HEPATIC DRUG-OXIDIZING ENZYME SYSTEMS AND URINARY D-GLUCARIC ACID EXCRETION IN PATIENTS WITH CONGESTIVE HEART FAILURE

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- 1 Drug-oxidizing enzyme systems in liver biopsy samples and the urinary excretion of D-glucaric acid were studied in two different groups of patients with cardiac insufficiency.
- 2 In one group of six patients, the activities of drug-metabolizing enzymes had decreased considerably as compared with the control values, but in four liver samples from patients treated with oral hypoglycaemic agents for their diabetes, activities were higher than in control samples from ten patients.
- 3 In the other group of seven patients, the urinary excretion of D-glucaric acid (isolated by ion-exchange chromatography) was 60% lower than in the control group of nine humans, whereas in four patients taking antiepileptic agents excretion rate was higher than control values.
- 4 Because the age distribution was markedly different between cardiac insufficiency and control groups, it is difficult to conclude, if the impairment of drug metabolism was a consequence of the old age or of the disease process. However, drug-oxidizing enzyme systems seem to be inducible also in old age.
- 5 The results support further the opinion that the urinary excretion of D-glucaric acid may be one useful index in assessing an individual's capacity to metabolize foreign compounds especially in the patients with lowered drug metabolizing capacity.

Introduction

It is well known that many intrinsic and extrinsic factors affect the activities of microsomal drug-metabolizing mixed-function oxidases located mainly in the liver. In animal studies it has been shown that many disease processes, e.g. chronic liver disease, starvation, hormone deficiencies, diabetes etc., have a decreasing effect on drug metabolism whereas many chemical agents, including many drugs, pesticides and carcinogens, stimulate drug metabolism (Conney, 1967; Mannering, 1968).

From several *in vivo* studies it can be concluded that overall drug metabolism is impaired in some but not in all patients with liver disease (Mawer, Miller & Turnberg, 1972; Leevy, 1974). The results of *in vitro* studies of Schoene, Fleischmann, Remmer & von Olderhausen (1972) and Auranen (1972) show that the activities of drug-metabolizing enzymes are clearly diminished in patients with severe liver disease.

The induction of hepatic microsomal drug-metabolizing enzymes in man following treatment

with different drugs, is well documented (Conney, 1967; Conney & Burns, 1972).

Cardiac insufficiency may lead to liver injury through liver congestion and the following degenerative changes. In order to elucidate the effects of cardiac insufficiency on drug metabolizing enzymes in man we measured the amounts of some components of the microsomal mixed-function oxidase system and the activities of the respective enzymes toward different test substrates in liver biopsy samples taken during abdominal surgery for cholecystectomy. We also measured, in another group of patients, the urinary excretion of D-glucaric acid, which has been thought to be one kind of indicator reflecting the level of drug-metabolizing capacity in an individual (Aarts, 1965; Conney, 1967; Hunter, Carella, Maxwell, Stewart & Williams, 1971; Notten & Henderson, 1973; Hunter, Burnham, Chasseaud & Down, 1974). Also we report some data on patients taking oral hypoglycaemic and antiepileptic agents in order to elucidate the possible inducibility of

hepatic drug-metabolizing enzymes and the urinary excretion of D-glucaric acid.

Methods

Liver biopsy samples

Biopsies of adult human liver were taken in connection with abdominal operations in the Department of Surgery, University of Oulu, predominantly from patients with uncomplicated cholelithiasis. Patients (nos. 1-10 in Table 1) suffering from cardiac insufficiency (duration of disease from 5 months to 20 years, mean 4.5 years) were in a stable, compensated stage of disease. All received digoxin (from 0.125-0.5 mg/day) and some of them were also receiving coronary vasodilators (3/10), reserpine, dihydralazine, and hydrochlorthiazide (1/10) and glycyllin (1/10). Four patients had maturity-onset diabetes mellitus which had lasted for 8-20 years and was treated by oral hypoglycaemic agents (Table 1) and diet at least the last year before operation. In thorax X-ray, an enlarged heart (6/10), and in electrocardiography, left ventricular hypertrophy (7/10) were usual findings. The samples were forwarded to the pathologist for histological examination and the rest (usually weighing 0.2-1 g) was transferred to the Department of Pharmacology for the determination of drug-metabolizing enzymes. The preparation of the liver samples was started within 30-60 min of biopsy.

Urine samples

Twenty-four hour urine samples were obtained from the Department of Medicine and Department of Neurology, University of Oulu. Five patients (nos. 1-5 in Table 2) suffered from chronic cardiac insufficiency which had lasted for 2-5 years (mean 2.8 years). In two patients (nos. 6 and 7 in Table 2) urine samples were obtained one week after digitalization. Digoxin (0.25-0.5 mg/day) was administered to all patients and some of them received furosemide (6/7) and spironolactone (3/7). In thorax X-ray, all patients had an enlarged heart and in electrocardiography of five patients, left ventricular hypertrophy was evident. Samples of normal human urine (controls nos. 17-20) were collected from the staff of our Department of Pharmacology and controls nos. 21-25 from patients who were under examination without any drugs in the Department of Neurology. Urines were collected without any preservative and examined immediately after collection or otherwise stored at -20°C .

Preparation of the liver samples

The biopsy samples were processed in a cold room at $+4^{\circ}\text{C}$. The samples were rinsed in ice-cold buffer, blotted dry and weighed. They were homogenized in 0.1 M potassium phosphate buffer, pH 7.4, with an all-glass Potter-Elvehjem homogenizer. Homogenates were centrifuged first at 12,000 g for 20 min and normally the supernatant was used for enzymic determinations. The remaining supernatant was centrifuged at 100,000 g for 60 min and the suspended microsomal pellet was used for the determination of cytochrome P-450 and NADPH-cytochrome c reductase.

The various fractions were used within 24 h of the starting of the preparation. During this time, when kept in a cold room in ice, there was no decrease in levels of activities of different microsomal components.

Enzyme assays

The composition of cofactor mixture and the conditions of incubations are given earlier (Pelkonen, Kaltiala, Larmi & Kärki, 1973). The methods used were the following: 3,4-benzpyrene: Kuntzman, Mark, Brand, Jacobson, Levin & Conney (1966); aminopyrine *N*-demethylation: Nash (1953); aniline *p*-hydroxylation: Kato & Gillette (1965); cytochrome P-450: Omura & Sato (1964) and NADPH-cytochrome c reductase: Masters, Williams & Kamin (1967).

Protein was measured by the Biuret method (Layne, 1954).

D-glucaric acid determination

The separation of urinary D-glucaric acid was done by our modification of the chemical method of Ishidate, Matsui & Okada (1965), in which pre-elution volume was increased from 150 to 250 ml and the end of pre-elution and the elution were fractionated into 5.0 ml samples. The ion-exchange resin columns were prepared as in the original method. We used each column only once and then the columns were packed with new resin and converted to borate form. From each urine sample we collected 14 fractions (3 before elution and 11 elution fractions). The first three fractions were taken to show that all interfering compounds were washed off before elution. These 5.0 ml fractions were used for determination of D-glucaric acid as described by Ishidate *et al.* (1965). The small modifications were done to give a better reliability and to exclude the possibility that some normal urinary components could interfere with the determination of D-glucaric acid.

Table 1 Hepatic drug-oxidizing enzyme systems in patients with cardiac insufficiency

Patient	Age (years)	Known inducers administered ¹	Liver histology	Liver function tests ²	Cytochrome P-450 (nmol/mg microsomal protein)	NADPH-cyt. c reductase (nmol mg ⁻¹ microsomal protein min ⁻¹)	3,4-Benz-pyrene hydroxylase (pmol g ⁻¹ liver min ⁻¹)	Aminopyrine N-demethylase (nmol g ⁻¹ liver min ⁻¹)	Aniline hydroxylase (nmol g ⁻¹ liver min ⁻¹)
Six cardiac insufficiency-patients without diabetes									
1 M	61	none	mild Fi ³	normal	0.21	50	85.8	33.8	5.3
2 F	64	none	cholangitis	ASAT,ALAT	0.48	52	72.4	23.0	4.7
3 F	67	none	normal	normal	0.28	101	49.5	21.5	5.6
4 F	46	none	cholestasis	ASAT,ALAT,B ⁴	0.33	64	57.6	28.2	3.8
5 F	66	none	normal	normal	0.25	57	42.2	22.3	4.6
6 F	70	none	mild Fi	normal	0.70	79	44.1	46.7	12.1
Four cardiac insufficiency-patients with diabetes									
7 F	67	Tolbutamide, phenformin	normal	normal	1.32	122	116.3	69.4	19.3
8 F	72	Phenformin, glibenclamide	mild Fi	normal	0.36	74	87.5	20.8	11.0
9 F	75	Tolbutamide, diazepam	normal	normal	0.80	101	316.7	38.0	12.0
10 M	77	Tolbutamide	normal	normal	0.31	60	141.7	80.5	9.2
Three patients with uncomplicated cholelithiasis									
11 F	60	none	normal	normal	0.73	101	72.5	67.4	13.1
12 F	63	none	mild Fi	normal	0.56	79	122.3	44.9	8.1
13 F	64	none	normal	normal	0.51	79	53.5	42.3	7.6

¹ According to the lists published by Conney (1967) and Mannerling (1968)² Tests, where pathological results are mentioned³ Fi = fatty infiltration⁴ B serum bilirubin, ASAT aspartate amino transferase, ALAT alanine amino transferase.

Results

Drug-metabolizing enzymes and D-glucaric acid excretion

Data on individual patients are presented in Tables 1 and 2. It can be seen that the levels of different drug-metabolizing enzymes in the group of cardiac insufficiency are decreased by about 30% as compared with the control group (Table 3). Similarly, the urinary excretion of D-glucaric acid is reduced to about 40% of the control value (Table 4). When patients used some drugs known

to be inducers of drug metabolism (anti-epileptic drugs, oral hypoglycaemic drugs) their ability to metabolize drugs *in vitro* as well as the excretion of D-glucaric acid were higher than in the control groups. (D-glucaric acid excretion was very high in patients treated with diphenylhydantoin only for short time, about two weeks.) In Table 1 three patients with cholelithiasis, over 60 years of age and without drug intake are shown. Their ability to metabolize drugs does not differ much from the control values. It appears in Table 2 and 4 that by our method the individual variations of D-glucaric acid excretion in normal persons are small.

Table 2 Urinary D-glucaric acid excretion in patients with cardiac insufficiency

Patient	Age (years)	Known inducers administered ¹	Liver function tests ²	D-glucaric acid (mg/day)
Seven cardiac insufficiency-patients				
1 F	42	none	normal	3.0
2 F	88	none	normal	3.0
3 M	68	alcohol	B	9.0
4 F	61	none		5.5
5 F	65	none	normal	6.3
6 F	69	none	normal	3.9
7 F	71	none	normal	2.1
Four patients with toxic hepatitis				
8 F	40	none	ASAT,ALAT,AFOS,B ³	1.2
9 F	59	promethazine	ASAT,ALAT,B	6.3
10 F	62	none	ASAT,ALAT,AFOS,B	4.5
11 F	28	none	ASAT,ALAT,AFOS,B	3.0
Five patients taking inducers without hepatic damage				
12 F	17	DPH ⁴		23.8
13 F	23	DPH ⁵		65.9
14 M	43	alcohol	normal	34.8
15 M	34	DPH ⁶		49.2
16 M	18	DPH, phenobarbital, Diazepam, nitrazepam		59.1
Nine control persons				
17 F	28	none		8.6
18 M	35	none		12.7
19 M	50	none		16.2
20 F	22	none		12.2
21 F	18	none		8.2
22 F	31	none		7.6
23 M	30	none		8.4
24 M	21	none		12.9
25 F	16	none		11.9

¹ According to the lists published by Conney (1967) and Mannering (1968)

² Tests, where pathological results are mentioned

³ B serum bilirubin, ASAT aspartate amino transferase, ALAT alanine amino transferase, AFOS alkaline phosphatase

⁴ Diphenylhydantoin for 6 months

⁵ Diphenylhydantoin for 10 days

⁶ Diphenylhydantoin for 2 weeks.

Table 3 Comparison of hepatic drug-oxidizing enzyme systems in various patient groups (mean $\pm$ s.d.)

Patient group	Age (years)	Cytochrome P-450 (nmol/ mg microsomal protein)	NADPH-cyt. c reductase (nmol mg ⁻¹ microsomal protein min ⁻¹)	3,4-Benz- pyrene hydroxylase (pmol g ⁻¹ liver min ⁻¹)	Aminopyrine N-demethylase (nmol g ⁻¹ liver min ⁻¹)	Aniline hydroxylase (nmol g ⁻¹ liver min ⁻¹)
Cardiac insufficiency without diabetes, <i>n</i> = 6 % of control	62.3 $\pm$ 8.6	0.38 $\pm$ 0.18* 59.4	67.2 $\pm$ 19.6 84.8	58.6 $\pm$ 17.3* 60.9	29.3 $\pm$ 9.7 74.2	6.0 $\pm$ 3.1 81.1
Cardiac insufficiency with diabetes, <i>n</i> = 4 % of control	72.8 $\pm$ 4.3	0.69 $\pm$ 0.48 107.8	89.3 $\pm$ 27.7 112.8	165.6 $\pm$ 103.2 172.0	52.2 $\pm$ 27.6 132.2	12.9 $\pm$ 4.5* 174.3
Uncomplicated cholelithiasis, <i>n</i> = 3 % of control	62.3 $\pm$ 2.2	0.60 $\pm$ 0.10 93.8	86.3 $\pm$ 12.7 109.0	82.8 $\pm$ 35.5 86.0	51.5 $\pm$ 13.8 130.4	9.6 $\pm$ 3.0 129.7
The control group, <i>n</i> = 10 Nine F One M	36.7 $\pm$ 11.3	0.64 $\pm$ 0.26	79.2 $\pm$ 15.9	96.3 $\pm$ 44.2	39.5 $\pm$ 18.6	7.4 $\pm$ 1.9

* Significantly different from control, *p* < 0.025.**Table 4** Comparison of urinary D-glucuronic acid excretion (mean $\pm$ s.d.) in various patient groups

Patient group	Age (years)	D-glucuronic acid (mg/day)	% of control
Cardiac insufficiency, <i>n</i> = 7	66.3 $\pm$ 13.7	4.7 $\pm$ 2.4*	42.7
Toxic hepatitis, <i>n</i> = 4	47.3 $\pm$ 16.1	3.7 $\pm$ 2.2*	33.6
Inducer users, <i>n</i> = 5	27.0 $\pm$ 11.2	46.6 $\pm$ 17.3*	423.6
Control group, <i>n</i> = 9	27.9 $\pm$ 10.5	11.0 $\pm$ 2.9	100.0

* Very significantly different from control, *p* < 0.0005.

Liver function tests and liver histology

The histology of the liver samples was studied in all biopsies and liver function tests were checked in all cases where data were available. No apparent relationship between mildly disturbed liver function or minor changes in liver histology and drug-metabolizing capacity or D-glucaric acid excretion was found.

Age and sex characteristics of different groups

As can be seen in Table 3 and 4, the mean ages in different groups differ considerably from each other. The mean age of the control groups were 37 years for biopsy-patients and 28 years for D-glucaric acid-subjects. Thus, the control groups are not 'real' controls for the groups of cardiac insufficiency. This was so because it was difficult to find patients over 60 with only uncomplicated cholelithiasis and without considerable drug usage. The rare cases are presented in Tables 1 and 3. In all groups there were more females than men.

Discussion

It has been known for a long time that cardiac failure has some effects also on the liver function, probably through the generalized congestion and the decreased availability of nutrients to the liver. Richman, Delman & Grob (1961) showed altered liver function in acute and chronic cardiac failure, but the majority of indices of hepatic function returned to normal within one to two weeks following cardiac compensation with the exception of those reflecting biosynthetic processes of the liver, which improved more slowly.

It could be expected that this decreased liver function is reflected also in the drug-metabolizing capacity of the body, because the liver is the main site-of biotransformation reactions of drugs and other foreign compounds (Parke, 1968). Thomson, Melmon, Richardson, Cohn, Steinbrunn, Cudihee & Rowland (1973) showed that in heart failure subjects, both the measured volume of distribution and plasma clearance of intravenously administered lidocaine were significantly reduced. This fact cannot be taken as a firm evidence of the reduced drug metabolizing capacity of the liver, because the data of Thomson *et al.* (1973) and Stenson, Constantino & Harrison (1971) suggest that clearance of lidocaine, which is principally by hepatic metabolism, is limited by hepatic blood flow. Accordingly, a low cardiac index, leading to a diminished hepatic blood flow, results in a low lidocaine clearance.

Nevertheless, in this study, decreased drug oxidation, whether measured by liver microsomal drug-oxidizing enzyme activities or by urinary D-glucaric acid excretion, was observed. All these patients suffered from chronic cardiac insufficiency, which had lasted for several years on average. It must be concluded that although the cardiac failure was compensated in these patients, the disease lasting several years had resulted in a permanent liver damage, as reflected by the decreased levels of drug-oxidation parameters. However, the drug-oxidizing enzymes were inducible, as shown in a group of four patients with cardiac insufficiency and old-age diabetes. Oral hypoglycaemic agents are known to be good inducers of drug oxidation and in fact, the levels of drug-metabolizing enzymes in the above mentioned group were higher than in the 'control' group. Thus one could conclude that drug oxidation in patients of cardiac insufficiency although decreased is nevertheless inducible by known inducing agents.

Since Marsh (1963) identified D-glucaric acid as a normal constituent of human urine, several investigators have shown that the urinary excretion of D-glucaric acid may be useful as an index of induced drug metabolism by e.g. oral contraceptive agents, barbiturates and anti-epileptics (Mowat, 1968; Hunter *et al.*, 1971; Latham, 1974; Sotaniemi, Medzihradsky & Eliasson, 1974). This study demonstrates that the urinary excretion of D-glucaric acid is decreased in patients with cardiac insufficiency and with toxic hepatitis, disease states in which also liver microsomal drug-oxidizing enzymes have decreased (this study and Schoene *et al.*, 1972). This finding supports indirectly an opinion that the urinary excretion of D-glucaric acid may serve as a useful index also for the decreased drug oxidation. The confirmation of this opinion must await the simultaneous, correlative studies of drug-oxidizing enzymes and D-glucaric acid excretion in the same patients. Although the results of Smith and Rawlins (1974), Cunningham & Price Evans (1974) on the urinary excretion of D-glucaric acid and drug half-lives implicate that the urinary excretion of D-glucaric acid does not necessarily reflect the level of drug-oxidizing enzymes in the liver microsomes, at least in 'control' humans, this does not exclude the possibility of finding reasonably good correlations between D-glucaric acid excretion and hepatic drug oxidation in the extreme disease states, with considerably decreased or increased drug metabolizing capacity.

In this study most patients suffering from cardiac insufficiency were women. In rat, the sex difference with respect to drug-oxidizing capacity is a well-known fact (Kato, 1974). In man,

O'Malley, Crooks, Duke & Stevenson (1971) have shown that the half-life of antipyrine was 30% longer in males. On the other hand, Jori, DiSalle & Quadri (1974) did not find any difference in the disappearance of amino-pyrine from plasma between female and male subjects. Some *in vitro* studies on hepatic drug-oxidizing enzymes in man (Gold & Ziegler, 1973; Pelkonen, Kaltiala, Larmi & Kärki, 1973; Pelkonen, Kaltiala, Larmi & Kärki, 1974) indicate no sex difference. Thus, as far as one can infer from data in the literature, the predominance of women in this study will have no significant influence on the results obtained.

Finally, this study again reveals several difficulties inherent in the study of drug metabolism in man. Hospital population where liver biopsies could be obtained from is necessarily very selected with respect to disease, age and sex characteristics, drug intake etc. Many of these

characteristics are unevenly distributed between different groups, e.g. age between the 'control' group and the cardiac insufficiency group. These differences make comparisons between different groups very difficult and uncertain, and it is difficult to say, whether the apparent decrease of drug metabolism and D-glucaric acid excretion in patients with cardiac insufficiency is a consequence of a natural physiological condition, (old age), or a consequence of a disease process, (cardiac insufficiency), or a consequence of the use of different drugs (e.g. digitalis preparations etc). As long as these different factors remain unexplored, one can only hold that the concept of the decreased drug oxidation in cardiac insufficiency is merely a useful working hypothesis.

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