J. Pharm. Pharmacol. 1993, 45 836–838
Communicated December 16, 1992

COMMUNICATIONS

Effects of turpentine oil pretreatment on β-blocker pharmacokinetic parameters in rats

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Abstract—Turpentine oil treatment (0·2 mL kg^{-1}, s.c.) was used to increase the plasma concentration of α_1-acid glycoprotein (0·13 mg mL^{-1} in control rats) to 1·72 mg mL^{-1} after 2 days, and allow assessment of its effects on the pharmacokinetics and stereoselective binding of three β-blockers. Racemates (5 mg kg^{-1}) were administered intravenously to control and turpentine oil-pretreated rats and the plasma concentrations were determined up to 90 min. Stereoselective analysis showed the apparent distribution volume and the area under plasma concentration-time curves (AUC) of *R*-(+)-propranolol to be, respectively, one-quarter and twice those of the *S*-(−)-enantiomer and differences in pharmacokinetic parameters between the two were magnified by turpentine oil pretreatment. Pharmacokinetic parameters of oxprenolol enantiomers were essentially similar for the controls but after turpentine oil pretreatment, a higher affinity of the *R*-(+)-enantiomer for plasma was observed. Acebutolol enantiomers behaved non-stereospecifically throughout. These results were consistent with predictions from the in-vitro stereospecific binding properties of these agents to purified rat α_1-acid glycoprotein.

Determination of blood concentrations of therapeutic drugs is an important aspect of evaluating the quality and level of biological effects. Drug concentrations in the blood depend on plasma protein binding, tissue binding, renal clearance and hepatic extraction. Therefore, a change in binding protein concentration is one of the major factors which can affect plasma drug concentrations as well as pharmacokinetic parameters. It has been shown that high plasma levels of propranolol are closely related to high binding to plasma proteins probably to α_1-acid glycoprotein (AAG) (Yasuhara et al 1985). AAG has a high affinity for basic drugs (Piafsky 1980: Belpaire et al 1984: Murai-Kushiya et al 1993) and its plasma concentration may be increased several times under some stress conditions (Aronsen et al 1972; Chio & Oon 1979; Piafsky 1980; Edwards et al 1982; Yasuhara et al 1985; Hasegawa et al 1989). Recently, AAG from both man and rat has been demonstrated to have a stereospecific binding property, not only for basic but also for acidic drugs (Albani et al 1984; Lima et al 1984; Vogelsang et al 1984; Brunner & Müller 1987; Murai-Kushiya et al 1993).

In an earlier experiment, we showed that stereospecific changes in the pharmacokinetic parameters of racemic pindolol occurred in endotoxin-pretreated rats with increased plasma AAG levels (Hasegawa et al 1989). We subsequently examined the stereospecific binding properties of three other β-blocking agents to purified rat AAG (Murai-Kushiya et al 1993). Racemic propranolol binds to AAG stereospecifically and both oxprenolol enantiomers bind equally, whereas neither enantiomer of acebutolol binds to any plasma protein.

In this communication we set out to examine the stereospecific binding of β-adrenergic drugs to rat AAG, in-vivo.

Materials and methods

Materials. Racemic propranolol hydrochloride and oxprenolol were purchased from Sigma Chemical Company (St Louis, MO, USA) and racemic acebutolol hydrochloride was a generous gift from Sandoz Pharmaceuticals Ltd (Tokyo, Japan). Horseradish peroxidase-linked anti-rabbit Ig F(ab′)$_2$ fragment (from donkey), and 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) were purchased from Amersham, Japan (Tokyo, Japan). α_1-Acid glycoprotein (AAG) was purified by the procedure of Charlwood et al (1976) from turpentine oil-pretreated rats. Anti-serum to AAG was raised in rabbits by injecting antigen (1 mg mL^{-1}) emulsified with 1 mL of Freund's complete adjuvant once a month for 4 months before collection. All other chemicals used were of analytical reagent or HPLC grade.

Animals and animal treatment. Rats, 190–220 g, obtained from Nippon SLC Co. (Hamamatsu, Japan) were divided into two groups: controls and turpentine oil-pretreated. The latter received an injection of 0·2 mL kg^{-1} turpentine oil subcutaneously and the former 0·4 mL olive oil. Two days later, all animals were anaesthetized with intraperitoneal pentobarbitone (35 mg kg^{-1}) before drug challenge. Propranolol, oxprenolol or acebutolol racemates (5 mg kg^{-1}), dissolved in sterile 0·9% NaCl, were injected intravenously and blood samples were withdrawn into heparinized syringes from the abdominal descending aorta at 5, 15, 30, 60 and 90 min thereafter and immediately centrifuged at 4°C to obtain plasma.

Determination of plasma proteins. The content of AAG in the plasma was determined using the enzyme-linked immunosorbent assay (ELISA) method. Plasma was applied to an Amberlite CG-50 Type I column (Organo Ltd, Tokyo, Japan) equivalent to 0·045 mM sodium citrate buffer (pH 5·1) and eluted with the same buffer to remove high amounts of other plasma proteins. After the pH was adjusted to 9·6, 50 μL of appropriately diluted samples were coated in microplates and immunoassayed non-competitively using anti-AAG rabbit serum, horseradish peroxidase-linked anti-rabbit Ig F(ab′)$_2$ fragment and ABTS as a substrate.

Albumin and total proteins were determined with a Hitachi 7050 Autoanalyzer (Tokyo, Japan).

Analysis of R-(+)- *and* S-(−)-*enantiomers in plasma.* Plasma concentrations of both enantiomers were determined by HPLC (Murai-Kushiya et al 1993) after derivatization by 2, 3, 4, 6-tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate (Hasegawa et al 1989). Peak positions of enantiomers on HPLC were confirmed by single enantiomer experiments as described previously (Murai-Kushiya et al 1993).

Calculation of pharmacokinetic parameters. The pharmacokinetic parameters in control and turpentine oil-pretreated rats of all enantiomers except *S*-(−)-propranolol were calculated according to the two-compartment open model. Hybrid constants (A, B, α, β) were then obtained by nonlinear regression analysis using MULTI (Yamaoka 1984) to fit the following equation:

$$c = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

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The classic pharmacokinetic parameters (c_o, V_1, V_2, AUC, CL and $t_{\frac{1}{2}}$) were calculated from hybrid constants. For *S*-(−)-propranolol in turpentine oil-pretreated rats, the one compartment open model was applied using the following equation and the parameters (c_o, V, AUC, CL and $t_{\frac{1}{2}}$) were calculated as described above:

$$c = c_o e^{-k_e t} \quad (2)$$

Results and discussion

Plasma concentrations of propranolol, oxprenolol and acebutolol enantiomers were determined from 5 to 90 min after intravenous administration of the racemates to control and turpentine oil-pretreated rats. Under the present experimental conditions, plasma concentrations of AAG were 0·129±0·020 and 1·72±0·37 mg mL^{-1} for control and turpentine oil-pretreated animals, respectively. The plasma concentration of albumin was in contrast decreased by turpentine oil-pretreatment (control, 34·3±1·7 mg mL^{-1}; turpentine, 23·9±1·1 mg mL^{-1}, $P<0·01$) without change in total protein levels (control, 42·7±2·1 mg mL^{-1}; turpentine, 41·4±1·1 mg mL^{-1}).

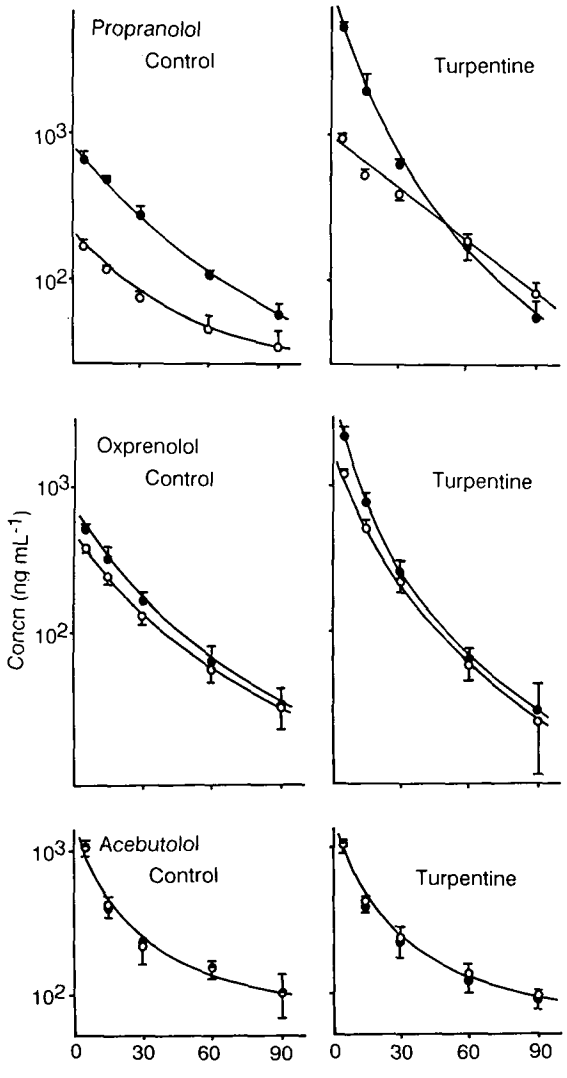

FIG. 1. Plasma concentrations of propranolol, oxprenolol and acebutolol enantiomers in control and turpentine oil-pretreated rats. The racemates (5 mg kg^{-1}) were injected intravenously into control and turpentine oil-pretreated rats under pentobarbitone anaesthesia. Open and closed circles indicate *S*-(−)- and *R*-(+)-enantiomers, respectively. Each point represents the average for data from four animals with the lines indicating standard deviation.

Plasma concentrations of *R*-(+)-propranolol were always higher than those of *S*-(−)-propranolol in control (Fig. 1) and both concentrations were significantly increased by turpentine oil-pretreatment (Fig. 1). For the *S*-(−)-enantiomer in turpentine oil-pretreated rats, pharmacokinetic parameters were calculated using the one-compartment open model because of a linear relationship in time-log plasma concentration plots.

Table 1 summarizes pharmacokinetic parameters for each enantiomer calculated from each value in Fig. 1.

The more marked response of the *R*-(+)-enantiomer to turpentine oil-pretreatment suggests it has a much higher affinity for AAG at the time of racemate injection than the *S*-(−)-enantiomer. In our previous experiment, the concentration range of the high stereospecific binding of propranolol racemate was 6–60 μg mL^{-1} at 1 mg mL^{-1} purified rat AAG (Murai-Kushiya et al 1993). Lower concentrations of racemate demonstrated nearly 100% binding for both enantiomers while 120 μg mL^{-1} resulted in around 20% binding that was still stereospecific. Presuming the total blood volume in the whole body to be 20 mL for a 200 g rat, the original racemate concentration would be 50 μg mL^{-1} in the blood just after the injection (5 mg kg^{-1}). For control rats, the high stereospecific range was calculated to be 0·8–8 μg mL^{-1} based on a 0·13 mg mL^{-1} plasma AAG level. For turpentine oil-pretreated rats, the high stereospecific range would be 10–100 μg mL^{-1} from the 1·7 mg mL^{-1} AAG level. Comparison of these two ranges with the calculated concentration (50 μg mL^{-1}) of racemate, indicates that 1·7 mg mL^{-1} AAG should induce higher stereospecific binding than 0·13 mg mL^{-1} AAG in this experiment.

As shown in Table 1, CL values of *S*-(−)- and *R*-(+)-propranolol were decreased by turpentine oil-pretreatment, to a lesser extent than V_1 values. This indicates that the high plasma concentrations induced by increased AAG might result in the higher clearance rates from plasma, as shown by approximately half of the turpentine oil-pretreated rats.

Plasma concentrations of oxprenolol enantiomers were similar in control rats and both were increased by turpentine oil-pretreatment (Fig. 1). Pharmacokinetic parameters were similar in control rats (Table 1), but after turpentine oil-pretreatment the pharmacokinetic analysis indicate the *R*-(+)-enantiomer to have a higher affinity for the plasma protein, confirming the in-vitro finding (Murai-Kushiya et al 1993). As for propranolol, CL values of *S*-(−)- and *R*-(+)-enantiomers were decreased to a lesser extent by turpentine oil-pretreatment, than were V_1 values.

Plasma concentrations of acebutolol enantiomers were similar in controls and after turpentine oil-pretreatment (Fig. 1), and there was no difference in pharmacokinetic parameters between enantiomers (Table 1).

AAG has stereospecific binding properties for various racemic drugs such as propranolol, disopyramide, verapamil and pindolol (Albani et al 1984; Lima et al 1984; Vogelsang et al 1984; Brunner & Müller 1987; Murai-Kushiya et al 1993). Furthermore, it can be increased up to 20 times in rats by laparotomy (Yasuhara et al 1985), turpentine oil-pretreatment (Charlwood et al 1976) and endotoxin-pretreatment (Hasegawa et al 1989). It is also elevated in man undergoing trauma (Edwards et al 1982), surgery (Aronsen et al 1972), cancer development (Chio & Oon 1979) and myocardiac infarction (Freilich & Giardina 1984). Because of these findings, we can conclude that plasma concentrations of racemic drugs should be determined stereoselectively. In addition, the present experiment has illustrated the applicability of in-vitro binding studies to predict in-vivo events.

Table 1. Pharmacokinetic parameters for propranolol, oxprenolol and acebutolol enantiomers

	A	α	B	β	c_o (ng mL^{-1})	V_1 (L Kg^{-1})	V_2 (L Kg^{-1})	AUC (ng mL^{-1} min)	CL (L kg^{-1})	$t_{\frac{1}{2}}$ (min)
Propranolol										
Control *S*-(−)	159·6	0·0607	58·7	0·005	218	11·45	17·48	14400	0·000174	45·6
R-(+)	495·3	0·0440	308	0·0225	804	3·11	0·36	24900	0·0001	21·5
Turpentine *S*-(−)	—*	—	—	—	950	2·63	—	35300	0·000071	25·8
Turpentine *R*-(+)	8265	0·124	1228	0·033	9493	0·263	0·123	10400	0·000024	13·5
Oxprenolol										
Control *S*-(−)	424·8	0·0529	62·2	0·00927	487	5·13	4·94	14700	0·00017	21·0
R-(+)	606·3	0·0534	66·3	0·00974	673	3·72	3·19	18200	0·000138	18·7
Turpentine *S*-(−)	1481	0·151	681	0·04	2162	1·16	0·54	26800	0·000093	8·60
Turpentine *R*-(+)	3419	0·137	650	0·0379	4068	0·615	0·28	42100	0·000059	7·18
Acebutolol	1674	0·147	285	0·0112	1959	1·28	3·05	36900	0·0000677	13·1
Control *S*-(−)	1676	0·170	330	0·0129	2005	1·25	2·81	35400	0·0000606	12·2
R-(+)	1606	0·161	359	0·0157	1965	1·27	2·25	32900	0·000076	11·6
Turpentine *S*-(−)	1606	0·171	340	0·0158	1947	1·28	2·41	31000	0·0000808	11·0
Turpentine *R*-(+)										

* Parameters for *S*-(−)-propranolol in turpentine oil-pretreated rats were calculated using the one-compartment open model.

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