Drug metabolism in diabetic subjects with fatty livers

H. I. PIRTIIAHO¹, P. I. SALMELA¹, E. A. SOTANIEMI¹, R. O. PELKONEN², U. PITKÄNEN³ & P. V. LUOMA¹

Clinical Research Unit, Department of Internal Medicine¹, and Departments of Pharmacology² and Radiation Therapy³, University of Oulu, Oulu, Finland

1 The effect of fatty degeneration of liver parenchyma on drug metabolism was investigated in 21 obese non-insulin-dependent diabetic subjects by measuring plasma antipyrine kinetics, hepatic cytochrome P-450, liver size and the extent of fatty infiltration.

2 The hepatic drug metabolising capacity, as measured by total antipyrine clearance and the estimated total amount of cytochrome P-450, was at the same level as in non-diabetic control subjects with normal livers.

3 Relative antipyrine clearance (per unit weight of liver) and cytochrome P-450 concentrations were significantly lower in the diabetics than in controls.

4 The extent of fatty infiltration correlated poorly with the indices of drug metabolism.

5 In non-insulin-dependent diabetics, slight to moderate hepatic fatty infiltration, without more serious structural distortion interfering with hepatic blood flow or hepatocellular function, seems to have only a minor influence on drug metabolism.

Keywords drug metabolism diabetes fatty liver

Introduction

Fatty liver is a common finding in middle aged overweight non-insulin-dependent diabetic subjects (NIDDs) (Creutzfeldt *et al.*, 1971). It is often asymptomatic, associated with hepatomegaly, and causes only minor disturbances in liver function tests (Salmela *et al.*, 1984), apparently having no great effect on the prognosis of diabetes (Thaler, 1975). For certain subjects, however, fatty liver might be harmful for other reasons. Such patients also have other diseases which are treated with multiple drug regimens. Since the liver is responsible for the elimination of many drugs, and hepatic drug metabolizing enzyme activities may be lowered in diabetic fatty liver (Salmela *et al.*, 1980), harmful consequences of drug therapy are possible in such subjects.

Only a few studies of the effects of diabetes associated fatty liver on hepatic drug elimination are available. Our recent results have shown

that lowering of hepatic drug-metabolizing capacity was most notable when assessed by *in vitro* assays, whereas the antipyrine elimination rate *in vivo* was generally within a low normal range or only slightly reduced (Salmela *et al.*, 1980). To evaluate the cause of these inconsistent findings, the present study was planned to also take account of the extent of fatty infiltration and the size of the liver. Antipyrine kinetics were used as an *in vivo* index and the concentration of cytochrome P-450 as an *in vitro* index of drug metabolism.

Methods

Patients

Twenty-one NIDDs were investigated. There were 18 women and three men, of age range

43–76 (mean $\pm$ s.d.: 58.7 ± 9.2) years. They were overweight, the body mass index (BMI) ranging from 28 to 36 (mean 32.0 ± 6.1 kg/m²). All of them were referred to the Clinical Research Unit for diagnostic liver biopsy due to previously noted altered liver function test results and/or overt hepatomegaly. The duration of diabetes was from 3 months to 18 years (mean 4.8 years). None had uncompensated heart failure during the study. Eight patients had hypertension treated with diuretics and/or clonidine. All patients had normal renal function as judged by the measurement of serum creatinine levels. The patients fulfilled the following criteria: in liver biopsy specimens 10% or more of the hepatocytes were fat laden; there were only slight non-specific inflammatory changes, mostly in the periportal area, and a less than 10% increase in fibrous trabeculae. Patients with overt hepatitis or cirrhosis were excluded from the study. Subjects with a history of alcohol abuse or therapy with drugs known to induce or impair the hepatic drug metabolising system were also excluded.

Eleven non-diabetic subjects, age range 23–56 (mean 36.1 ± 12.8 s.d.) years with normal or near normal liver histology were used as controls for the drug metabolism studies. They were selected from our material of over 500 consecutive diagnostic liver biopsies. The exclusion criteria were the same as for the diabetic subjects.

Informed consent from the patients was obtained before the antipyrine test. Tissue surplus from the histological examinations was approved to be used for drug metabolism studies by the local ethical committee.

Protocol

All subjects were studied in the Clinical Research Unit. Blood samples for the liver function tests were taken after an overnight fast. Three to four hours later, a percutaneous liver biopsy was performed with a Thru-Cut needle. All biopsy samples were used for histological study and part of each specimen was utilised for the determination of cytochrome P-450 content (P-450). The next morning, after an overnight fast, antipyrine (20 mg/kg dissolved in 100 mg fruit juice) was administered. Venous blood samples were drawn at zero time and 1, 3, 6, 12, 24 and 48 h after antipyrine ingestion.

Methodology

The extent of fatty degeneration was quantified by a morphometric method as previously described (Arranto & Sotaniemi, 1981). In short,

randomly selected slides of biopsy sections were projected upon a point count test screen, the points falling over the fat vacuoles and non-fatty parenchyma were counted, and the volume density of fat laden hepatocytes was expressed as percentages of liver tissue. Liver volume was measured from ^{99m}Tc-sulphur colloid scans performed as a part of routine diagnostic studies using the method of Rollo & DeLand (1968) as described previously (Pirttiaho & Pitkänen, 1977). Assuming the specific gravity to be unity, the volume was equal to liver mass. Relative liver weight was calculated by dividing total liver weight (g) by body weight (kg). Cytochrome P-450 concentrations in the whole homogenates of biopsy samples were measured by the method of Greim *et al.* (1970) as used earlier (Salmela *et al.*, 1980). The total amount of hepatic cytochrome P-450 was counted by multiplying its concentration in biopsy (nmol/g) by estimated liver weight (g).

Plasma antipyrine concentrations were analysed by a gas-liquid chromatography method with phenacetin as an internal standard (Prescott *et al.*, 1973). The antipyrine clearance rate (CR) was calculated from the equation $CR = D/AUC$ where D is the antipyrine dose and AUC the area under the plasma concentration curve. The AUC was calculated by the trapezoidal rule and the area to infinite time added by integration of C_t/k where C_t is the last antipyrine concentration and k the elimination rate constant calculated from the equation $k = \ln 2/t_{1/2, z}$. The antipyrine half-life ($t_{1/2, z}$) was obtained from the linear part of the time-concentration curve on a semilog graph. The apparent volume of distribution (V) was calculated from the equation $V = D/(AUC \times k)$. The relative antipyrine clearance rate was calculated by dividing the absolute clearance (ml/min) by liver weight (g).

Serum concentrations of albumin and the activities of alanine amino transferase and alkaline phosphatase were measured by standard autoanalyser techniques. Plasma coagulation factors were assessed by using a commercial test (Thrombotest®, Medica Co., Finland).

Statistical analysis of the data was performed by Student's *t*-test and regression analysis.

Results

Relevant clinical data and the results of the drug metabolism studies are shown in Figure 1 and Tables 1 and 2. The diabetic subjects with fatty liver eliminated antipyrine with a slightly, though not significantly slower rate than the controls. However, the relative antipyrine

Figure 1 Liver size and indices of drug metabolism in control subjects (○) and in diabetics with fatty liver (●).

Table 1 Clinical data in diabetics with fatty liver and in control subjects.

Subjects	Age (years)	BMI (kg/m ²)	Fatty infiltration (%)	Liver function test results			
				Alb (g/l)	TT (%)	ALAT (u/l)	A-P (u/l)
Diabetics (n = 21)							
Mean	58.7*	32.0*	27.7	43.6	98.2	58.8	217.0
s.d.	9.2	6.1	13.9	3.4	6.8	41.7	84.8
Controls (n = 11)							
Mean	36.1	21.3	—	41.9	85.3	35.9	206.9
s.d.	12.6	2.5	—	4.2	18.5	24.2	126.8

P value for differences between means: * < 0.001.

Reference values for liver function tests: Alb (serum albumin concentration) 40–54 g/l, TT (Thrombotest) 60–100%, ALAT (serum alanine aminotransferase activity) less than 40 U/l, A-P (serum alkaline phosphatase activity) 60–250 U/l.

Table 2 Liver size and indices of drug metabolism in diabetics with fatty liver and in control subjects.

Subjects	Liver size		Antipyrine clearance		V (l/kg)	Cytochrome P-450	
	Absolute	Relative	Total	Relative		Content	Total
	(g)	(g/kg)	(ml/min)	$\frac{\text{ml/min}}{1000 \text{ g}}$		(nmol/g)	(μmol)
<i>Diabetics</i>							
Mean	2734*	35.2*	32.9	12.4*	0.40	5.4*	14.2
s.d.	701	8.6	13.9	4.9	0.08	3.0	7.7
<i>Controls</i>							
Mean	1481	25.8	37.9	25.7	0.43	11.8	17.3
s.d.	264	2.4	13.0	7.2	0.10	1.6	3.0

P value for differences between means: * < 0.001.

V = apparent volume of distribution.

Figure 1 Liver size and indices of drug metabolism in control subjects (○) and in diabetics with fatty liver (●).

clearance rate, as counted per unit weight of the liver, was significantly decreased in the diabetics. The cytochrome P-450 contents in their biopsy specimens were consistently significantly lowered, whereas the estimated total amount of cytochrome P-450, reflecting the hepatic drug metabolizing capacity as a whole, was at the control level. The indices of drug metabolism showed only a poor relationship with the extent of histological change. The closest correlation was seen between the concentration of cytochrome P-450 and the number of fat-laden hepatocytes ($r = -0.41$, $0.05 < P < 0.1$).

Most diabetic subjects showed overt hepatomegaly, the liver size correlating with the extent of fatty infiltration ($r = 0.63$, $P < 0.01$). Their liver size also correlated with body weight ($r = 0.49$, $P < 0.05$), although the relationship was weaker than in the controls ($r = 0.89$, $P < 0.001$). Hepatomegaly was not due to the greater body weight of the diabetics since their relative liver weight (g/kg body weight) was also significantly increased compared with the controls (Table 1).

Conventional tests of liver function showed minor changes both in diabetic subjects and in controls. The values of serum albumin and Thrombotest, known to be impaired in severe liver damage only, were within normal limits in both groups.

Discussion

Many factors can affect hepatic drug disposition in diabetes-associated fatty liver. We have previously shown that the hepatic microsomal enzyme activity in NIDDs is often impaired and that the extent of impairment is related to the severity of hepatic parenchymal changes (Salmela *et al.*, 1980). However, the results of *in vivo* and *in vitro* studies have been divergent, possibly due to the fact that *in vitro* studies fail to take the total hepatic capacity for drug

metabolism into account. The results of the present study confirm our previous findings and show that NIDDs with fatty liver have lowered hepatic drug metabolizing enzyme capacity as assessed per unit weight of liver tissue. Thus, the relative antipyrine clearance rate (ml/min per 1000 g of liver tissue) was significantly slower and the hepatic cytochrome P-450 concentration lower than in non-diabetic controls. On the other hand, both the total antipyrine clearance rate and the total amount of hepatic cytochrome P-450 were at control levels. These findings suggest that a moderate fatty infiltration has only a minor influence on hepatic drug metabolism as a whole but its main consequence is a 'dilution' effect appearing in those parameters measured per unit weight or volume of the liver. These observations in diabetics are comparable with earlier results indicating fairly normal drug metabolism in alcoholics with fatty liver (Sotaniemi *et al.*, 1977). Consistent with earlier studies (Homeida *et al.*, 1979), the results also show the superiority of relative antipyrine clearance over absolute clearance in discriminating subjects with normal liver from those with altered liver parenchyma.

Antipyrine is considered to be a suitable marker drug to reflect hepatic oxidative drug metabolism in man (Branch, 1982). However, it is not a model for all drugs (Kadar *et al.*, 1973; Hepner *et al.*, 1977). Thus, fatty liver may have a more extensive influence on the disposition of some other drugs with different metabolic destinies. Moreover, the architectural distortions secondary to severe fatty infiltration and ballooning of the hepatocytes may disturb hepatic blood flow by interfering with the hepatic microcirculation and by shunting blood away from sinusoidal perfusion (Branch & Shand, 1976). Nevertheless, the results of this study suggest that relative antipyrine clearance and hepatic cytochrome P-450 concentrations may be valuable tools in quantifying hepatic parenchymal function in NIDDs.

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