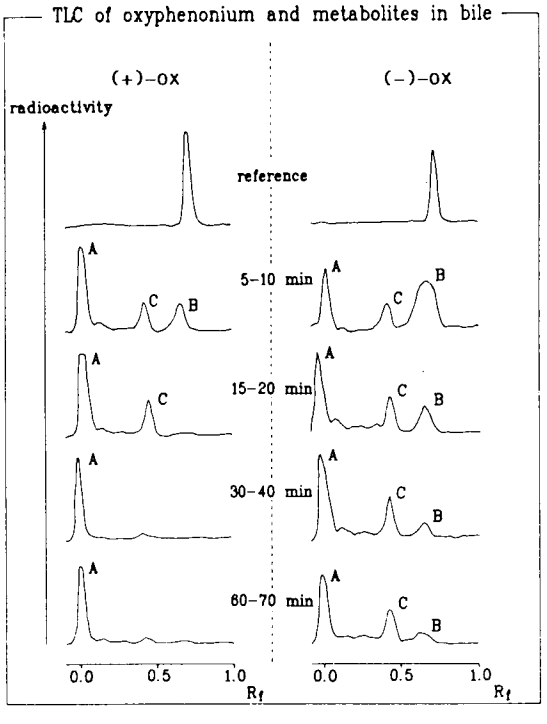


FIG. 2. TLC radioscans of bile samples after administration of [^{14}C]oxyphenonium enantiomers to a rat isolated perfused liver. Blank bile was spiked with some [^{14}C]oxyphenonium. Peak areas are not corrected for differences in scan speed. Conditions as described in experimental section.

Protein binding

The binding of (+)- and (−)-oxyphenonium to BSA showed no enantioselectivity and was 15% for both enantiomers.

Metabolism

In Fig. 2 radioactivity scans of TLC-plates spotted with bile from liver perfusion experiments are presented. At least three zones could be detected: one with an R_F 0·0 (A), one with R_F 0·3 (C) and one with R_F 0·7 (B) being comparable to that of oxyphenonium. The relative amounts of the three spots were different for (+)- and (−)-enantiomers, as were their rates of disappearance during the perfusion. Bile was incubated with β-glucuronidase-sulphatase and subjected to TLC-analysis before and after this procedure. No differences were seen in the chromatograms of treated compared with untreated bile.

Discussion

There are considerable differences in the disposition of (+)- and (−)-oxyphenonium in the rat isolated perfused liver, especially with respect to biliary excretion.

The hepatic uptake of the drugs is rapid with a plasma disappearance half-life of 3·3 and 2·6 min for the (+)- and (−)-isomers, respectively. As the perfusate flow was maximally 28 mL min^{-1} and the distribution volume 100 mL, the minimum half-life that can be anticipated for this phase is 2·5 min ($t_{\frac{1}{2}}$=(0·7 * V)/Cl, the maximum plasma clearance Cl being the perfusate flow). Therefore, it can be inferred that hepatic uptake during the first 6 min is predominantly flow-limited and a different behaviour in membrane transport itself might be obscured by rate limitation of the supply to the liver. For a further study of the hepatic uptake, use of isolated hepatocytes or membrane vesicles would be preferable, since the influence of flow is excluded in such preparations.

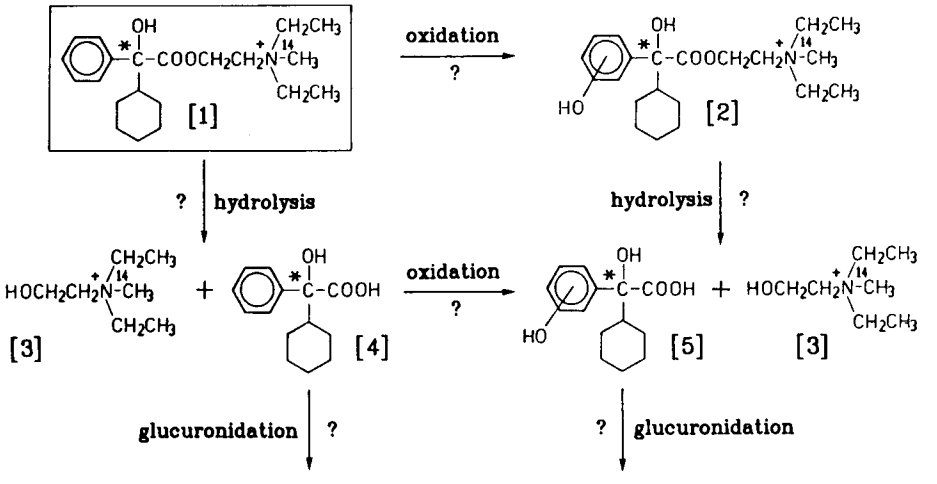
Bovine serum albumin was the only protein present in the perfusate. The binding to BSA in the perfusion medium was low: about 15% and no enantioselectivity was observed. This observation is consistent with data from the literature: only a few drugs tend to be enantioselectively bound to albumin, e.g. oxazepam (Müller & Wollert 1975), coumarin derivatives (Veronich et al 1979) and various acid compounds (Pettersson et al 1986). The binding of oxyphenonium to BSA cannot contribute to differences in the kinetics of the enantiomers in the rat isolated perfused liver.

After about 20 min the concentration of radioactivity in the perfusate increased and it is conceivable that a metabolite(s) appeared in the medium. Ion pair extraction of the radioactive compound in the perfusate at 120 min could not be achieved, also indicating the presence of other compounds than unchanged oxyphenonium.

Large differences in biliary excretion of radioactivity are seen between the (+)- and the (−)-isomers. The biliary excretion rate exhibited first order kinetics, the half-lives for (+)- and (−)-oxyphenonium of the descending phase of the curve being 19 and 29 min, respectively. After 5–10 min the maximum percentage of dose in the liver was calculated to be 70% and did not differ between (+)- and (−)-oxyphenonium. Consequently, the driving force for metabolism and/or excretion of both compounds in the liver can be considered to be identical. The differences in biliary excretion rate can then be caused by differences in rate of metabolism and/or in bile canalicular transport of the parent compounds or the metabolites. But we have to keep in mind that this extrapolation has to be done with great care, because most of data refer to total ^{14}C with no resolution of drug from its metabolites.

From Fig. 3 it is clear that metabolites are excreted in the bile in different ratios and with different appearance/disappearance times. The relative abundance of "substance" A is larger for (+)- than for (−)-oxyphenonium in all fractions analysed. "Substance" B, probably the parent compound, is present in bile from (+)-oxyphenonium experiments only during the first 15 min, whereas this compound remains in the bile from the (−)-oxyphenonium experiments even after 70 min. "Substance" C also disappears more rapidly following (+)-oxyphenonium than with (−)-oxyphenonium.

One (or more) metabolite(s) formed was (were) also excreted in the perfusate. The radioactive compounds in the perfusate at 120 min as well as the radioactive compounds in the liver homogenates cannot be due to the parent compound as it (they) could not be extracted with dichloromethane by ion pair complexation with perchlorate. The radioactive metabolites in bile are not conjugates of glucuronic acid or



Scheme I. Supposed main metabolic pathways of oxyphenonium.

sulphuric acid, because treatment of bile with β-glucuronidase-sulphatase did not result in changes in mobility on the TLC plates.

Thus, the question remains which process(es) may actually be responsible for the observed differences in biliary metabolite excretion for (+)- and (−)-oxyphenonium. The supposed main metabolic pathways are presented in Scheme I.

If hydrolysis occurs, the formed CHPGA [4] is no longer radioactive and cannot be followed any further (UV-absorbance is very low). The alcohol with the quaternary ammonium group, β-hydroxyethyl diethyl methylammonium (HEDMA) [3] resembles choline ($HOCH_2$-$CH_2N(CH_3)_3^+$) and might enter the hepatic metabolic pathways of choline (Lehninger 1979). If this metabolic pathway does indeed occur, "substance" C would correspond to HEDMA and "substance" A might be a conversion product of it with an even more polar, perhaps phosphorylcholine-like structure.

On the other hand oxidative metabolic processes may also play a role. Enantioselective hydroxylations have been described for many compounds (Honma et al 1985; Vukusic et al 1985; Ochs et al 1986). The product [2], after oxidative metabolism of oxyphenonium may have an R_F-value close to that of oxyphenonium, but it can be hydrolysed.

An alternative might be that enantioselective canalicular transport occurs for the parent compounds which may explain some of the differences. It is then conceivable that the (+)-isomer is more efficiently transported and that this, together with a more efficient metabolism rapidly exhausts the hepatic pool of the parent compound. This idea is supported by the observation that the initial amounts of (+)- and (−)-oxyphenonium in the liver are similar, but that, in spite of the more rapid metabolism of the (+)-isomer, about equal amounts of material with an R_F-value similar to unchanged oxyphenonium are initially excreted in the bile.

It remains to be clarified which of these mechanisms is the major determinant of the observed differences in the hepatic disposition of the oxyphenonium enantiomers.

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