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# In-vitro biotransformation of antipyrine, lignocaine and propranolol in the liver of rats with turpentine-induced inflammation

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In rats with inflammation induced by turpentine injection, changes in drug disposition occur in-vivo and in the perfused isolated liver. Therefore the biotransformation of a low extraction drug, antipyrine, and of two high extraction drugs, lignocaine and propranolol, has been evaluated in the 9000*g* supernatant fraction of the liver of turpentine-treated rats. Aminopyrine *N*-demethylase activity and cytochrome P450 content were also measured. Turpentine treatment significantly reduced the in-vitro breakdown of the three drugs; aminopyrine *N*-demethylase activity and cytochrome P450 content were also decreased. Similar results were found in the proadifen-treated rats, except that in those, the cytochrome P450 content was slightly increased. The changes in drug disposition seen after turpentine-induced inflammation, could therefore be due in part to a change in hepatic enzymatic activity.

In man, rats and dogs with inflammation, plasma concentrations of propranolol and other drugs that are mainly bound to $\alpha_1$-acid glycoprotein ($\alpha_1$-AGP), are increased (Schneider & Bishop 1982). This could be due to the increased serum binding of the drugs as a result of increased $\alpha_1$-AGP concentrations. However, a decrease of metabolism or hepatic blood flow could also be involved. In rats with adjuvant-induced arthritis, a reduction of hepatic microsomal enzymatic activity and of cytochrome P450 content were found (Whitehouse & Beck 1973).

We have previously studied the increase in propranolol plasma concentrations in rats with turpentine-induced inflammation (Belpaire et al 1986). In their perfused livers, the clearance of propranolol and lignocaine, two highly extracted drugs, was not significantly affected, whereas that of antipyrine, a low extraction drug, was reduced (Chindavijak et al 1987). To evaluate more precisely the hepatic enzymatic activity of rats with turpentine-induced inflammation, we investigated the in-vitro metabolism of antipyrine, lignocaine and propranolol using the liver 9000*g* supernatant fraction. Cytochrome P450 content and aminopyrine *N*-demethylase activity were also measured. For comparative purposes, the influence of proadifen (SKF 525A), an inhibitor of drug metabolizing enzymes, was studied.

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## MATERIALS AND METHODS

### *Chemicals*

Propranolol HCl (ICI, UK), lignocaine HCl (Astra Nobelpharma, Belgium) and proadifen (SKF 525A; SKF, USA) were gifts. *N*-methyl [$^{14}$C]antipyrine (Amersham, Belgium; specific activity: 50 mCi mmol$^{-1}$), NADP glucose 6-phosphate (Sigma, UK) and turpentine oil (Bossuyt, Belgium) were purchased.

### *Animals*

Male Wistar rats, 320–410 g, were purchased from the Rega Institute (Louvain, Belgium). Inflammation was induced in eight rats by two injections of 0·5 mL turpentine oil intramuscularly in each hind limb (48 and 24 h before death). Eight other rats received a single intraperitoneal injection of proadifen, 100 mg kg$^{-1}$, 1 h before death. Eight control rats were not pretreated. The animals were allowed free access to food and water until killed.

### *Preparation of 9000g supernatant fraction*

Rats were decapitated and exsanguinated. Livers were rapidly removed, rinsed, weighed and homogenized in a Potter Elvehjem Homogenizer with a Teflon pestle after addition of ice-cold 0·01 M potassium phosphate buffer containing 1·15% potassium chloride, pH 7·4 (4 mL g$^{-1}$ liver). The homogenates were centrifuged at 9000*g* for 20 min at 4 °C, the

supernatant fractions were separated and kept at −20 °C until analysis. One mL of 9000*g* supernatant fraction corresponded to 200 mg liver.

*Antipyrine, lignocaine and propranolol metabolism*
For the three drugs, the metabolism was studied in a 1 mL incubation mixture containing 0·5 mL 9000*g* supernatant fraction, 1 μmol NADP, 5 μmol G6P and 5 μmol $MgCl_2$ in buffer (0·1 M potassium phosphate-1·15% potassium chloride, pH 7·4 for antipyrine and 0·1 M Tris HCl, pH 7·4 for lignocaine and propranolol). This mixture was preincubated for 5 min at 37 °C, after which the substrate was added and incubation was done under air in a shaking water bath at 37 °C. For the three drugs, linearity of the enzymatic activity with time and enzyme concentration was ascertained. In blank determinations, the cofactors were omitted and replaced by an equal volume of buffer. The enzymatic activity was expressed as amount of substrate disappearing $g^{-1}$ liver $min^{-1}$.

For antipyrine the procedure of McManus & Ilett (1979) was followed. Antipyrine, 10 μmol (containing $2{\cdot}2 \times 10^5$ d $min^{-1}$ *N*-methyl [$^{14}C$]antipyrine), and semicarbazide, 10 μmol, to trap the formaldehyde formed, were added to the mixture and incubated for 60 min; the reaction was stopped by 1 mL NaOH 0·5 M. Unchanged antipyrine was extracted with chloroform. The radioactivity remaining in the aqueous phase, corresponding to the metabolites, was counted and gave the extent of antipyrine metabolism.

For lignocaine the procedure of Kawai et al (1985) was followed. Lignocaine, 15 nmol, was added to the incubation mixture and incubated for 10 min; the reaction was stopped by the addition of 1 mL NaOH 1 M. Unchanged lignocaine was determined in 2 mL assay mixture by HPLC, using trimecaine as internal standard, 5 ODS column (Chrompack) as stationary phase, a mixture of 0·04 M sodium phosphate buffer (pH 3)–acetonitrile (82·5 : 17·5) as mobile phase, and UV detection at 215 nm. The between-run variation coefficient of the assay method was 2·6% and the analytical recovery was 95·0% at a concentration of 2 μg lignocaine per mL assay mixture (n = 12).

For propranolol the procedure of Ishida et al (1985) was followed. Propranolol, 40 nmol, was added to the incubation mixture and incubated for 30 min; the reaction was stopped by the addition of 1 mL NaOH 1 M. Unchanged propranolol was determined in 2 mL assay mixture by spectrofluorimetry (Shand et al 1970). The between-run variation coefficient of the assay method was 2·5% and the analytical recovery was 100·5% at a concentration of 20 nmol propranolol $mL^{-1}$ assay mixture (n = 7).

*Aminopyrine* N-*demethylase*
The enzymatic activity was determined according to Mazel (1971) with some modifications. A 2 mL incubation mixture contained 0·5 mL 9000*g* supernatant fractions, 1 μmol NADP, 10 μmol G6P, 10 μmol $MgCl_2$ and 25 μmol semicarbazide in 0·1 M potassium phosphate-1·15% potassium chloride buffer (pH 7·4). After 5 min preincubation, the reaction was initiated by addition of aminopyrine, 10 μmol, and was carried out in air in a shaking water bath at 37 °C for 40 min. The reaction was stopped by addition of trichloroacetic acid, 1 mL, 10%, and the formaldehyde formed was determined by spectrophotometry as described by Nash (1953) and expressed as amount formed $g^{-1}$ liver $min^{-1}$.

*Cytochrome P450*
Cytochrome P450 content in the 9000*g* supernatant fraction was measured by the method of Omura & Sato (1964) and calculated from the CO difference spectra of dithionite-reduced samples, assuming a value of 91 $cm^{-1}$ $mM^{-1}$ for the molar extinction increment between 450 and 490 nm.

*Statistical analysis*
The data were expressed as mean ± s.e.m. and analysed using the Mann-Whitney U-test. $P < 0{\cdot}05$ was considered as the level of significance.

## RESULTS

Injection of turpentine oil, 0·5 mL i.m., twice, led to marked swelling of the hindlimbs. There were no differences in body weight, liver weight or gross liver appearance between treated and control rats.

The influence of administration of turpentine oil on the enzymatic activities studied and on the cytochrome P450 content, is shown in Fig. 1. The disappearance rate of antipyrine, lignocaine and propranolol was decreased to 36, 42 and 74% of the control values, respectively ($P < 0{\cdot}001$ for antipyrine and lignocaine and $P < 0{\cdot}01$ for propranolol). Aminopyrine *N*-demethylase activity and cytochrome P450 content decreased respectively to 36 and 65% of the control values ($P < 0{\cdot}001$).

The influence of proadifen administration is also shown in Fig. 1. In the proadifen-treated group, the metabolism of antipyrine, lignocaine and propranolol decreased to 25, 20 and 55% of the control values respectively ($P < 0{\cdot}001$) and the aminopyrine *N*-demethylase activity was 53% of the control value

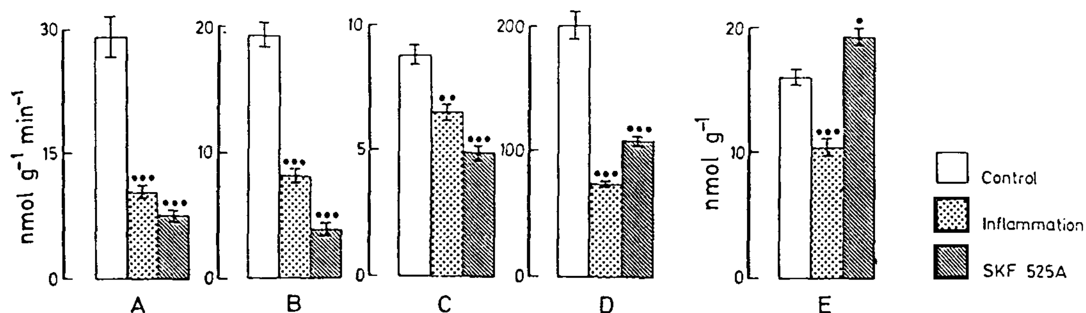


FIG. 1. Disappearance rate of (A) antipyrine, (B) lignocaine and (C) propranolol, expressed as amount of drug disappearing $g^{-1}$ liver $min^{-1}$, (D) aminopyrine *N*-demethylase activity expressed as formaldehyde formed and (E) cytochrome P450 content in the 9000*g* supernatant fraction of rat liver. Influence of administration of turpentine (0·5 mL, 48 and 24 h before death) or proadifen (100 mg $kg^{-1}$ 1 h before death). Data are expressed as means ± s.e.m. (n = 8 for each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: different from controls (Mann-Whitney U-test). Key: blank, control; stippled, turpentine administration; hatched, proadifen administration.

($P < 0.001$). Cytochrome P450 content was slightly but significantly ($P < 0.05$) increased by proadifen.

## DISCUSSION

The serum concentrations of drugs that are bound to $\alpha_1$-AGP are increased in patients with inflammatory diseases and in animals with experimental inflammation (Schneider & Bishop 1982). This was found for the β-adrenoceptor drug propranolol in rats with turpentine-induced inflammation (Belpaire et al 1986) and a decrease in the hepatic biotransformation of propranolol could be involved. We previously studied this problem in the perfused isolated liver of such rats; we have now compared the in-vitro biotransformation of drugs in rats with turpentine-induced inflammation with that in control rats.

Several in-vitro studies reported a reduced capacity of the liver of rats with adjuvant-induced arthritis to metabolize drugs such as aminopyrine and aniline which are demethylated and hydroxylated, respectively; in those studies cytochrome P450 was found to be lower (Morton & Chatfield 1970; Beck & Whitehouse 1973; Cawthorne et al 1976; Mathur et al 1978; Ishizuki et al 1983). For propranolol, the in-vitro metabolism was not changed (Barber et al 1982).

Previous studies in the rat have shown that turpentine causes an increase in $\alpha_1$-AGP concentrations (Jamieson et al 1972), an increase in serum binding and total concentration of propranolol (Belpaire et al 1986). That inflammation was present in the rats studied here can be inferred from the increased serum binding of oxprenolol in these rats (unpublished results). The use of turpentine has the advantage of inducing inflammation much more rapidly than mycobacterial inoculation.

Our in-vitro biotransformation results in turpentine-treated rats are largely in agreement with those of most studies in rats with adjuvant-induced arthritis. Indeed, in our turpentine-treated rats there was a lower cytochrome P450 content and a lower aminopyrine *N*-demethylase activity, and the disappearance rate of the three drugs studied was decreased. In contrast, Barber et al (1982) using rats with adjuvant-induced arthritis, found only a slight, non-significant decrease in propranolol metabolism. A possible explanation for this discrepancy could be the method of inducing inflammation. This could also be true for the finding of Cawthorne et al (1976) that, in mycoplasma-induced arthritic rats, there was a decrease in aminopyrine *N*-demethylase activity without decrease in cytochrome P450 content, while we found a decrease in both.

Proadifen treatment slowed the disappearance rate of the three drugs studied and lowered the aminopyrine *N*-demethylase activity as expected, while there was a slight increase in cytochrome P450. The effect of proadifen on the disappearance rate of the three drugs was larger than that of turpentine, whereas the aminopyrine *N*-demethylase activity was more influenced by turpentine than by proadifen. We have no explanation for that, nor do we know why cytochrome P450 content was slightly increased in the proadifen-treated rats. As the intramuscular injection of turpentine oil causes only local lesions and turpentine itself does not reach the liver (Kaplan & Jamieson 1977), the changes in metabolic activity probably reflect the influence of inflammation, and not a direct hepatotoxic effect.

After completion of this manuscript, a paper by Kobusch et al (1986) appeared, showing that when turpentine was administered subcutaneously, sero-

mucoids (i.e. the serum protein fraction consisting mainly of $\alpha_1$-AGP) increased, the rate of *N*-demethylation, *O*-dealkylation and hydroxylation, and the hepatic cytochrome P450 content decreased. When, on the other hand, turpentine was administered orally, seromucoids remained unchanged, but the enzymatic activities and the cytochrome P450 content increased, probably due to enzyme induction.

Our results show that in turpentine-induced inflammation, there is a decreased in-vitro metabolism of both high and low extraction drugs. Changes in intrinsic clearance will, however, have more impact on systemic clearance of low extraction drugs such as antipyrine, than on that of high extraction drugs such as propranolol and lignocaine; indeed, for high extraction drugs, systemic clearance is mainly determined by the liver blood flow. In our liver perfusion experiments, where flow is constant, there was in turpentine-treated rats a clearcut decrease in hepatic clearance for antipyrine, but not for lignocaine or propranolol (Chindavijak et al 1987). The influence of proadifen in our in-vitro experiments is more pronounced than that of turpentine; this probably explains why in the perfused rat liver, a decrease in hepatic clearance is found even for the high extraction drugs.

As the area under the plasma concentration time curve (AUC) after oral administration of high extraction drugs is inversely related to the free fraction and intrinsic clearance (Wilkinson & Shand 1975), it can therefore be concluded that the increase in the AUC of the high extraction drug, propranolol, observed after oral administration in man and in rats with inflammation (Schneider & Bishop 1982), can be explained by both a decreased free fraction of the drug and a decreased intrinsic clearance.

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