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# Effect of inflammation and proadifen on the disposition of antipyrine, lignocaine and propranolol in rat isolated perfused liver

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The effect of inflammation, induced in rats by injection of turpentine oil, on drug disposition has been evaluated in rat isolated perfused livers. The drugs studied were a low extraction drug, antipyrine, and two high extraction drugs, lignocaine and propranolol. Turpentine significantly increased the half-life of antipyrine and of propranolol, but not that of lignocaine. Proadifen (SKF 525A) significantly increased the half-life of all three drugs. Turpentine decreased the clearance of antipyrine significantly by about 50% and that of propranolol non-significantly by about 20%, but did not affect the clearance of lignocaine. Proadifen significantly decreased the clearance of all three drugs, but this was most pronounced for antipyrine. In both turpentine- and proadifen-treated rats a significant increase in volume of distribution of propranolol was observed. The results show that, as with proadifen, turpentine-induced inflammation affects the hepatic clearance of antipyrine in the rat isolated perfused liver. With both high extraction drugs, the effect of inflammation on their clearance was low or absent, in contrast to the effect of proadifen. This suggests that a possible effect of inflammation on intrinsic clearance is not large enough to influence the hepatic clearance of the high extraction drugs.

It is established that the serum concentrations of substances bound to $\alpha_1$-acid glycoprotein ($\alpha_1$-AGP) are increased in patients with inflammatory disease (see e.g., for β-adrenoceptor blocking agents, Schneider & Bishop 1982) and in rats with experimental inflammation (Bishop et al 1981; Barber et al 1983; Terao & Shen 1983; Mugabo et al 1985; Yasuhara et al 1985; Belpaire et al 1986; Walker et al 1986). The increase of the area under the curve (AUC) is mainly seen after oral administration of such substances. As the AUC after oral administration is inversely related to the free fraction of drug in plasma and to the intrinsic clearance, changes in both factors could be involved. The free fraction is decreased in inflammation, due to the increased $\alpha_1$-AGP concentrations (Piafsky et al 1978). The idea that intrinsic clearance could be decreased, is strengthened by the finding that in rats with experimental inflammation, hepatic drug metabolizing activity and cytochrome P450 content are decreased (see e.g. Whitehouse & Beck 1973). Yasuhara et al (1985) and Walker et al (1986) found a decreased systemic clearance of propranolol in rats subjected to laparotomy and in rats with adjuvant-induced arthritis.

We recently described alterations in kinetics and effect of propranolol, a drug bound to $\alpha_1$-AGP, in rats with inflammation induced by injection of turpentine oil (Mugabo et al 1985; Belpaire et al 1986). The study of drugs in the isolated perfused liver preparation permits the evaluation of intrinsic clearance, as hepatic flow and protein binding are kept constant. We have therefore examined the disposition of several substances in the isolated perfused liver of rats pretreated with turpentine oil. Antipyrine, a drug with a low hepatic extraction ratio, and propranolol and lignocaine, drugs with a high hepatic extraction ratio, were studied. For comparative purposes, the influence of proadifen (SKF 525A), a known enzyme inhibitor, was evaluated.

*Chemicals*: Propranolol HCl (ICI, UK), lignocaine HCl (Astra Nobelpharma, Belgium) and proadifen (SKF 525A: Smith Kline and French, USA) were gifts. Turpentine oil (Bossuyt, Belgium) was purchased.

## METHODS

### *Animals*

Male Wistar rats, 320–450 g, purchased from the Rega Institute (Louvain, Belgium), were allowed free access to food and water until death subsequent to anaesthesia. Inflammation was induced by intramuscular injection of turpentine oil: 0·5 mL was given in one hindlimb at 48 h, and 0·5 mL in the other hindlimb 24 h before death; control animals

* Correspondence.

were not treated. The erythrocyte sedimentation rate was measured using the Westergren method. In another series of experiments, proadifen (100 mg $kg^{-1}$) was injected intraperitoneally 1 h before isolation of the liver; control rats were given 0·9% NaCl (saline). For the study of antipyrine, the turpentine- and proadifen-treated rats were compared with one group of seven control animals, as the experiments were done concurrently; three of the rats were injected with saline 1 h before death and four animals received no injection. For lignocaine and propranolol there was a separate control group for turpentine- and for proadifen-treated rats.

*Isolated perfused liver*

The livers were removed under ether anaesthesia and perfused using a standard technique (Miller 1973). After 30 min of 'once through' perfusion with Krebs-bicarbonate solution, perfusion was continued with 100 mL of recirculating blood medium (37 °C, pH 7·4, oxygenated with 95:5 oxygen:carbon dioxide) consisting of 11·5% washed bovine erythrocytes and 2% bovine serum albumin in Krebs-bicarbonate solution. The bovine erythrocyte preparations were made weekly; the last washing was with Krebs-bicarbonate containing ampicillin (100 μg $mL^{-1}$) as a preservative. After 30 min perfusion with blood medium, antipyrine (5 mg), lignocaine (1 mg) or propranolol (5 mg) was added to the perfusion reservoir. (With this dose of propranolol, non-linear kinetics could be present (Shand et al 1973), but with lower doses it was difficult to evaluate the β-phase.)

By altering the inflow pressure, if necessary, the perfusion flow rate was kept constant at 20 mL $min^{-1}$ for the experiments with propranolol and antipyrine; the flow rate was kept at 14 mL $min^{-1}$ for lignocaine because it disappeared from the medium too rapidly to allow an accurate assessment of its disposition. Perfusate samples were collected from the reservoir at specified times. Each sample volume was less than 5% of the reservoir volume (1 mL for antipyrine and propranolol, 0·7 mL for lignocaine).

*Drug concentration assay in perfusate plasma*

Perfusate samples were centrifuged immediately, and the supernatant kept at −20 °C until analysis.

Antipyrine was measured by gas-liquid chromatography as described by Van Peer et al (1981), except that aminopyrine was used as internal standard. The between-run variation coefficient was 10·2%, and the analytical recovery was 109% at a concentration of 32 μg $mL^{-1}$ (n = 18).

Lignocaine was determined by high performance liquid chromatography using a Spherisorb 5 ODS column (Chrompack) and a mixture of 0·04 M sodium phosphate buffer (pH 3)–acetonitrile (82·5:17·5) as mobile phase, with UV detection at 215 nm. Lignocaine and its internal standard trimecaine were extracted from the sample with hexane. The between-run variation coefficient was 6·1% and the analytical recovery was 98% at a concentration of 5 μg $mL^{-1}$ (n = 25).

Propranolol was assayed by spectrofluorometry (Shand et al 1970). The between-run variation coefficient was 9·4% and the analytical recovery was 106% at a concentration of 5 μg $mL^{-1}$ (n = 27).

*Calculation of pharmacokinetic parameters*

The drug concentrations in the perfusate plasma were plotted against time on a semilogarithmic scale and analysed by linear regression. For antipyrine and lignocaine, the decline of the concentrations was mono-exponential, and the elimination rate constant and the half-life were determined from the curve. The volume of distribution was calculated by dividing the dose by the concentration at time zero, obtained by extrapolation from the disposition curve. The clearance was calculated from the volume of distribution and the half-life ($Cl = 0{\cdot}693\ Vd/t_{\frac{1}{2}}$).

Propranolol concentrations declined biexponentially. The slow disposition rate constant (β) was calculated by linear regression analysis of the post-distribution perfusate plasma concentrations. The elimination half-life was obtained from 0·693/β and the clearance from dose/$AUC_{0\rightarrow\infty}$ where $AUC_{0\rightarrow\infty}$ is the area under the concentration-time curve. $AUC_{0\rightarrow\infty}$ is the sum of $AUC_{0\rightarrow40}$ calculated by trapezoidal rule and $AUC_{40\rightarrow\infty}$ calculated from the plasma concentration at 40 min divided by β. The volume of distribution was calculated from dose/β $AUC_{0\rightarrow\infty}$. Statistical analysis was performed using the Mann-Whitney U-test; significance was assumed when $P < 0{\cdot}05$.

## RESULTS

There was no difference in final body weight between treated and non-treated rats nor was relative liver weight significantly higher or bile formation rate significantly lower in the treated compared with control rats. However, the erythrocyte sedimentation rate was increased markedly in turpentine-treated rats (Table 1). The viability of the liver was indicated by constant bile formation rates,

Table 1. Comparison of experimental parameters of control rats, turpentine-treated rats and proadifen-treated rats. Data are given as means ± s.e.m. for the pooled groups.

| | Controls (n = 33) | Turpentine-treated (n = 20) | Proadifen-treated (n = 18) |
|---|---|---|---|
| Erythrocyte sedimentation rate (mm $h^{-1}$) | ≤3 | 23·6 ± 10·5 | ≤3 |
| Body weight (g) | 379 ± 25 | 370 ± 30 | 387 ± 35 |
| Relative liver weight (%) | 3·00 ± 0·19 | 3·43 ± 0·18 | 3·0 ± 0·28 |
| Bile formation* (μL $min^{-1}$ $g^{-1}$) | 0·97 ± 0·23 | 0·71 ± 0·29 | 0·93 ± 0·26 |

* Measured over 60 min.

the gross appearance of the liver and the constancy of the inflow pressure. The increase of perfusion pressure needed to maintain constant flow never exceeded 4·5 cm water.

*Antipyrine*

The decline in plasma perfusate concentrations of antipyrine was mono-exponential but much slower than that of either lignocaine or propranolol. As is apparent from Fig. 1 and Table 2, turpentine treatment, but even more so proadifen treatment, prolonged the half-life of antipyrine. As the volume of distribution was unchanged, a marked decrease in clearance could be calculated in the two groups of rats.

In the control group, there was no difference in pharmacokinetic parameters between the three rats injected with saline and the four rats receiving no injection.

*Lignocaine*

The decline of the lignocaine concentrations with time was also mono-exponential (Fig. 1). Turpentine treatment did not influence this decline (Table 2). Proadifen did slow down the decline of the lignocaine concentrations, with a significant increase in

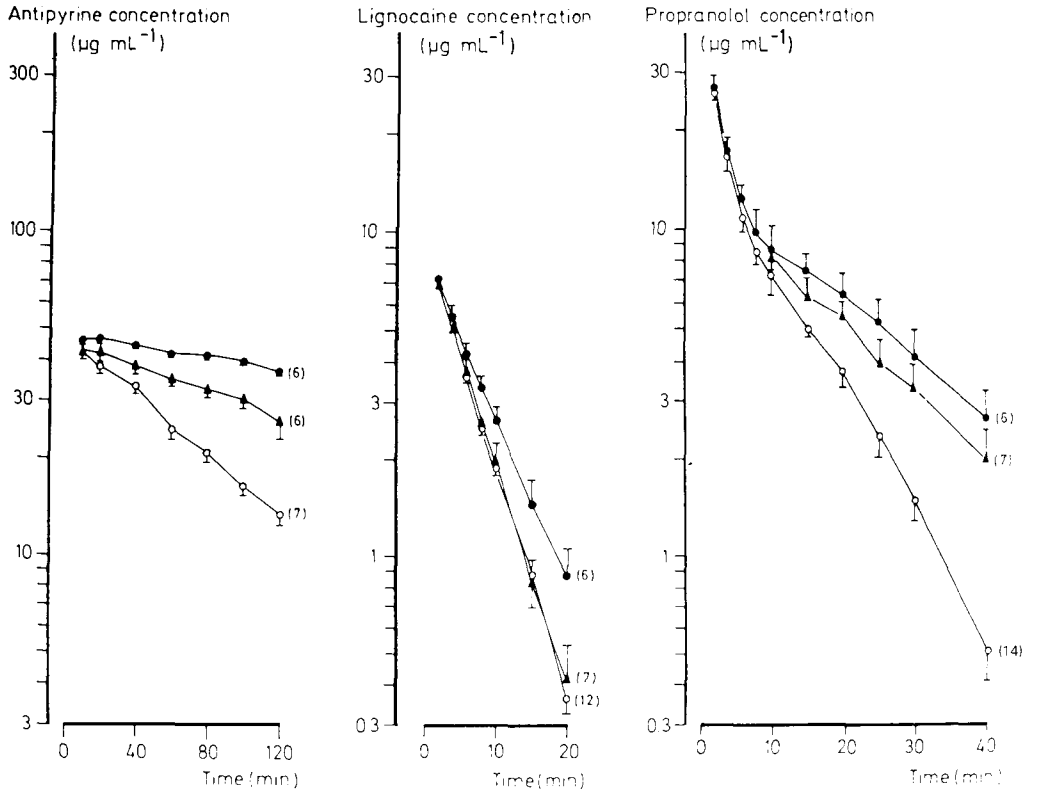


FIG. 1. Concentration-time profiles in plasma perfusate of isolated perfused livers from control rats (○), turpentine-treated rats (▲) and proadifen-treated rats (●). Antipyrine (5 mg), lignocaine (1 mg) or propranolol (5 mg) were added to 100 mL perfusate medium at time 0. Data are expressed as means ± s.e.m. The number of experiments is given in parentheses. For lignocaine and propranolol, the two control groups have been pooled for the sake of clarity.

half-life while the volume of distribution was increased slightly, but non-significantly, and the clearance was moderately but significantly decreased (Table 2).

Table 2. Pharmacokinetic values for antipyrine, lignocaine and propranolol in control rats, turpentine-treated rats and proadifen-treated rats. Turpentine oil: i.m. 0·5 mL 48 h and 0·5 mL 24 h before death; proadifen: 100 mg kg$^{-1}$ i.p. 60 min before death. Antipyrine (5 mg), lignocaine (1 mg) or propranolol (5 mg) were added to 100 mL perfusate medium. Data are presented as means ± s.e.m.

| | $t_{\frac{1}{2}}$ (min) | $V_d$ (mL) | Cl (mL min$^{-1}$) |
|---|---|---|---|
| Antipyrine | | | |
| Control (n = 7) | 65·2 ± 1·8 | 104·9 ± 3·7 | 1·13 ± 0·07 |
| Turpentine (n = 6) | 160·9 ± 12·2[c] | 109·8 ± 4·1 | 0·48 ± 0·05[c] |
| Proadifen (n = 6) | 374·2 ± 45·1[c] | 103·7 ± 2·1 | 0·23 ± 0·02[c] |
| Lignocaine | | | |
| Control (n = 6) | 4·1 ± 0·3 | 102·0 ± 8·5 | 17·1 ± 0·7 |
| Turpentine (n = 7) | 4·3 ± 0·3 | 99·0 ± 5·8 | 16·3 ± 0·5 |
| Control (n = 6) | 4·2 ± 0·2 | 98·1 ± 6·7 | 16·0 ± 0·3 |
| Proadifen (n = 6) | 6·0 ± 0·5[b] | 114·6 ± 4·7 | 13·6 ± 0·9[a] |
| Propranolol | | | |
| Control (n = 8) | 7·2 ± 0·5 | 188·7 ± 9·4 | 18·5 ± 1·1 |
| Turpentine (n = 7) | 14·7 ± 2·3[b] | 286·6 ± 25·5[b] | 14·8 ± 1·7 |
| Control (n = 6) | 6·7 ± 0·6 | 191·0 ± 17·2 | 20·2 ± 1·5 |
| Proadifen (n = 6) | 15·5 ± 1·9[b] | 286·2 ± 31·8[a] | 13·5 ± 1·6[a] |

[a] $P < 0·05$, [b] $P < 0·01$, [c] $P < 0·001$: Different from controls (Mann-Whitney U-test).

*Propranolol*

The concentrations of propranolol as a function of time are shown in Fig. 1. In both control and treated rats, the decline of the perfusate concentrations was bi-exponential. In all individual rats, the decline was linear after 20 min, and half-lives were calculated from that time on. Half-life, volumes of distribution and clearances are listed in Table 2.

In both proadifen-treated and turpentine-treated rats, the second phase showed a much slower decline than in control rats, with a half-life which was approximately double, and the volume of distribution also much increased. The clearance in the turpentine-treated rats, while less than in the control rats, was not significantly different, but in the proadifen-treated rats the clearance was markedly decreased ($P < 0·05$).

## DISCUSSION

In patients and animals with inflammation, the serum concentrations of drugs that bind to $\alpha_1$-AGP are increased. While this could be a consequence of the $\alpha_1$-AGP increase occurring during inflammation, a decrease in hepatic biotransformation should also be considered. A decreased in-vitro hepatic biotransformation for several drugs was found in rats with experimental inflammation (see e.g. Whitehouse & Beck 1973). Furthermore, Bishop et al (1981), Yasuhara et al (1985), and Walker et al (1986) found in-vivo a decrease of the systemic clearance, although for a drug with a high hepatic extraction ratio, changes in intrinsic clearance would not be expected to influence systemic clearance. Our purpose was to evaluate, using the isolated perfused liver where hepatic blood flow and protein binding are kept constant, whether changes in hepatic clearance would occur as a result of induced inflammation.

Rats with inflammation caused by turpentine injection were used because we had found the kinetics and the pharmacodynamic effect of propranolol to be altered in this model (Mugabo et al 1985; Belpaire et al 1986). The turpentine-treated rats had an increased erythrocyte sedimentation rate and the serum binding of oxprenolol was increased (unpublished results), which provides an indirect proof of an increase of serum $\alpha_1$-AGP concentrations (Belpaire et al 1984).

For antipyrine and lignocaine, a mono-exponential decay of the perfusate plasma concentrations was found, corresponding to literature data (Rowland 1972; Pang & Rowland 1977; Lennard et al 1983; Webster et al 1984). For propranolol, the perfusate plasma concentrations declined bi-exponentially with time, as also seen by others (Shand et al 1973; Anderson et al 1978; Katayama et al 1984; Iwamoto et al 1985, 1986). The β-phase approximated an exponential decay, but there was a slight downward curvature, which was also seen by Shand et al (1973). The values of the pharmacokinetic parameters found for the three drugs in our control rats were in the same range as those obtained by those authors.

Antipyrine and propranolol half-lives were significantly increased in turpentine-treated rats and in animals treated with proadifen the half-life of all three drugs increased significantly, the increase being more pronounced for antipyrine and propranolol. Since half-life is determined by clearance and volume of distribution, the changes of these parameters have to be considered. In turpentine-treated rats, the clearance of antipyrine decreased by about 50%, and that of propranolol by about 20%, while the clearance of lignocaine did not change. As hepatic blood flow and protein binding were constant, the changes in the hepatic clearance can only be explained by changes in enzymatic activity (Wilkinson & Shand 1975). As with proadifen, inflammation markedly lowered the clearance and thus the metabolism of antipyrine, in agreement with several in-vitro studies reporting a decreased metabolism of

low extraction drugs and a lower cytochrome P450 content in the rat with acute inflammation (Whitehouse & Beck 1973; Cawthorne et al 1976; Swingle et al 1978; Müller & Hirschelmann 1981; Ishizuki et al 1983). We also found that the aminopyrine *N*-demethylase activity in the 9000*g* supernatant fraction of the liver at the end of the perfusion was decreased in both the turpentine- and proadifen-treated rats (unpublished results). We found inflammation to have no effect on lignocaine and a slight effect on propranolol hepatic clearance and thus on hepatic enzymatic activity. It has to be emphasized that small changes in hepatic enzymatic activity will not decrease the clearance of high, flow-dependent clearance drugs (Wilkinson & Shand 1975). Nevertheless, using constant flow perfusion, we could detect the inhibitory effect of proadifen on the clearance of lignocaine and propranolol. The effect of turpentine on hepatic clearance is probably not direct since according to Kaplan & Jamieson (1977) little turpentine reaches the liver when it is administered subcutaneously and enzymatic activity does not change when turpentine is added in-vitro.

A significant increase in the volume of distribution of propranolol was found in turpentine- and in proadifen-treated rats. We have no explanation for the higher hepatic uptake of propranolol. There is some evidence that proadifen alters the distribution of some compounds, possibly due to changes in membrane permeability (Marchand & Nadeau 1973; Clark & Krieger 1976). Venkataramanan & Axelson (1980) have found a higher volume of distribution for tocainide in proadifen-treated rats. On the other hand the volume of distribution of lignocaine and antipyrine was not altered by turpentine or by proadifen treatment. This suggests the existence of different drug binding sites in the liver. It has, in fact, been shown that large concentrations of unchanged propranolol, up to 25 times those in the reservoir, are present in the isolated perfused liver (Anderson et al 1978), whereas most lignocaine entering the liver is rapidly metabolized (Lennard et al 1983).

Our study was set up to evaluate whether in the rat with inflammation induced by turpentine injection, changes in hepatic clearance could be seen in the isolated perfused liver, where hepatic blood flow and protein binding are constant. The clearance of the low extraction drug, antipyrine, was slowed down considerably, pointing to a decreased intrinsic clearance. For the high extraction drugs propranolol and lignocaine, no such changes in clearance were seen as a result of inflammation which is in contrast to rats treated with proadifen. It is not clear how far the changes in volume of distribution seen for propranolol in the isolated set-up affect the in-vivo disposition.

From a study in liver 9000 g supernatant fraction, we know that the enzymatic breakdown of antipyrine, lignocaine and propranolol is decreased after turpentine treatment (Chindavijak et al 1987). However, in the isolated perfused liver, flow is constant and therefore the decrease in intrinsic clearance will only affect systemic clearance of the low extraction drugs such as antipyrine, as found in this study.

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