The Impact of Liver Cirrhosis and Cholelithiasis on Phase I and II Reactions Using Antipyrine and Trimethadione as a Model Substrate

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Five male patients with extrahepatic cholelithiasis and ten male patients with liver cirrhosis were administered simultaneously 4 mg/kg trimethadione (TMO) and 500 mg/man antipyrine (AP). Changes in phase I (AP and TMO) and phase II (AP) reactions of the model substrates in liver disease were examined. The patients with liver cirrhosis exhibited prolonged half-life (t1/2) and decreased metabolic clearance (CL) of AP when compared with cholelithiasis patients, while no change was found in the apparent volume of distribution (Vd). The serum dimethadione (DMO)/TMO ratio at 4 hr after oral administration of TMO was apparently decreased in the cirrhotic patients. The cirrhotic patients also exhibited significant decreases in urinary excretion and clearance for production of three major AP metabolites (4-hydroxyantipyrine, 3-hydroxymethyl-3-norantipyrine, and 3-hydroxymethylantipyrine), while no significant differences between the cirrhotic patients and cholelithiasis patients were noted in percentages of either total or individual glucuronidation of these metabolites. These findings suggest that AP and TMO metabolisms (phase I) are severely impaired in cirrhotic patients, whereas glucuronidation of AP is unaffected (phase II).

Key words : antipyrine, trimethadione, conjugation, pharmacokinetics, liver cirrhosis

Introduction

Drug metabolism reactions can generally be divided into two phases: phase I (functionalization reaction) and phase II (conjugation reaction). Both phase I and phase II enzymes are influenced by liver damage.

Antipyrine (AP) is currently used as a marker of human liver function with respect to drug metabolism1,2, because it is likely that different isoenzymes of the mixed-function oxygenase system are involved in the formation of the different metabolites of AP in man3. There are three major AP metabolites, 4-hydroxyantipyrine (OHA), 3-hydroxymethyl-3-norantipyrine (NORA) and 3-hydroxymethylantipyrine (HMA), which are separately conjugated and excreted in the urine. Only few reports have concerned simultaneous assessment of the influence of liver disorders on the phase I and phase II reactions of AP metabolism3, indicating that liver disease may affect the activity of different drug-metabolizing enzymes to a different extent.

We have been using trimethadione (TMO), as
well as AP, as a marker of the drug-oxidizing capacity of the human liver by measuring the ratio of the blood concentration of TMO to its only metabolite, dimethadione (DMO), in a single sample obtained after TMO administration. TMO metabolism is only phase I reaction.

It has been reported that in patients with extrahepatic cholelithiasis (cholelithiasis) the elimination half-life (t₁/₂) of AP is prolonged when compared with control subjects, while serum clearance (CL) remains unchanged. In our previous study of TMO metabolism we also reported that there was no difference in the serum DMO/TMO ratios of the two groups at 4 hr after oral TMO administration. So, we used patients with cholelithiasis as a control in this study.

The objective of the present study was to elucidate any changes in the phase I (AP and TMO) and phase II (AP) reactions of AP and TMO metabolism, mainly AP, in patients with cholelithiasis and those with liver cirrhosis after coadministration of these two probes.

Materials and Methods

Materials

TMO (Mino-Aleviatin®) and AP were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. OHA, NORA and HMA were obtained from Chikoh Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

Human experiments

Five male patients with cholelithiasis (aged 36–74 yr; body weight 39–57.5 kg) and ten male patients with cirrhosis (aged 47–71 yr; body weight 43.3–72 kg), who had given their informed consent, participated in the study. They were all non-smokers. Diagnosis was established by clinical and biochemical tests in all patients and confirmed histologically in patients with cirrhosis.

The mean clinical and liver characteristics are listed in Tab. 1. Most of the patients were treated with one or more (up to 5) of the following drugs: glycyrrhizin, glutathione, Sho-saiko-to (Chinese herbal medicine), spironolactone, magnesium hydroxide, synthetic aluminium salicate, ursodeoxycholic acid, ranitidine, famotidine and vitamins.

All patients were administered TMO 4 mg/kg and AP 500 mg/man in aqueous solution, at 06:30 after overnight fast. Blood was sampled before drug administration and after 1, 2, 4, 8, 12 and 24 hr. Urine samples collected over a 48-hr period. Serum and urine samples were frozen at −80°C until analysed.

Assay of TMO and AP in blood

TMO and AP in blood were assayed according to Tanaka and Misawa and Teunissen et al., respectively.

Assay of AP and its metabolites in urine

They were measured according to Teunissen et al. Total concentrations of free plus conjugated HMA were measured after hydrolysis of the urine samples with β-glucuronidase-sulphatase (Limpet Acetone Powder type I, Sigma Chemical Co., St Louis, U.S.A.), during 3 hr at 37°C. Concentrations of free HMA, NORA and OHA were measured in urine without enzymic hydrolysis.

Data analysis

The slope (β) of the terminal log-linear phase of the serum AP concentration was determined by linear regression analysis and were used to calculate the serum elimination t₁/₂ of AP. The apparent volume of distribution (Vd) was calculated by

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Mean±SD, * P<0.05, ** P<0.01
dividing the given dose by the extrapolated value of AP concentration at time zero. Total AP serum clearance (CL) was calculated according to:

$$CL = \frac{\ln 2 \cdot V_d}{t_{1/2}}$$

The rates of formation of AP metabolites were expressed as CLm and calculated according to Danhof et al. Results are presented as mean (SD), and statistical analysis employed the paired Student's t-test.

Results
Pharmacokinetic parameters in patients with liver disease who had received TMO and AP concomitantly are shown in Tab. 2A. AP t1/2 was about 4 times longer in patients with cirrhosis than in patients with cholelithiasis, while no difference in mean Vd was observed. CL was reduced by about 50% in the patients with cirrhosis when compared with cholelithiatsis. The serum DMO/TMO ratios at 4 hr after oral administration of TMO in cirrhotic patients were significantly decreased by about 60% compared to the patients with cholelithiasis. Cumulative urinary excretion, clearance for production, and percent glucuronidation of AP and its major metabolites after coadministration of AP and TMO in patients with liver disease are shown in Tab. 2B. The excretion of the three main metabolites of AP in cirrhotic patients was reduced compared to the patients with cholelithiasis, whereas the excretion of unchanged AP was increased. Clearance for production of the major metabolites of AP was substantially impaired in patients with cirrhosis. There was no significant difference between the two groups in mean values for total percentages of glucuronidation of AP metabolites (91.7±9.4 vs 85.4±4.5).

Discussion
The results of the present study indicate a reduced drug-metabolizing capacity of the liver in cirrhotic patients as demonstrated by prolonged t1/2 and decreased CL of AP and significantly decreased serum DMO/TMO ratios. These results are in agreement with our previous findings and reports by other workers. The recovery rate of AP metabolites (% of dose) in urine and CLm of these metabolites were significantly reduced in cirrhotic patients when compared with those having...
cholelithiasis, the reduction being in the following rank order: NORA>OHA>HMA. Thus, N-demethylation reactions may be more readily affected than hydroxylations. The oxidation of these metabolites is known to be catalyzed by different cytochrome P-450 (P-450) isozymes. The above results, therefore, indicate selective inhibition of individual P-450 isozymes involved in AP metabolism. In other words, liver disease affects oxidative drug-metabolizing enzyme activity differently. These findings are consistent with the results of studies by Teunissen et al. in patients with alcoholic cirrhosis.

We have already reported that CL and t1/2 of TMO and AP did not differ after each drug alone or in combination. In addition TMO metabolism and CLm of NORA are mediated by closely related form(s) of the P-450 system in healthy subjects after coadministration of TMO and AP. It was showed that enzymes involved in TMO metabolism as well as the formation of NORA in this study are more strongly inhibited in patients with liver cirrhosis. More importantly, the degree of impaired N-demethylation may have implications with regard to liver disease.

The percentages of HMA, NORA and OHA glucuronidated were determined by analysis of urine samples with and without enzymic hydrolysis. Without hydrolysis hardly any OHA and NORA were detected in the urine of the two groups. The changes reported in the conjugation reaction, which differ depending on the substrate in liver disease, may be attributable to interindividual differences in glucuronidation of AP metabolites in urine revealed no significant differences between patients with cholelithiasis and those with liver cirrhosis. The above fact indicates impaired AP oxidizing capacity and intact conjugation capacity in cirrhotic patients. Furthermore, no significant difference was found between the two groups in percent glucuronidation of each metabolite in urine. Similar findings were reported by Teunissen et al.

These results indicate that the phase I reactions of AP and TMO are severely impaired in patients with liver cirrhosis, whereas the phase II reactions of AP metabolites are scarcely affected.

References


