Biochemical Pharmacology, Vol. 37, No. 13, pp. 2667–2673, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

EFFECT OF AGING ON HEPATIC ELIMINATION OF CIMETIDINE AND SUBSEQUENT INTERACTION OF AGING AND CIMETIDINE ON AMINOPYRINE METABOLISM*

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(*Received* 24 *August* 1987; *accepted* 14 *December* 1987)

Abstract—Aging and cimetidine may each impair hepatic microsomal drug metabolism. To test if and by what mechanisms advanced age may increase sensitivity to the inhibitory effects of cimetidine, the interaction of these two factors with aminopyrine metabolism in the rat was studied using a correlative approach. Initial studies using the aminopyrine breath test indicated that a 40 mg/kg dose of cimetidine, i.p., impaired the $^{14}CO_2$ exhaled by up to 76% more in aged (26-month) than in young (3- to 4-month-old) rats. Using an isolated liver perfusion to dissect out hepatic components of this phenomenon, it was found that various doses of cimetidine impaired aminopyrine clearance to a greater degree ($P < 0.05$) in aged than in young livers. However, cimetidine metabolism in this system ranged from 36 to 78% less in aged versus young livers ($P < 0.05$). Subsequent *in vitro* studies indicated that microsomes isolated from aged livers also averaged a 76% lower rate of cimetidine metabolism ($P < 0.05$). A *fixed* cimetidine concentration, however, inhibited aminopyrine demethylation to the same degree in aged versus young rats ($P < 0.05$). *In vivo* pharmacokinetics showed an age-related decrease in both aminopyrine and cimetidine systemic clearance. In the young rat the liver contributed about 30% to total systemic clearance of cimetidine. In the aged rat, all clearance was renal. Despite a decrease in glomerular filtration rate, net tubular cimetidine secretion was well-maintained. Despite this, absence of the hepatic component resulted in decreased overall systemic clearance of the drug in aged rats. It is concluded that (1) the aged rat liver exhibits impaired cimetidine metabolism, resulting in decreased overall systemic clearance of the drug despite normal net renal tubular secretion, (2) there is no age-related enhanced sensitivity to cimetidine of the hepatic microsomal oxidizing system using aminopyrine as the probe drug, and (3) the larger inhibition of aminopyrine metabolism in aged rats following various doses of cimetidine is due to decreased overall cimetidine clearance, resulting in higher concentrations of the inhibitor in the liver of aged rats.

The incidence of adverse drug reactions in the elderly is three to seven times higher than in the young [1, 2]. The origin of this phenomenon is likely multifactorial. First, the degree of polypharmacy in the aged is significantly higher than in the young [3], increasing the likelihood of adverse reactions caused by *multiple* drug interactions. Second, the elderly experience an increased incidence of side effects from *individual* drug therapies [4–8]. Likely causes for this include increased tissue and organ sensitivity (pharmacodynamic effect) to specific drugs [9–12] and age-related decrease in drug elimination (pharmacokinetics) [2, 6, 13]. Furthermore, a number of factors may contribute to age-dependent alterations in pharmacokinetics. Among these may be decreased gut absorption [2, 13], altered volume of distribution [2], decreased drug binding in plasma [6], reduced renal clearance [2], and decreased hepatic removal of drugs [13]. The latter can be due to diminished blood flow to the liver (for high clearance, flow-dependent drugs) and/or to a reduction in hepatic microsomal drug-metabolizing enzyme activity (intrinsic clearance) with aging [2, 6, 9, 14] for low extraction drugs. Mechanisms of age-related adverse drug–drug interactions in humans exhibit, therefore, complex pharmacologic patterns that do not lend themselves easily to clinical investigation. Our studies assess the individual interactive components in an animal model of aging. They focus on the hypothesis that *some* adverse multiple drug responses in the elderly are caused by a diminished hepatic metabolizing capacity of one drug which can, in turn, alter hepatic elimination of other agents. More specifically, we report an age-related change in elimination of cimetidine by the liver and kidneys of the rat, and the effect of this on hepatic metabolism of a second marker drug, aminopyrine.

* Supported by VA Research Funds.

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MATERIALS AND METHODS

Animal models. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were used. Aged rats were between 24 and 26 months, and the young were 120–160 days of age. Body weights averaged 422 ± 64 g (SE) and 517 ± 19 g (SE) for young and aged rats, respectively, for breath tests, *in vivo* aminopyrine pharmacokinetics, and animals used as a source of liver for perfusion or microsomes. Body weights (in grams) for the cimetidine infusion studies were 348 ± 22 and 598 ± 56 for young and aged rats respectively.

Aminopyrine breath test. The aminopyrine breath test, as previously described [15, 16], was used to approximate N-demethylation *in vivo*. [^{14}C]Aminopyrine (sp. act. 73.8 μCi/μmol, Amersham, Arlington Heights, IL) was administered intraperitoneally in tracer doses at 0.5 μCi/100 g body weight, and $^{14}CO_2$ was collected at 15-min intervals for 3 hr. Results are expressed both as percent of injected dose exhaled by 30 min after injection (control and post-cimetidine) and as the absolute rate of $^{14}CO_2$ exhaled at 30 min (pmol/min).

In vivo *aminopyrine clearance.* For aminopyrine studies, rats were injected i.p. with aminopyrine (45 mg/kg), and blood samples were taken from the tail vein of unanesthetized animals at various intervals as previously described [16]. Concentrations of aminopyrine in whole blood were determined by HPLC by the method of Lockwood and Houston [17]. Blood levels showed a rapid initial rise followed by a monoexponential decay. This beta phase of elimination (β) was analyzed by an interactive nonlinear regression with proportional standard deviation weighing [18]. AUC was estimated by the trapezoidal rule and extrapolated to infinity. Blood clearance (Cl) was determined from Cl = dose/AUC and apparent volume of distribution (Vd_β) from $Vd_\beta = Cl/\beta$.

In vivo *cimetidine clearance.* Young and aged male rats were anesthetized with pentobarbital (5 mg/100 g body weight i.p.), and polyethylene tubing (Clay Adams Intramedic PE-10) was placed in a femoral artery and vein. A low abdominal incision was made, the ureters were identified and then cannulated with PE-10 tubing. The animals were maintained at normal body temperature with a heating pad. A bolus of cimetidine (1 mg/100 g) was given through the femoral vein catheter and then perfusion was started with a solution containing saline (0.9%), mannitol (5%), inulin (0.2%) and cimetidine. The cimetidine perfusion rate was 40 μg/min and the volume was 0.123 ml/min. After 60 min of perfusion to achieve steady-state cimetidine and inulin levels, as well as constant urine flow, three serial 10-min urine collections were started and an arterial blood sample was collected at the beginning and end of each urine collection. Each blood sample was transferred to a heparinized microcentrifuge tube, and plasma was separated. Plasma and urine were analyzed for cimetidine by HPLC [19] after the extraction technique of Lorenzo and Drayer [20], and inulin was quantitated spectrophotometrically by the method of Heyrovsky [21]. Cimetidine and inulin renal clearances were calculated by the UV/P* method, and inulin clearance was considered to be equivalent to glomerular filtration. Cimetidine systemic clearance was calculated as the dosing rate/steady-state plasma concentration.

Isolated liver perfusion. Liver perfusions utilized standard techniques [22]. The liver was exposed and the bile duct was cannulated. The portal vein and its tributaries were tied off, and the portal vein was cannulated with perfusate flowing. Adequate perfusion (Krebs–Ringer solution containing Ca^{2+}, pH 7.4) was identified by blanching of all lobes of the liver. After equilibration, a closed system was established, and washed human red blood cells were added to a relative concentration of 20% (v/v). Oxygenation (95% O_2/5% CO_2) was accomplished with a rolling drum oxygenator, and the oxygen content of the effluent was monitored continuously. Flow rate was maintained at 30 ml/min with infusion pressure, and bile flow was monitored. Drugs were added via the oxygenator reservoir, and perfusate samples were taken immediately proximal to the liver. Liver viability (which could be maintained over a 2.5-hr period) was monitored by steady bile flow and normal O_2 consumption.

Microsomal preparations and in vitro *assay for cimetidine metabolism and aminopyrine N-demethylation.* Hepatic microsomes were prepared by a standard technique [23]. Aminopyrine *N*-demethylase activity was determined as previously described [16] by a modification of the radiometric method of Poland and Nebert [24]. [^{14}C]Aminopyrine was repurified by chloroform extraction prior to use. For assay, [^{14}C]aminopyrine was adjusted to 10^{-5} M and the incubation period was 60 min, a time previously determined to yield linearity of [^{14}C]formaldehyde production. When aminopyrine demethylation was determined in the presence of cimetidine, concentrations of the inhibitor remained constant throughout the 1-hr incubation, since the high sensitivity of the radiometric demethylase assay allowed us to reduce microsome concentration in the reaction mixture to a level which did not detectably alter cimetidine levels. Cimetidine metabolism was determined in an identical reaction mixture (minus aminopyrine) as used above, except that a fifty times higher concentration of microsomes was included. The rate of metabolism of cimetidine was determined by the rate of disappearance of the compound over a 1-hr incubation period. Cimetidine was assayed as described above [19, 20]. Additionally, all *in vitro* values are expressed per unit microsomal protein since age did not alter total microsomal protein content [74.36 ± 3.71 (SE) vs 73.07 ± 2.67 mg protein/g microsomal pellet, aged vs young respectively].

Other assays and chemicals. Protein was assayed by the method of Hartree [25]. Unlabeled aminopyrine and other chemicals were purchased from Sigma, St. Louis, MO.

Statistical methods. Statistical significance ($P < 0.05$) was established by the unpaired, two-tailed Student's *t*-test (between age group comparisons) as well as by the two-way ANOVA for Tables 1–5.

* U = urine concentration; V = urine volume; and P = plasma concentration.

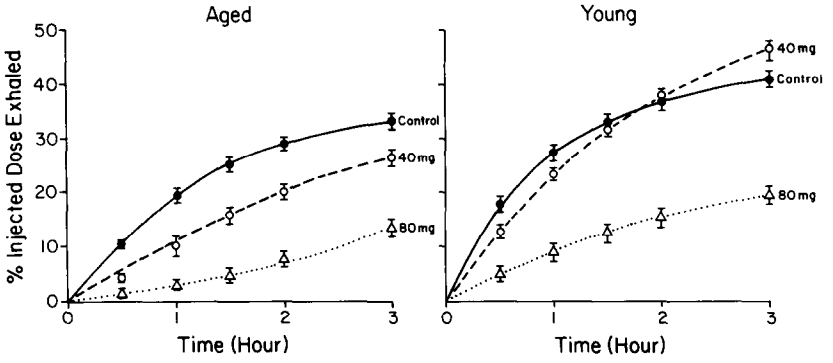

Fig. 1. Effect of cimetidine on the aminopyrine breath test in young and aged rats. Rats were administered cimetidine i.p. 1 hr prior to injection (i.p.) of [^{14}C]aminopyrine. Each curve represents the mean value ± SE of six rats. The same six animals in each age group were used for each of the three curves at 5-day intervals.

RESULTS

Cimetidine inhibition of the aminopyrine breath test. Initial studies utilized the aminopyrine breath test to compare first aminopyrine demethylation in the absence of inhibitor and then the inhibitory effects of two doses of cimetidine on aminopyrine demethylation in young versus aged rats (Fig. 1). At 30 min, following [^{14}C]aminopyrine, the young control rats exhaled $^{14}CO_2$ at the rate of 233.6 ± 11.2 (SE) pmol/min, while the exhalation rate for aged controls was essentially equal at 275 ± 15.2 pmol/min ($P > 0.05$, Table 1). On the other hand, when the control data is expressed as *percent* of the administered dose exhaled as $^{14}CO_2$ by 30-min post-injection, young rats had exhaled 18.0 ± 1.4 (SE) versus 10.5 ± 0.5% in aged animals ($P < 0.05$). Cimetidine (40 and 80 mg/kg) was administered 1 hr prior to the labeled aminopyrine. Age-related differences were apparent in response to cimetidine pretreatment with both methods of data expression (Table 1). A 40 mg/kg dose of cimetidine reduced breath test yield in young rats by 15% (absolute rate) and 28% (percent yield), while in aged rats corresponding reductions were 38 and 46% respectively ($P < 0.05$). This age-related difference was more striking at a higher (80 mg/kg) dose of cimetidine (Table 1). Additionally, Fig. 1 illustrates that, at the 40 mg/kg dose of cimetidine, the breath test normalized to control values within 1.5 hr in the young, but not in the aged rats.

Aminopyrine clearance by the isolated perfused liver. Since factors other than altered hepatic clearance can affect the breath test, aminopyrine and cimetidine clearances were studied in isolated perfused livers from young and aged rats. Overall hepatic aminopyrine clearance, while slightly lower, was not affected significantly by aging, but inhibition of its clearance at a fixed perfusion rate and a given dose of cimetidine was greater in aged than in young livers by ANOVA ($P < 0.05$) (Table 2). Infusion of three "doses" of cimetidine (1, 2.5, or 5 mg) into the perfusate resulted in inhibition of aminopyrine clearances of 21, 34 and 70%, respectively, for young compared to 47, 45 and 77% for aged livers ($P < 0.05$ for the 1 and 5 mg dose, $P = 0.07$ for the 2.5 mg dose). It follows that this greater degree of inhibition of aminopyrine clearance in the aged liver could be due to either enhanced intrinsic sensitivity to cimetidine of the microsomal demethylating enzymes and/or to higher cimetidine levels due to impaired cimetidine clearance by the aged liver. This point is addressed in Fig. 2, which shows that when individual percent inhibitions of aminopyrine clearance are plotted as a function of corresponding cimetidine area under the curve (AUC, total cimetidine in the

Table 1. Amount of administered aminopyrine exhaled as $^{14}CO_2$

	Absolute rate (pmol/min) at 30-min post-injection			Percent dose exhaled by 30 min		
	Control	Cimetidine dose† 40 mg/kg	80 mg/kg	Control	Cimetidine dose† 40 mg/kg	80 mg/kg
Young	233.6 ± 11.2	198.6 ± 5.8	119.1 ± 29.0	18.0 ± 1.4	13.0 ± 0.3	5.0 ± 1.1
Cimetidine / Control		0.85	0.51		0.72	0.28
Aged	275.7 ± 15.2	170.8 ± 29.7	34.0 ± 5.8‡	10.5 ± 0.5‡	5.7 ± 1.1‡	1.1 ± 0.2‡
Cimetidine / Control		0.62	0.12		0.54	0.10

Values are means ± SE; N = 6 rats per group with control and cimetidine breath tests run on the same rats at 1-week intervals.

† Cimetidine was administered i.p. 1 hr prior to a single i.p. dose of [^{14}C]aminopyrine.

‡ $P < 0.05$, aged vs young.

Table 2. Effect of cimetidine on aminopyrine clearance by isolated perfused livers of young and aged rats

Age	Cimetidine dose* (mg)	Hepatic clearance of aminopyrine (ml/min/g liver)	% Inhibition
Young	0	0.64 ± 0.08†	
	1.0	0.50 ± 0.02	21.4 ± 3.0
	2.5	0.42 ± 0.01	33.8 ± 2.2
	5.0	0.19 ± 0.02	69.6 ± 2.8
Aged	0	0.52 ± 0.06	
	1.0	0.27 ± 0.05‡	47.3 ± 8.9
	2.5	0.30 ± 0.03§	44.9 ± 5.9
	5.0	0.11 ± 0.01‡	77.4 ± 1.8

* Milligrams of cimetidine added to the perfusate (250 ml total volume), along with 5.0 mg of aminopyrine.
† Mean ± SE, N = 5 livers/group.
‡ $P < 0.05$, young vs aged.
§ $P = 0.07$.

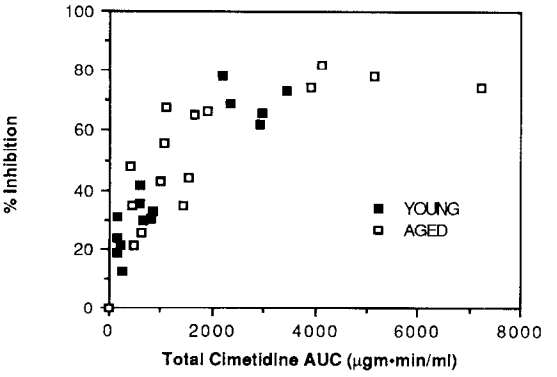

Fig. 2. Relationship between inhibition of aminopyrine clearance by the isolated perfused liver and total perfusate cimetidine. Each point represents the percent inhibition of aminopyrine clearance and its corresponding cimetidine AUC (area under the curve for total perfusate cimetidine).

perfusate), a similar correlative curve is defined by values from young and aged livers. Additionally, a given dose of cimetidine added to the perfusate resulted in significantly higher ($P < 0.05$) cimetidine AUCs for the aged liver perfusions. The aged versus young AUC values, respectively, were 809 ± 251 (SE) vs 185 ± 23 (SE) for the 1 mg dose, 1255 ± 186 (SE) vs 702 ± 52 (SE) for the 2.5 mg dose, and 5106 ± 764 (SE) vs 2774 ± 224 (SE) for the 5 mg dose of cimetidine. The latter data suggest that the observed cimetidine-induced impairment of aminopyrine clearance with aging in this perfusion system may be due to reduced hepatic cimetidine clearance resulting in higher inhibitor levels rather than to a greater intrinsic sensitivity of liver microsomes to the inhibitor with age. To examine this possibility further, hepatic clearance of cimetidine at different ages was determined (Table 3). Clearance of cimetidine by livers from aged rats ranged from 36 to 78% less than by young livers with a significant difference ($P < 0.05$) in clearance at all cimetidine doses. This differential effect on the two age groups could be due to varying hepatic metabolism of cimetidine, its biliary excretion, or both.

Cimetidine and aminopyrine metabolism by isolated hepatic microsomes. More direct evidence for an effect of aging on aminopyrine and cimetidine hepatic metabolism was obtained using isolated hepatic microsomes. Table 4 illustrates that aminopyrine N-demethylation was 53% less per unit protein by microsomes isolated from aged than from young livers (66.4 vs 144.3, $P < 0.05$). In addition, four *fixed* concentrations of cimetidine were included in microsomal reaction mixtures resulting in inhibition of aminopyrine demethylation from 14 to 64% (Table 4). A statistically significant ($P < 0.05$) difference in the degree (percent) of inhibition between the two age groups occurred only at the lowest cimetidine level (3.3 μg/ml). No differences on a percent basis ($P > 0.05$) occurred at the three higher cimetidine concentrations (11.1 to 44.1 μg/ml) and, in absolute terms, the inhibition was actually less in the aged liver.

Identical incubation conditions, albeit with a fifty times higher concentration of liver microsomes, were utilized to determine age-related changes in hepatic cimetidine metabolism (Table 5). Such higher concentrations of microsomal enzymes were required for accurate measurements of cimetidine metabolism. Three concentrations of cimetidine were used (3.3 to 70.6 μg/ml), and microsomes isolated from aged livers showed an average of 42% lower rate of cimetidine metabolism than those from young livers, $P < 0.05$. Thus, whatever the contribution of biliary excretion may be, aged hepatic microsomes in male rats metabolized cimetidine less than those obtained from young rat liver.

Comparison of in vivo *pharmacokinetics of aminopyrine and cimetidine in young and aged rats.* To determine if the age-related changes in aminopyrine

Table 3. Comparative cimetidine clearance by the isolated perfused livers of young and aged rats

Age	Hepatic clearance (ml/min/liver)			Hepatic clearance (ml/min/g liver)		
	Cimetidine dose* (mg)			Cimetidine dose* (mg)		
	1.0	2.5	5.0	1.0	2.5	5.0
Young	5.72 ± 0.66†	3.64 ± 0.26	1.85 ± 0.15	0.40 ± 0.04	0.25 ± 0.2	0.16 ± 0.02
Aged	1.71 ± 0.40‡	2.28 ± 0.42‡	1.04 ± 0.13‡	0.09 ± 0.02‡	0.16 ± 0.03‡	0.07 ± 0.01‡

* Milligrams of cimetidine added to the perfusate (250 ml).
† Mean ± SE, N = 8 livers/group.
‡ $P < 0.05$, young vs aged.

Table 4. Effect of aging on cimetidine inhibition of aminopyrine demethylation by isolated hepatic microsomes

Age	Concn of cimetidine (μg/ml)	Aminopyrine demethylation (pmol formaldehyde/ mg protein/min)	% Inhibition	Absolute inhibition
Young	0	144.3 ± 11.7*		
	3.3	123.2 ± 8.7	13.6 ± 1.4	21.1 ± 2.2
	11.1	96.0 ± 6.1	31.9 ± 2.4	48.3 ± 4.3
	22.2	71.0 ± 3.9	49.0 ± 2.4	73.3 ± 8.4
	44.1	53.2 ± 4.0	61.4 ± 2.9	91.1 ± 9.7
Aged	0	66.4 ± 13.3†		
	3.3	51.9 ± 11.1	22.4 ± 2.9†	14.5 ± 1.9
	11.1	46.8 ± 8.7	32.2 ± 5.1	21.3 ± 5.2†
	22.2	36.7 ± 7.3	43.9 ± 8.0	29.7 ± 7.7†
	44.1	27.5 ± 5.6	63.9 ± 8.2	41.9 ± 9.3†

* Mean ± SE, N = 13 rats/young group, N = 9 rats/aged group, 5 assays/value.
† $P < 0.05$, young vs aged.

Table 5. Effects of aging on cimetidine metabolism by isolated hepatic microsomes

Age	Cimetidine metabolism (ng metabolized/mg protein/min)		
	Cimetidine concentration (μg/ml)		
	3.3	22.2	70.6
Young	5.77 ± 0.52*	40.2 ± 1.8	107.0 ± 3.5
Aged	4.11 ± 1.00	20.0 ± 4.1†	55.6 ± 11.8†

* Mean ± SE, N = 13 rats/young group, N = 9 rats/aged group, 1 assay/liver.
† $P < 0.05$, young vs aged.

and especially cimetidine metabolism observed *in vitro* correspond to those occurring *in vivo*, pharmacokinetics of these two drugs were studied *in vivo*. *In vivo* clearance of aminopyrine (without inhibitor) in the aged versus young rat was not significantly different $P > 0.05$) when expressed as ml/min, but was reduced by 35% ($P < 0.05$) when expressed as ml/min/kg body weight (Table 6). This was accompanied by a 40% increase in $T_{\frac{1}{2}}$ and a 63% increase in AUCs as compared to the young rats, while volume of drug distribution was not altered.

Overall *systemic* clearance of cimetidine per kg body weight was decreased by aging. This was due to the marked fall of nonrenal clearance of the drug

Table 6. Comparison of *in vivo* pharmacokinetics of aminopyrine in young and aged rats

Age	Clearance (ml/min)	Clearance (ml/min/kg)	$T_{\frac{1}{2}}$ (min)	Volume of distribution (ml)	Volume of distribution (ml/kg)	AUC $(mg \cdot min \cdot ml)^{-1}$
Young	14.2 ± 1.5*	35.6 ± 3.6	39.5 ± 1.7	776.5 ± 70.6	1941 ± 171	1452 ± 94
Aged	12.1 ± 1.4	23.2 ± 2.7†	55.4 ± 5.8†	864.4 ± 87.7	1618 ± 146	2374 ± 241†

* Mean ± SE, N = 6 young rats, N = 11 aged rats. Aminopyrine was administered i.p. at 45 mg/kg.
† $P < 0.05$, young vs aged.

Table 7. Comparison of *in vivo* systemic and renal clearance of cimetidine in young and aged rats

Age	Steady-state plasma concentrations (μg/ml)	Systemic clearance (ml/min)	Systemic clearance (ml/min/kg)	Renal clearance (ml/min/kg)	Non-renal clearance (ml/min/kg)	% Renal clearance
Young	3.25 ± 0.07*	12.32 ± 0.26	35.56 ± 1.40	24.30 ± 1.13	11.26 ± 0.82	68.34 ± 1.86
Aged	2.80 ± 0.23	15.36 ± 0.91†	26.05 ± 1.40†	26.37 ± 1.70	0	99.57 ± 0.82†

* Mean ± SE, N = 6 young rats, N = 5 aged rats.
† $P < 0.05$, young vs aged.

Table 8. Effect of age on renal function and cimetidine secretion

Age	Urine flow (μl/min)	GFR (ml/min)	GFR (ml/min/kg)	Secretory clearance (ml/min/kg)	Cimetidine renal clearance/GFR
Young	68.29 ± 3.40*	2.09 ± 0.11	6.02 ± 0.38	18.27 ± 0.78	4.07 ± 0.10
Aged	58.58 ± 2.53	1.63 ± 0.14†	2.71 ± 0.15†	23.66 ± 3.59	9.77 ± 1.36†

* Mean ± SE, N = 6 young rats, N = 5 aged rats.
† $P < 0.05$, young vs aged.

in the aged animal. In the young rat renal drug removal contributed 68% to total clearance, while in the aged animals this was essentially 100%. Renal clearance of cimetidine was unchanged ($P > 0.05$) in the aged rats (Table 7). Although the glomerular filtration rate (GFR) was decreased ($P < 0.001$), renal tubular secretion of cimetidine (Table 8), which accounts for most of cimetidine renal elimination, was preserved.

DISCUSSION

Our data in male rats provide evidence for (1) an age-related decrease in systemic clearance of aminopyrine likely due to reduced hepatic microsomal oxidation, (2) decreased metabolism of cimetidine by the aged liver with preservation of *net* renal tubular secretion of the drug, and (3) evidence for greater inhibition by cimetidine of aminopyrine metabolism in aged rats. This last finding was likely due to increased cimetidine levels rather than to increased sensitivity of the aminopyrine demethylase enzyme(s) to a fixed concentration of cimetidine.

Our demonstration of decreased aminopyrine elimination in aged male rats using the breath test, liver microsomes, and, most importantly, *in vivo* drug clearance, is consistent with data of others that in male rats the activity of hepatic demethylating microsomal enzymes falls with aging [26, 27]. This may be attributed, at least in part, to feminization of the cytochrome P-450 system, the latter showing a lower rate of drug oxidation [28–30].

The H_2-receptor antagonist, cimetidine, is largely excreted in unchanged form by the kidneys with a substantial tubular secretory component. In healthy humans only about 30–50% of the drug is extracted by the liver [31, 32], and in rats similar, but less extensive, data have been obtained [32, 33]. Thus, with decreased renal function in the aged, one might anticipate a decreased clearance of the drug since cimetidine clearance is usually reflected by creatinine clearance. Cimetidine secretion, however, appears spared in the elderly, whereas procainamide secretion is not [34, 35]. The role of aging liver in cimetidine clearance has not been assessed previously. Our data in rats clearly confirm the importance of renal excretion of cimetidine in overall (systemic) clearance of the drug. In young rats the kidneys accounted for about 70% of all drug removal. Moreover, tubular secretion contributed in a major way to this. In the aged rats, however, there was a marked decrease in hepatic clearance of the drug. This was consistently shown in liver microsomes, perfused liver and, most importantly, in animals *in vivo*. The latter finding assumes that systemic clearance corresponds primarily to *hepatic* drug clearance. The basis for impaired hepatic elimination of cimetidine with aging is uncertain. It clearly is not due to altered blood flow since the same data were obtained in isolated liver microsomes and perfused liver with a fixed perfusion rate. Thus, this appears to be due to decreased cimetidine metabolism, although the metabolites have not been quantitated by us as yet. GFR decreased in the aged rat. However, net *renal* clearance of cimetidine was not impaired since secretory clearance was unchanged with aging (Table 8). Since it is generally accepted that the entire nephron is lost with aging (i.e. filtration and secretion/reabsorption), there would appear to be enhanced cimetidine secretion in the remaining nephrons of the aged rat. This is reflected by the large increase in the ratio of cimetidine renal clearance to GFR (Table 8). This may be construed teleologically as a compensatory phenomenon for decreased hepatic drug removal and a lower GFR. This compensation, however, was insufficient to prevent a decrease in overall systemic clearance of cimetidine with aging. Whether a similar decrease in hepatic cimetidine clearance occurs in aged humans remains to be determined.

Since its approval for use in 1977, cimetidine has become one of the most widely prescribed drugs. However, in addition to its inhibitory effect on gastric acid secretion, it has been found also to inhibit the oxidative metabolism of many drugs in the liver [36, 37]. Since hepatic metabolism of cimetidine by the aged rat liver was impaired, it is not surprising that we found a greater inhibition of aminopyrine metabolism in perfused aged rat liver and the aminopyrine breath test exposed to the same cimetidine doses as young rats. The explanation of this likely was decreased cimetidine degradation and, hence, a higher cimetidine concentration in liver from aged rats. This was supported by two series of experiments. First, in perfused liver, the relationship between percent inhibition and cimetidine AUC was the same in young and aged livers (Fig. 2). Second, when liver microsomes from young and aged liver were exposed to the *same* cimetidine concentrations, inhibition of aminopyrine demethylation was essentially equal. On a percentage basis, there was no difference in inhibition (except for an increase in aged liver with the lowest cimetidine dose), whereas on an absolute basis, the aged tissue actually was inhibited less (Table 4). These data very clearly show that the aged rat liver microsomal oxidizing system

in vitro is not more susceptible to the inhibitory effects of cimetidine, as long as comparisons are made at the same concentrations of the inhibitor and are not based on initial dose given. This lack of altered sensitivity to cimetidine in aged rat liver is paralleled by recent observations in aged patients who also failed to show such effects *in vivo* [38–40].

A preliminary study *in vivo* of the effects of cimetidine on systemic aminopyrine clearance in young versus aged rats was not assessable. While the peak cimetidine blood levels at a dose of 40 mg/kg, i.p. were higher in the aged versus young rats, 31.3 ± 7.7 vs 12.9 ± 1.5 mg/ml, $P < 0.05$, as expected (Tables 7 and 8), this dose of cimetidine gave about a 75% inhibition of aminopyrine clearance, consistent with saturation of the hepatic demethylating enzyme system for this drug (Table 4). Comparative studies of drug–drug interaction with this high degree of inhibition are not likely to be helpful. Studies at a lower cimetidine dose (5 mg/kg) gave unmeasurable blood cimetidine levels. This and the lack of aged rats of this species precluded further studies.

In conclusion, the salient findings in these studies are the demonstrations (1) that aged rat liver had impaired cimetidine metabolism but preserved net renal tubular secretion of the drug, and (2) that there was no evidence for enhanced sensitivity of aged rat liver microsomal oxidizing system to cimetidine, using aminopyrine as a probe drug, although there was greater inhibition of its metabolism at a given dose of cimetidine due to decreased overall cimetidine clearance.

REFERENCES

1. N. Hurwitz, *Br. med. J.* **1**, 536 (1969).
2. D. L. Schmucker, *Pharmac. Rev.* **30**, 445 (1979).
3. World Health Organization, *Drugs* **22**, 279 (1981).
4. D. J. Greenblatt and M. D. Allen, *Br. J. clin. Pharmac.* **5**, 407 (1978).
5. D. H. Lawson, *Q. Jl Med.* **43**, 433 (1974).
6. D. Richey and A. Bender, *A. Rev. Pharmac. Toxic.* **17**, 49 (1977).
7. H. Jick, D. Slone, I. T. Borda and S. Shapiro, *New Engl. J. Med.* **279**, 284 (1968).
8. U. Klein, M. Klein, H. Sturm, M. Rothenbukler, R. Huber, P. Stucki, I. Gekalov, M. Keller and R. Hoigne, *Int. J. clin. Pharmac.* **13**, 187 (1976).
9. I. H. Stevenson, S. A. M. Salem and A. M. M. Shepherd, in *Drugs and the Elderly* (Eds. O. S. Crooks and I. H. Stevenson), p. 51. Macmillan, London (1979).
10. C. M. Castleden, C. F. George, D. Marcer and C. Hallett, *Br. med. J.* **1**, 10 (1977).
11. K. Morgan, *Lancet* **1**, 516 (1982).
12. M. M. Reidenberg, M. Levy, H. Warner, C. B. Coutinho, M. A. Schwartz, G. Yu and J. Cheripko, *Clin. Pharmac. Ther.* **23**, 371 (1978).
13. U. Klotz and K. W. Brukel, in *Liver and Aging* (Ed. K. Kitani), p. 289. Elsevier Biomedical Press, New York (1982).
14. K. O'Malley and J. Kelly, in *Liver and Aging* (Ed. K. Kitani), p. 303. Elsevier Biomedical Press, New York (1982).
15. P. V. Desmond, R. Patwardhan, R. Parker, S. Schenker and K. V. Speeg, Jr., *Life Sci.* **26**, 1261 (1980).
16. G. Henderson, J. Secor, D. Heitman and S. Schenker, *Devl Pharmac. Ther.* **9**, 201 (1986).
17. G. F. Lockwood and J. B. Houston, *J. Pharm. Pharmac.* **31**, 787 (1979).
18. R. F. Duggleby, *Analyt. Biochem.* **110**, 9 (1980).
19. W. C. Randolph, V. L. Osborne, S. S. Walkensten and A. P. Intoccia, *J. pharm. Sci.* **66**, 1148 (1977).
20. B. Lorenzo and D. E. Drayer, *J. Lab. clin. Med.* **97**, 545 (1981).
21. A. Heyrovsky, *Clin. chim. Acta* **1**, 470 (1956).
22. M. S. Lennard, G. T. Tucker and H. F. Woods, *Xenobiotica* **14**, 885 (1984).
23. M. R. Franklin and R. W. Estabrook, *Archs Biochem. Biophys.* **143**, 318 (1971).
24. A. P. Poland and D. W. Nebert, *J. Pharmac. exp. ther.* **184**, 269 (1973).
25. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
26. R. Kato, P. Vassanelli, G. Frontino and E. Chiesara, *Biochem. Pharmac.* **13**, 1037 (1964).
27. W. Klinger, *Archs int. Pharmacodyn. Thér.* **180**, 309 (1969).
28. S. Fujita, T. Uesugi, H. Kitagawa, T. Suzuki and K. Kitani, in *Liver and Aging* (Ed. K. Kitani), p. 55. Elsevier Biomedical Press, New York (1982).
29. H. Kitagawa, S. Fujita, T. Suzuki and K. Kitani, *Biochem. Pharmac.* **34**, 579 (1985).
30. T. Kanataki, K. Maeda, M. Shimada, K. Kitani, T. Nagai and R. Kato, *J. Pharmac. exp. Ther.* **233**, 222 (1985).
31. M. C. Gerber, G. Tejwani, N. Gerber and J. R. Bianchine, *Pharmac. Ther.* **27**, 353 (1985).
32. D. C. Taylor, P. R. Cresswell and D. C. Bartlett, *Drug Metab. Dispos.* **6**, 21 (1978).
33. I. M. Weiner and L. Roth, *J. Pharmac. exp. Ther.* **216**, 516 (1981).
34. M. M. Reidenberg, M. Camacho, J. Kluger and D. E. Drayer, *Clin. Pharmac. Ther.* **28**, 732 (1980).
35. D. E. Drayer, J. Romankiewicy, B. Lorengo and M. M. Reindenberg, *Clin. Pharmac. Ther.* **31**, 45 (1981).
36. K. V. Speeg, Jr., R. V. Patwardhan, G. R. Avant, M. C. Mitchell and S. Schenker, *Gastroenterology* **82**, 89 (1982).
37. R. G. Knodell, J. L. Holtzman, D. L. Crankshaw, N. M. Steele and L. N. Stanley, *Gastroenterology* **82**, 84 (1982).
38. J. F. Kelly, D. S. Tang-Liu, S. Lazo, R. A. Upton and R. L. Williams, *Clin. Pharmac. Ther.* **31**(C9), 238 (1982).
39. M. Divoll, D. R. Abernethy and D. J. Greenblatt, *Clin. Pharmac. Ther.* **31**, 218 (1982).
40. J. Feeley, L. Pereira, E. Guy and N. Hockings, *Br. J. clin. Pharmac.* **17**, 77 (1984).