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Quantitative Liver Function in the Elderly Assessed by Galactose Elimination Capacity, Aminopyrine Demethylation and Caffeine Clearance

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Summary

Hepatic function was assessed in 13 healthy elderly subjects, 71–88 years of age, with three quantitative tests of liver function. The galactose elimination capacity was significantly ($P < 0.05$) lower in the elderly (6.08 ± 1.30 $mg \cdot min^{-1} \cdot kg^{-1}$, mean ± SD) than in a group of 70 subjects under 40 (7.48 ± 0.94 $mg \cdot min^{-1} \cdot kg^{-1}$) and 11 subjects between the age of 40 and 70 (7.08 ± 0.68 $mg \cdot min^{-1} \cdot kg^{-1}$). The demethylation of aminopyrine as assessed by the aminopyrine breath test, and the systemic clearance of caffeine, two measures of microsomal function, demonstrated a comparable decrease but showed much more interindividual variation. Caffeine clearance decreased from 1.49 ± 0.44 $ml \cdot min^{-1} \cdot kg^{-1}$ in young adults to 0.97 ± 0.39 $ml \cdot min^{-1} \cdot kg^{-1}$ ($P < 0.01$) in the elderly, and the demethylation of aminopyrine decreased from 0.79 ± 0.15 to 0.62 ± 0.20% $dose \cdot kg \cdot mmol^{-1}$ ($P < 0.05$). Our data indicate that aging is associated with a loss of the functioning mass of hepatocytes. The decrease in drug metabolism parallels the loss of functional mass but shows more interindividual variation probably reflecting the many genetic and environmental factors influencing these tests of microsomal function.

Introduction

The elderly constitute a growing portion of our society. Because of age-related pathological processes this age group requires more medication than younger subjects. The more frequent use of drugs may in part explain the high incidence of adverse side-effects in older people [1]. In addition, age-related changes in the metabolism of drugs may be responsible for some of the problems with pharmacotherapy in old age. Because the liver plays a major role in the biotransformation of many drugs a better understanding of the physiological changes in hepatic function occurring with advancing age is, therefore, es-

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sential for safer pharmacotherapy.

Most of our knowledge about quantitative aspects of hepatic function in old age is based on studies of drug metabolism in the elderly. The majority of these studies demonstrate highly variable decreases in the systemic clearance of drugs with increasing age, particularly of drugs the elimination of which depends on the biotransformation by the microsomal drug metabolizing enzyme system (for review see [2]). However, observations based on the activity of microsomal enzymes at an unknown degree of substrate saturation are substantially influenced by genetic and environmental factors and concomitant drug therapy that may obscure age-related changes in hepatic function.

In contrast to model drugs used to probe hepatic function galactose is metabolized by cytosolic enzymes, the rate-limiting step probably being the phosphorylation of the sugar by galacto-kinase [3]. Under appropriate conditions the elimination of galactose follows zero order kinetics indicating saturation of the enzyme system involved [4]. Unlike the tests based on the metabolism of model compounds by microsomal enzymes where the concentrations of substrate are well below the K_m of the enzymes involved, the disappearance of galactose from plasma, thus, reflects the capacity of the liver to metabolize galactose which in turn depends on the available mass of functioning hepatocytes and, therefore, might provide a more reliable index of hepatic function.

In the present study, we have estimated the functioning mass of hepatocytes in various age groups by determining the capacity to eliminate an intravenous load of galactose and compared the results with the effects of age on two established tests of hepatic function reflecting the activity of microsomal enzymes, the demethylation of aminopyrine and the clearance of caffeine [5,6].

Subjects and Methods

Subjects

Thirteen elderly volunteers (6 males/7 females) ranging in age from 71 to 88 (mean 78) years participated in this study. All subjects were free of liver disease and in good health for their age based on an interview, physical examination and screening clinical laboratory tests (haemoglobin, leucocytes, prothrombin time, serum creatinine, bilirubin, bile acids, the activities in serum of aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyltransferase [γ-GT] and alkaline phosphatase). All were independent and living in their own home except subject No. 5 who lives in a home for the elderly. Four subjects were taking medication prescribed by their family physician for the treatment of hypertension, mild congestive heart failure or a sleeping problem. Two subjects were smokers (pipe and cigars) and none reported more than occasional alcohol consumption (Table 1). All subjects gave their written consent to participate in the study after the procedure had been fully explained to them.

A population of younger subjects without liver disease provided the reference values that had in part been published previously [7,8]. These subjects were either healthy volunteers participating in other studies or patients who were seen in our clinic and who were found to be free of liver disease by recognized clinical and laboratory criteria. The subjects were non-smokers and consumed less than 10 g of ethanol per day.

Quantitative tests of liver function

Galactose elimination capacity. A butterfly-needle was placed in an antecubital vein and the volunteers were kept at bed rest for the duration of the determination of the galactose elimination capacity and the [^{14}C]aminopyrine breath test. The two tests were always started at 9 and 10.30 a.m., respectively. The galactose elimination capacity was assessed according to Tygstrup's procedure [4,9] using venous blood samples drawn every 5 min between 20 and 60 min after injection of 0.5 g/kg body weight of galactose in saline. Urine was collected for 2 h after the administration of galactose. The concentrations of galactose in plasma and urine were measured enzymatically (Test-Combination Galactose, Boehringer Mannheim, GmbH).

TABLE 1

CLINICAL AND LABORATORY DATA OF THE ELDERLY SUBJECTS STUDIED

Subject No.	Sex	Age (yr)	Body weight (kg)	Haemoglobin (g/dl)	Leucocytes ($\times 10^9$/l)	Prothrombin time (%)	Serum creatinine (μmoles/l)	Total serum bilirubin (μmoles/l)	AST (U/l)	ALT (U/l)	γ-GT (U/l)	Alkaline phosphatase (U/l)	Fasting serum bile acids (μmoles/l)	Concomitant drug[a] treatment and smoking history
1	m	88	59	15.1	5.4	76	119	9	18	10	20	51	2.2	1
2	m	85	65	13.8	3.4	83	113	16	15	12	36	45	2.5	–
3	f	82	82	15.4	4.2	90	85	9	17	9	31	43	2.4	2
4	f	81	67	14.2	4.3	93	101	12	15	23	18	50	1.3	2.3.4
5	m	81	72	12.7	4.2	88	102	5	14	13	18	84	3.2	–
6	f	80	45	15.4	5.3	100	74	9	15	14	27	46	1.7	5
7	f	78	64	13.1	4.8	88	85	9	14	13	14	74	0.6	6
8	f	77	60	14.9	5.7	96	79	26	14	15	14	68	2.6	–
9	m	76	62	14.1	3.6	85	91	18	19	12	15	49	1.1	–
10	f	75	71	14.3	5.1	100	81	11	13	14	16	84	2.5	–
11	f	71	59	14.0	4.5	100	78	9	14	14	20	72	4.4	7
12	m	71	86	16.6	6.2	92	82	14	12	11	23	68	2.5	–
13	m	71	69	14.2	6.5	100	76	8	18	17	10	39	1.9	1
Mean		78	66	14.4	4.9	92	89	12	15	14	20	59	2.2	
SD		5	10	1.03	0.95	8	13	5.5	2	3.5	7	16	0.96	
Normal values:				12.1–15.4	3.2–9.0	70–130	59–116	3.4–25.7	0–23	0–23	0–46	30–100	0–6	

[a] Numbers denote the following: 1 = current smoker; 2 = digoxin; 3 = flunitrazepam; 4 = reserpine + dihydralazine + hydrochlorthiazide; 5 = diazepam; 6 = hydralazine; 7 = bromazepam.

TABLE 2

KINETIC PARAMETERS OF CAFFEINE SALIVA CLEARANCE, GALACTOSE ELIMINATION CAPACITY AND [^{14}C]AMINOPYRINE BREATH TEST IN ELDERLY SUBJECTS

Subject No.	Caffeine saliva clearance			Galactose elimination capacity						[^{14}C]Aminopyrine breath test (% dose × kg/mmoles CO_2)
	k_e (h^{-1})	$t_{1/2}$ (h)	Cl (ml/min/kg)	GEC (mg/min/kg)	V_d (l)	Urine galactose (g)	Urine galactose (% dose)	c_0[a] (mg/dl)	c_{45}[a] (mg/dl)	
1	0.106	6.6	1.06	3.7	12.2	1.95	7.9	200	108	0.646
2	0.080	8.7	0.80	5.8	18.7	2.09	6.4	173	67	0.481
3	0.053	13.2	0.53	5.6	17.3	4.93	11.9	238	92	0.586
4	0.098	7.1	0.98	5.0	14.0	2.53	7.5	240	113	0.471
5	–[b]	–	–	4.8	18.8	3.20	8.9	190	94	0.886
6	–[b]	–	–	7.2	15.1	2.30	10.1	150	33	0.940
7	–[b]	–	–	6.3	13.0	3.26	10.1	248	80	0.445
8	0.158	4.4	1.58	6.9	15.6	2.95	9.8	192	49	0.747
9	0.105	6.6	1.05	7.0	20.0	1.48	4.8	154	43	0.540
10	0.092	7.5	0.92	6.1	15.0	3.84	10.7	237	78	0.524
11	–[b]	–	–	4.8	13.2	2.87	9.7	224	115	0.949
12	0.036	19.2	0.36	7.4	17.5	3.84	8.9	246	52	0.332
13	0.148	4.7	1.48	8.4	21.7	3.65	10.5	160	19	0.485
χ	0.097	8.6	0.97	6.1	16.3	2.99	9.0	204	72	0.618
SD	0.039	4.7	0.39	1.3	2.9	0.94	1.9	37	31	0.202

[a] Extrapolated concentration of galactose at time zero and measured plasma concentration at time 45 min.

[b] Determination of clearance not possible because caffeine was either ingested between the two saliva collections, or not enough caffeine was consumed resulting in unmeasurable concentration of caffeine in saliva.

Caffeine saliva clearance. On the day before the examination in the Clinical Pharmacology Unit the subjects were asked to drink 3 cups of coffee (approximately 330 mg of caffeine) between 2 and 4 p.m. and not to ingest any caffeine-containing foods and beverages thereafter. They collected the first sample of saliva into a plastic tube before going to sleep. After an overnight fast the volunteers came to the clinic the next morning where the second sample of saliva was collected. The caffeine concentrations in saliva were determined by an automated enzyme immunoassay [10]. The clearance of caffeine was calculated from the rate of decrease of the concentration of caffeine in the two timed samples of saliva and a volume of distribution assumed to be 0.6 $l \cdot kg^{-1}$ [11,12].

[^{14}C]Aminopyrine breath test. Following the collection of a baseline sample of respiratory CO_2 a tracer dose of 1.6 μCi dimethyl[^{14}C]aminopyrine was administered intravenously [13]. After 30 min breath was directly collected in a counting vial containing 2 mmol of hyamine in ethanol together with phenolphthalein as a pH indicator. The collection of breath was terminated when 2 mmols of CO_2 were trapped in the scintillation vial as indicated by the change in colour. The radioactivity of the exhaled CO_2 was measured by liquid scintillation spectrometry. The specific activity of $^{14}CO_2$ corrected for body weight and expressed as a percentage of the dose (% dose·kg·mmol CO_2^{-1}) was used as an index of aminopyrine demethylation.

Group comparisons were made by Peritz' *F*-test [14]. Differences between groups were considered statistically significant if $P < 0.05$. All results are given as mean ± standard deviation (SD). Correlations between variables were assessed by linear regression analysis.

Results

The individual results of the liver function studies in the elderly subjects and the kinetic parameters derived from plasma, saliva and urine data are summarized in Table 2. Mean values of the results of the various tests for different age groups are indicated in Table 3.

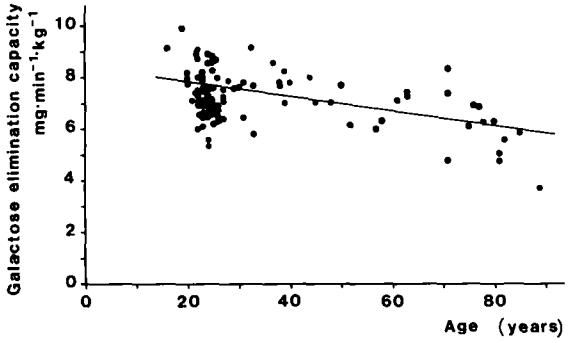

Fig. 1. Galactose elimination capacity as a function of age. Each point represents a healthy, normal subject. The hepatic capacity to eliminate galactose decreases significantly with increasing age ($P < 0.01$).

TABLE 3

QUANTITATIVE LIVER FUNCTION AND AGE

	Galactose elimination capacity (mg/min/kg)	Caffeine saliva clearance (ml/min/kg)	[^{14}C]Aminopyrine breath test (% dose × kg/mmoles CO_2)
Young adult subjects (<40 yr)	7.48 ± 0.94 (25 ± 5 yr, n = 70[a])	1.49 ± 0.44 (29 ± 4 yr, n = 15)	0.79 ± 0.15 (29 ± 6 yr, n = 20)
Middle-aged subjects (40–70 yr)	7.08 ± 0.68 (53 ± 8 yr, n = 11)	1.46 ± 0.55 (54 ± 8 yr, n = 14)	0.74 ± 0.21 (55 ± 8 yr, n = 11)
Elderly subjects (>70 yr)	6.08 ± 1.30 (78 ± 5 yr, n = 13) $P < 0.05$ vs young and middle-aged subjects	0.97 ± 0.39 (78 ± 6 yr, n = 9) $P < 0.01$ vs young, $P < 0.05$ vs middle-aged subjects	0.62 ± 0.20 (78 ± 5 yr, n = 13) $P < 0.05$ vs young subjects

[a] Age in years and number of subjects.

Galactose elimination capacity

Figure 1 depicts the galactose elimination capacity as a function of age. The galactose elimination capacity decreased with increasing age ($r = 0.466$, $P < 0.01$). Compared to a group of 70 young adults ranging in age from 16–39 years the galactose elimination capacity was on the average 19% lower ($P < 0.05$) in the group of elderly subjects (Table 3). The galactose elimination capacity in the elderly was also significantly lower ($P < 0.05$) than in a group of 11 middle-aged subjects ranging in age from 40–63 years. The difference between the young and the middle-aged subjects did not reach statistical significance.

The average volume of distribution of galactose did not change with age (16.3 ± 2.7 l and 16.3 ± 2.9 l in the group of young adults and elderly subjects, respectively). The percentage of the administered dose of galactose excreted in urine was identical in both age groups (10.8 ± 3.8% and 9.0 ± 1.9% in young adults and elderly subjects, respectively), indicating that the slower elimination of galactose in the elderly reflects a decreased hepatic clearance.

Caffeine saliva clearance

Figure 2 shows the caffeine saliva clearance as a function of age. Although substantial interindividual variation is evident the caffeine saliva clearance was significantly lower ($P < 0.05$) in the elderly than in the younger population (Table 3). The clearance decreased by 35% in the elderly as compared to the young and thus demonstrated the most marked age-related change of the three quantitative liver function tests. Two of the 4 elderly subjects with clearance values above 1 $ml \cdot min^{-1} \cdot kg^{-1}$ were smokers (Table 1). Two of the 13 elderly subjects drank caffeine-containing beverages between the two collections of saliva and, therefore, had higher concentrations of caffeine in the second sample. Two additional subjects did not ingest enough caffeine and therefore, ended up having very low concentrations of caffeine in the first sample of saliva and unmeasurable concentrations in the second.

[^{14}C]Aminopyrine breath test

Figure 3 shows the result of the [^{14}C]aminopyrine breath test as a function of age. Aminopyrine demethylation decreased with increasing age ($r = 0.377$, $P < 0.05$). Consequently the values in the elderly were significantly lower than in young adults ranging in age from 18 to 39 years ($P < 0.05$, Table 3). As was the case with the caffeine clearance the test result exhibited much more interindividual variation than the result of the galactose elimination capacity.

There was no difference in the result of any of the three tests between elderly males and females. The average decrease in the test results in the elderly was not different for the three tests (95% confidence limits: 8–29% decrease compared to the young refer-

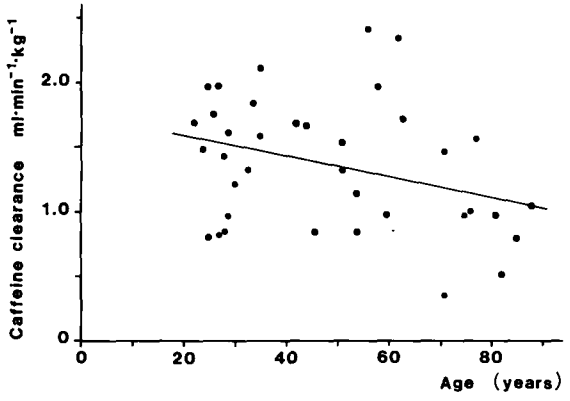

Fig. 2. Caffeine saliva clearance in healthy normal subjects as a function of age. The systemic clearance of caffeine decreases significantly with age ($P < 0.05$).

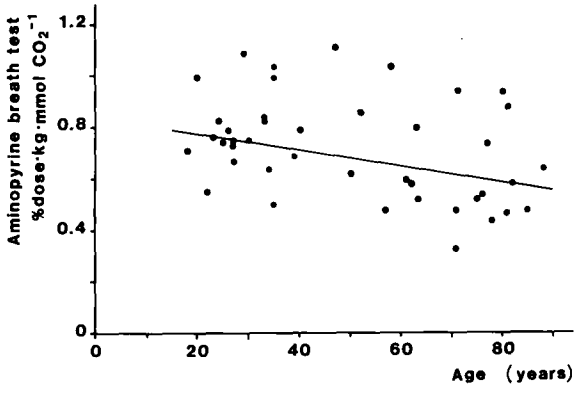

Fig. 3. Aminopyrine breath test in healthy, normal subjects as a function of age. The demethylation of aminopyrine decreases significantly with increasing age ($P < 0.05$).

ence population for the galactose elimination capacity, 14–55% decrease for the caffeine clearance, 6–37% decrease for the demethylation of aminopyrine).

Discussion

Our data demonstrate that not only the biotransformation of xenobiotics by the microsomal drug metabolizing enzyme system but also the capacity to metabolize galactose decreases with increasing age. The delayed elimination of galactose in elderly subjects (Table 3) cannot be explained by changes in the distribution or renal elimination of the test compound (Table 2). Therefore, the significantly decreased galactose elimination capacity in the elderly most likely reflects a loss of functioning hepatocyte mass. Assuming that approximately 2 $mg \cdot min^{-1} \cdot kg^{-1}$ are eliminated by extrahepatic processes [15] the loss of functioning mass amounts to about 25% from age 20–80 (Fig. 1). This loss of function agrees well with the loss of volume of the liver as determined by sonography and at autopsy [16–18] suggesting that the shrinkage of the liver with age affects functioning and supporting structures to the same extent. Our data (Fig. 1) suggest that the rate of decrease between the age of 70 and 90 (0.15 ± 0.06 $mg \cdot kg^{-1} \cdot min^{-1}$ per year) is higher than the yearly loss of functioning liver cell mass between the age of 20 and 65 (0.015 ± 0.009 $mg \cdot kg^{-1} \cdot min^{-1}$ per year). The biological significance of this accelerated loss of hepatocyte mass in old age which is confirmed by necropsy data [18] is not clear at present.

The two tests thought to probe microsomal function both demonstrate a progressive decrease with advancing age, albeit with much more interindividual variation than the elimination of galactose. Because of their pharmacokinetic properties characterized by a complete and rapid absorption, constant volume of distribution and low hepatic extraction both compounds are well suited to assess the activity of the hepatic monooxygenase system [5,6,11,19]. In the case of caffeine protein binding has been shown not to change with age [20], and excellent agreement between the clearance values derived from plasma and saliva data has been reported [21]. The relative decrease in the systemic clearance of caffeine and the demethylation of aminopyrine found in our elderly subjects is consistent with the decreased biotransformation by monooxygenases of a number of compounds in old age. Thus, the clearance of theophylline [22] and imipramine [23] has been shown to decrease by 30–40% and the clearance of antipyrine by 20–45% [16,24]. The two tests of microsomal function paralleled the decrease in the capacity to metabolize galactose suggesting that the decreased hepatic clearance of many drugs in old age is related to the loss of liver volume rather than changes in the function of microsomal enzymes. Indeed there is no difference in drug metabolism in vitro by microsomes isolated from young and aged livers [25,26]. Nevertheless the decrease in functioning cell mass alone could contribute to the high incidence of adverse side-effects of drugs in old age.

The aminopyrine breath test and the systemic clearance of caffeine are both influenced by a number of genetic and environmental factors and concomitant drug therapy that can either induce or inhibit the microsomal drug metabolizing enzyme system. This may explain the substantial interindividual variation of the two tests seen in the present and other studies. In contrast, the galactose elimination capacity does not appear to be as markedly influenced by genetic and environmental factors and, therefore, may be a more reliable index of hepatic function.

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