Biochemical Pharmacology, Vol. 36, No. 13, pp. 2238–2239, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

The pharmacokinetics of (+)- and (−)-primaquine in the isolated perfused rat liver preparation

(*Received* 24 *June* 1986; *accepted* 18 *December* 1986)

Many compounds exist in more than one isomeric form and exhibit stereoselective differences with respect to their pharmacological activity, metabolism and elimination, e.g. warfarin [1, 2] and propranolol [3]. The antimalarial drug primaquine possesses an asymmetric carbon atom in the alkylamino side chain which confers optical activity upon the (+)- or (−)-stereoisomers.

A comparison of the curative and toxic activity of (+)-, (−)- and racemic primaquine showed that all three forms are equipotent antimalarials [4]. The isomers, however, differ significantly with respect to toxicity [4]. Acute toxicity studies in the mouse showed the (+)-isomer to be three to four times more toxic than the (−)-isomer, while subacute toxicity studies in the rhesus monkey showed the (−)-isomer to be three times more toxic than (+)-primaquine. In both experimental animals the toxicity of the racemate lay between that of each isomer. It is nonetheless unknown whether the disparity in therapeutic indices reflects pharmacodynamic and/or pharmacokinetic differences between the isomers.

The ability of equivalent doses of racemic, (−)- and (+)-primaquine to inhibit drug metabolism in the isolated perfused rat liver preparation (IPRL) has been reported recently [5]. Although all three compounds are equipotent inhibitors of drug metabolism, the perfusate levels of (−)-primaquine fall more rapidly than (+)-primaquine, whereas concentrations of the racemate decline at a rate between those of the two isomers.

The present study has examined, in greater detail, the disposition of (+)- and (−)-primaquine in the IPRL. Earlier studies in this experimental model have shown that primaquine exhibits dose-dependent pharmacokinetics [6]. Therefore, the disposition of the isomers of primaquine was studied after low (0.5 mg) and high (2.5 mg) doses of each isomer.

Rats were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.). Livers were isolated using standard techniques and then perfused in a constant flow (15 ml/min) recirculating system containing 10% washed human red cells and 1% human serum albumin at 37°, as previously described [7]. The optical purity of each stereoisomer was determined by measurement of the optical rotation of an aqueous solution of each isomer (10 mg/ml; 5 ml). The values obtained were in agreement with those previously reported: (+) α^{22} + 28.7°, (−) α^{22} − 25.8° [8]. The elimination of primaquine isomers from the IPRL was studied over 3 hr after administration of either the (+)- or (−)-isomers of primaquine as a 0.5 and 2.5 mg bolus dose of the diphosphate salt. Due to the limited supply of (+)- and (−)-primaquine (≤15 mg), only three IPRL preparations could be carried out in each group. Primaquine isomers were added as aqueous solutions (50–250 μl) directly into the perfusate reservoir, thereby simulating systemic dosage. Samples (1.5 ml) were taken from the perfusate reservoir pre-dose and at 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min. Perfusate primaquine concentrations were determined by a selective and sensitive HPLC method previously reported [9].

Perfusate plasma primaquine concentrations declined biexponentially following the administration of (+)- or (−)-primaquine at doses of 0.5 and 2.5 mg (Fig. 1). Perfusate drug concentrations fell rapidly over the first 15 min after drug administration, followed by a more gradual rate of decline to the end of each experiment (3 hr). There were no significant differences in any of the pharmacokinetic parameters for the (+)- and (−)-isomers of primaquine after the 0.5 mg dose (Table 1). By contrast, there was a marked disparity in the elimination profiles for (+)- and (−)-primaquine after a 2.5 mg dose of each isomer with the perfusate levels of the (−)-isomer falling more rapidly than those of the (+)-isomer (Fig. 1). The clearance of the (−)-isomer (4.7 ± 1.1 ml/min) was significantly greater than the clearance of the (+)-isomer (1.4 ± 0.6 ml/min). This difference in the hepatic elimination efficiency was translated into a significantly shorter half-life (81.6 ± 37.8 min) and smaller area under the curve (310 ± 84 μg·min ml^{-1}) for (−)-primaquine when compared with the half-life (245 ± 122 min) and area under the curve (1105 ± 400 μg·min·ml^{-1}) for the (+)-isomer. At this dose size there was no significant difference in the volume of distribution of the (−)- and (+)-isomers of primaquine (522 ± 115 and 475 ± 207 ml respectively). The disparity in the elimination efficiency of the two isomers is likely to be due to either differences in the affinity of metabolising enzymes for the two substrates or to the involvement of different isoenzymes in the metabolism of the two compounds, although stereoselective binding may be a contributing factor. It is interesting to note that it is the more slowly eliminated (+)-isomer that is most toxic in rodents [4].

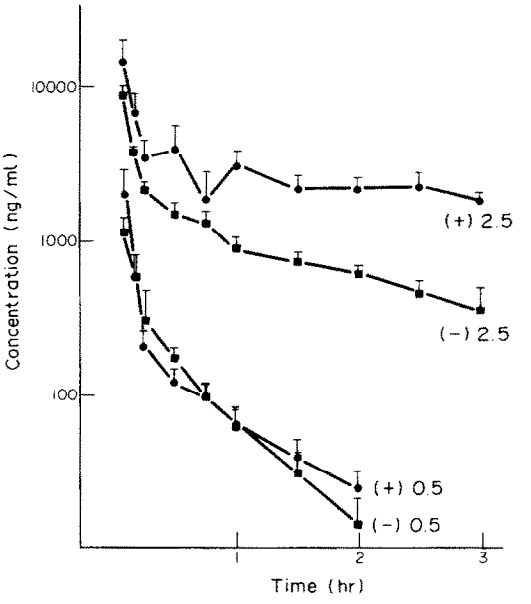

Fig. 1. Plasma concentration time profiles for (−)- and (+)-primaquine after administration to the isolated perfused rat liver preparation at a dose of 0.5 and 2.5 mg. Primaquine exhibited stereoselective differences in disposition only after the 2.5 mg dose.

The value for half-life and area under the curve for each isomer following a dose of 0.5 mg in this study was similar to that obtained after the administration of the racemate

Table 1. Pharmacokinetics of the (−)- and (+)-isomers of primaquine after administration of 0.5 and 2.5 mg to the isolated perfused rat liver preparation

Dose	Cl (ml/min)	V_d* (ml)	$T_{1/2}$ (min)	AUC ($\mu g \cdot min \cdot ml^{-1}$)
(−) 0.5 mg	11.9 ± 1.6	358 ± 64	21.2 ± 6.0	24.1 ± 3.5
(+) 0.5 mg	11.6 ± 3.7	499 ± 106	32.9 ± 14.1	26.2 ± 9.2
	NS†	NS	NS	NS
(−) 2.5 mg	4.7 ± 1.1	522 ± 115	81.6 ± 37.8	310 ± 84
(+) 2.5 mg	1.4 ± 0.6	475 ± 207	245 ± 122	1105 ± 400
	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$

* Volume of distribution.
† Not significant.

at an equivalent dose [6]. The volume of distribution for both the (+)- and (−)-isomer was considerably greater than the physical volume of the system (i.e. 100 ml + liver volume) suggesting extensive tissue uptake of both isomers, as was shown to be the case for the racemate [6]. The values for clearance indicate that both (+)- and (−)-primaquine behaved as high clearance compounds at this low dose (clearance for each isomer being more than 75% of liver blood flow, which was 15 ml/min in these experiments); once again these findings are consistent with those obtained for the racemate [6]. As the elimination of such high clearance compounds is primarily dependent on liver blood flow [10], it is conceivable that subtle stereoselective differences in the intrinsic clearance of low doses of primaquine cannot be distinguished in this experimental model.

The dose-dependent stereoselectivity in drug handling was an intriguing finding. However, our earlier studies in the isolated perfused rate liver have shown that there is a difference in the metabolic fate of primaquine at the two dose levels we have studied [6].

At the low dose, high-affinity, low-capacity processes that show an equal affinity for both isomers are responsible for metabolism; however, these routes became saturated at the higher dose and elimination proceeded via higher capacity but lower affinity processes that appeared to be able to discriminate between the two isomers.

The relevance of these findings to humans, where primaquine behaves as a low clearance compound at doses throughout the therapeutic range [11], is currently under investigation as are studies to investigate the toxicity and antimalarial activity of the two isomers at therapeutic concentrations.

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