635

Antipyrine kinetics in liver disease and liver transplantation

Antipyrine kinetics were studied in seven normal subjects, 10 patients with liver disease, and 13 clinically stable patients who received a liver transplant. Five patients were studied both before and after liver transplantation. Antipyrine concentrations in saliva after oral dosing were measured by HPLC. The antipyrine $t_{1/2}$ was significantly longer ($P < 0.05$) in patients with liver disease than in patients undergoing liver transplantation and normal subjects. Antipyrine clearance was not significantly different between patients undergoing liver transplantation and normal subjects, but it was significantly reduced ($P < 0.05$) in patients with liver disease. In five patients who were studied before and after liver transplantation, there was a significant ($P < 0.05$) increase in the antipyrine clearance and a marked reduction in its $t_{1/2}$ after liver transplantation. These results indicate that liver transplantation improves the drug metabolizing ability of patients with liver disease and that the oxidative metabolizing capacity of the liver in clinically stable patients after liver transplantation is similar to that of normal subjects. (CLIN PHARMACOL THER 1986;39:372-7.)

Mehul U. Mehta, M.S., Raman Venkataramanan, Ph.D., Gilbert J. Burckart, Pharm.D., Richard J. Ptachcinski, Pharm.D., Shuin L. Yang, M.D., Jeffrey A. Gray, M.D., David H. Van Thiel, M.D., and Thomas E. Starzl, Ph.D., M.D. *Pittsburgh, Pa.*

Liver disease alters the absorption and disposition of a variety of drugs.[1,2] Liver transplantation is considered to be an effective therapeutic option in certain liver diseases such as biliary atresia, sclerosing cholangitis, alcoholic cirrhosis, and primary biliary cirrhosis and in certain metabolic disorders such as α_1-antitrypsin deficiency.[3,4] After liver transplantation, patients receive a number of drugs including immunosuppressants, antihypertensives, antivirals, antifungals, and antibiotics. However, very little is known about the drug metabolizing capacity in patients after successful liver transplantation.

Our primary objective was to determine the oxidative metabolizing capacity of the liver in patients after liver transplantation and to compare this with normal subjects and patients with liver disease. We also compared oxidative drug metabolizing capacity before and after liver transplantation in the same patient by determining antipyrine kinetic parameters.

From the School of Pharmacy and School of Medicine, University of Pittsburgh.

Supported by United States Public Health Service Grant No. AM33475-01A1 and a Research and Development Grant from the University of Pittsburgh.

Received for publication Oct. 9, 1985; accepted Nov. 25, 1985.

Reprint requests to: Dr. Raman Venkataramanan, 718 Salk Hall, University of Pittsburgh, Pittsburgh, PA 15261.

METHODS

All participants gave informed, written consent before entering the study. Participants were nonsmokers and refrained from alcohol consumption for at least 1 week before and during the study. Biochemical profiles including albumin, total protein, bilirubin (total and direct), SGOT, SGPT, γ-guanosine triphosphate (γ-GTP), and alkaline phosphatase levels were determined before the study. Studies were conducted in seven normal subjects (four men and three women), 10 patients with liver disease (seven men and three women), and 13 patients (eight men and five women) who were clinically stable (total bilirubin <2.0 mg/dl) after liver transplantation. In all of the latter patients the study was conducted 1 to 2 months after transplantation, except in one patient who was studied 7 months after transplantation.

Before drug dosing blank saliva and urine samples were collected from all participants. After an overnight fast, antipyrine (600 mg) was taken by mouth with 200 ml water. Serial saliva samples (2.0 ml) were collected 2, 3, 6, 9, 12, 24, 30, and 36 hours after drug dosing

Table I. Characteristics of the participants

Participant No.	Age (yr)	Sex	Body weight (kg)	Albumin (gm/dl)	Total protein (gm/dl)	Bilirubin (mg/dl)		SGOT (IU/L)	SGPT (IU/L)	γ-GTP (IU/L)	Alkaline phosphatase (IU/L)
						Total	Direct				
Normal subjects											
1	28	M	71.0	4.5	7.5	0.6	0.1	40	27	33	93
2	33	M	51.0	4.5	7.5	1.5	0.5	33	20	22	97
3	27	M	72.5	4.9	7.6	0.7	0.2	22	14	8	60
4	35	M	72.7	4.5	7.9	0.8	0.2	38	16	12	74
5	24	F	62.0	—	—	—	—	—	—	—	—
6	23	F	59.0	4.7	7.8	1.3	0.3	19	21	10	46
7	25	F	56.8	4.7	7.5	0.7	0.3	40	14	10	37
$\bar{X}$	27.9		63.8	4.6	7.6	0.9	0.3	32	19	16	69
±SD	4.6		8.6	0.2	0.2	0.4	0.2	9	5	10	25
Patients with liver disease											
8	23	M	74.0	1.9	6.1	5.4	1.0	35	28	21	101
9	22	F	73.0	3.1	7.4	3.8	1.9	42	35	180	466
10	37	M	84.6	2.4	5.5	13.1	9.1	127	79	156	217
11	19	M	56.6	2.9	5.3	0.9	0.4	47	23	441	286
12	46	M	73.4	3.3	6.3	3.5	1.2	86	55	55	205
13	38	F	47.2	—	—	5.0	3.7	140	162	746	3635
14	57	F	86.0	—	—	2.2	0.6	121	25	219	101
15	33	M	72.0	3.0	6.5	16.6	10.0	254	161	148	240
16	44	M	100.0	2.6	6.3	5.7	4.5	141	51	256	264
17	20	M	71.7	3.4	6.2	1.1	0.3	26	44	174	129
$\bar{X}$	33.9		73.9	2.8	6.2	5.7	3.3	102	66	239	564
±SD	12.8		14.8	0.5	0.6	5.2	3.6	70	53	212	1084
Patients after liver transplantation											
8*	24	M	65.9	2.8	5.7	1.0	0.5	21	27	102	78
9*	23	F	64.7	3.7	6.2	1.6	0.9	29	64	111	108
10*	37	M	61.5	3.1	5.8	1.4	1.0	59	25	86	103
11*	19	M	56.5	2.8	5.4	0.8	0.4	27	16	357	257
12*	46	M	70.3	2.8	5.4	1.0	0.5	13	14	38	44
18	33	F	50.0	3.0	5.5	0.8	0.7	22	23	71	147
19	26	F	51.8	3.7	6.5	1.5	1.1	32	37	228	225
20	23	F	57.2	2.4	4.9	1.0	0.9	33	60	86	212
21	26	F	46.0	3.4	5.8	1.2	1.0	12	32	173	74
22	33	F	49.1	3.8	6.0	0.7	0.4	23	117	75	50
23	17	M	88.3	3.8	6.4	1.6	0.5	14	28	33	55
24	48	F	63.9	2.9	8.1	1.3	0.9	26	42	79	209
25	45	F	57.2	3.8	5.8	1.1	0.7	25	33	46	114
$\bar{X}$	30.8		60.2	3.2	6.0	1.2	0.7	26	40	114	129
±SD	10.5		11.1	0.5	0.8	0.3	0.3	12	28	91	74

M = Male; F = female.
*Also studied before transplantation as part of the liver disease group.

in all participants. Additional samples were collected at 48 and 72 hours in patients with liver disease. Salivation was stimulated by having the person chew on a piece of parafilm. Urine was collected for 72 hours. All salivary and urinary samples were stored at −20° C until analyzed for unchanged antipyrine. Antipyrine was quantitated by modifications of the HPLC procedure of Campbell et al.[5] Saliva (1.0 ml) was pipetted into 1.5 ml polypropylene vials, which were centrifuged at 15,600 × g for 5 minutes. The clear supernatants (100 to 200 μl) were pipetted into new vials to which 50 μl phenacetin solution in 5% methanol (12.5 μg/ml) and 1.0 ml methanol were added. The vials were mixed on a vortex for 20 seconds and centrifuged for

5 minutes. The clear supernatant (100 to 200 μl) was then injected onto the column. Before analysis, urine samples were centrifuged at 2000 × g for 5 minutes. One hundred microliters of a phenacetin solution in 5% methanol (0.1 mg/ml) was added to 1 ml of the clear supernatant and the mixture was shaken with 10 ml methylene chloride for 5 minutes. The resultant mixture was centrifuged at 2000 × g for 5 minutes, after which the aqueous layer was removed and the organic layer was evaporated under a stream of nitrogen. The residue was dissolved in 1.0 ml mobile phase and 100 μl of this solution was injected onto the column.

Samples were chromatographed at ambient temperature (21° to 24° C) on a 25 cm × 4 mm id stainless steel column packed with μ-Bondapak C-18 (10 μm; Waters Associates Inc.) fitted with a guard column of the same packing material. The mobile phase was acetonitrile and acetic acid (1% in water; 25:75) at a flow rate of 1.0 ml/min. Retention times for antipyrine and phenacetin were 6 and 8 minutes, respectively. The instruments used consisted of a model M-45 pump, a model U6K injector, and a model 441 fixed wavelength detector from Waters Associates Inc. Ultraviolet absorption was monitored at 254 nm. Peak heights were measured by a Hewlett-Packard Instruments integrator model 3390A and the peak height ratios were plotted against known concentrations to obtain a standard curve.

The saliva antipyrine AUC from time zero to infinity and the elimination rate constant (λ_z) from the terminal linear segment of the concentration-time curve were obtained by NONLIN '74.[6] Because antipyrine is rapidly and completely absorbed from the gastrointestinal tract,[7] it was assumed that bioavailability was 100% and that the absorption rate constant was much greater than the λ_z. Antipyrine clearance (CL) was calculated as: CL = Dose/AUC, and the apparent volume of distribution (V_{area}) was calculated as: $V_{area} = CL/\lambda_z$.

In most of the participants, analysis of the saliva samples revealed that urine was collected for approximately 7 $t_{1/2}$s. However, because of the long $t_{1/2}$ of antipyrine, urine collection in patients with liver disease was carried out only for about 2.5 $t_{1/2}$s. Based on the amount of antipyrine excreted unchanged, the duration of urine collection, and the antipyrine λ_z, the amount of antipyrine excreted unchanged up to infinity was calculated with standard pharmacokinetic equations.[8] Normality of the data obtained was tested by the Wilk-Shapiro test. Homogeneity of variances was tested by the F ratio test; the data analyzed were compared between groups by Student's t test. A P value <0.05 was considered to be significant.

RESULTS

Table I shows the demographic and biochemical profile of all the participants. Average age and body weight were similar in each of the groups studied. Comparison of the measures of hepatic injury obtained (such as bilirubin, SGOT, SGPT, γ-GTP, and alkaline phosphatase levels) between the control subjects and the patients with liver disease demonstrates the severity of the hepatic disease in these patients. However, when a comparison is made for these parameters between normal subjects and the transplant group, the functional status of the transplanted liver is very close to that in the normal subjects. Only four patients (Nos. 8, 10, 11, and 24) received drugs known to induce (phenytoin or phenobarbital), inhibit (cimetidine), or otherwise modify (levothyroxine) antipyrine kinetics.

The linear range of the antipyrine standard curve was 5 to 25 μg/ml. The coefficients of variation and accuracy were 4.0% and 106%, respectively, at 2.5 μg/ml and 5.3% and 103%, respectively, at 20 μg/ml ($n = 10$). Antipyrine was found to bind negligibly (<4%) to the polypropylene tubes used in sample preparation.

The pharmacokinetic parameters of antipyrine in the three groups are listed in Table II, along with the results of the statistical comparisons made between the three groups. Five patients (Nos. 8 to 12) were studied both before and after liver transplantation. Harmonic means were calculated for $t_{1/2}$ and CL and arithmetic means were calculated for V_{area}. The harmonic mean $t_{1/2}$ was 11.4 hours (range 9.6 to 13.5 hours) for subjects, 25.8 hours (range 12.1 to 39.2 hours) for patients with liver disease, and 10.2 hours (range 6.5 to 14.7 hours) for patients after liver transplantation. The harmonic mean CL values were 37.9 ml/min (range 27.0 to 50.7 ml/min) for subjects, 16.7 ml/min (range 12.8 to 28.0 ml/min) for patients with liver disease, and 36.6 ml/min (range 30.6 to 57.4 ml/min) for patients after liver transplantation. The antipyrine λ_z and CL were significantly smaller ($P < 0.05$) in the patients with liver disease as compared with the subjects and the patients after liver transplantation. V_{area} did not differ significantly between the three groups. Also, no differences were observed in the $t_{1/2}$ and CL between the control and the transplant groups.

Fig. 1 shows the dramatic decrease in $t_{1/2}$ in patient 8 after liver transplantation. In this patient the $t_{1/2}$ was 39.2 hours before transplantation and 13.7 hours after transplantation. In the five patients who were studied before and after transplantation, the mean CL and $t_{1/2}$ values were 16.6 ml/min and 28.3 hours before and 36.0 ml/min and 12.7 hours after transplantation. There

Table II. Antipyrine pharmacokinetics*

Participant No.	$t_{1/2}$ (hr)	CL		V_{area}	
		ml/min	ml/min/kg	L	L/kg
Normal subjects					
1	10.0	43.4	0.61	39.6	0.56
2	13.5	27.0	0.53	25.5	0.50
3	11.8	43.8	0.60	44.0	0.61
4	10.3	50.7	0.70	43.4	0.60
5	9.6	38.1	0.62	27.5	0.44
6	10.9	34.8	0.59	36.1	0.61
7	10.2	37.1	0.65	32.8	0.58
$\bar{X}$†	11.4	37.9	0.61	35.6 ± 7.3	0.56 ± 0.06
Patients with liver disease					
8	39.2	13.9	0.19	47.1	0.64
9	32.7	16.9	0.23	47.8	0.65
10	16.5	28.0	0.33	40.0	0.47
11	34.8	12.8	0.23	38.7	0.68
12	31.8	16.0	0.34	48.9	0.67
13	12.1	21.7	0.46	22.7	0.48
14	44.0	12.8	0.15	44.0	0.51
15	35.9	15.8	0.22	49.3	0.68
16	38.1	18.6	0.19	61.2	0.61
17	19.8	20.3	0.28	34.8	0.49
$\bar{X}$	25.8	16.7	0.24	43.5 ± 10.3	0.59 ± 8.09
Patients after liver transplantation					
8‡	13.7	35.6	0.54	42.1	0.64
9‡	11.4	30.6	0.47	30.2	0.47
10‡	11.8	34.8	0.57	35.6	0.63
11‡	10.9	57.4	1.0	54.1	0.77
12‡	14.7	31.1	0.44	39.7	0.56
18	11.5	31.5	0.63	32.6	0.65
19	6.5	44.5	0.86	23.5	0.45
20	9.9	37.2	0.65	29.3	0.51
21	8.9	38.6	0.84	29.6	0.64
22	11.6	31.0	0.63	27.9	0.57
23	12.9	36.9	0.42	41.1	0.49
24	6.6	54.8	0.86	31.1	0.49
25	10.5	32.0	0.56	27.2	0.48
$\bar{X}$†	10.2	36.6	0.61	33.5 ± 8.9	0.57 ± 0.1

Data are $\bar{X}$ ± SD.
*Harmonic mean for $t_{1/2}$ and CL; arithmetic mean for V_{area}.
†$P < 0.05$ compared with patients with liver disease.
‡Also studied before transplantation as part of the liver disease group.

was a significant increase in CL ($P < 0.05$) and the λ_z after liver transplantation, but there was no such change in V_{area}. Table III lists the results of the urinalyses, presented as the percentage of dose excreted as unchanged drug. This information could not be obtained in some of the patients because of incomplete urine collection or the presence of compounds that interfered with the assay procedure. There was no significant difference between the transplant group and the control group in the cumulative amount of antipyrine excreted in the urine. Compared with values before transplantation, the amount of antipyrine excreted unchanged in the urine is smaller after transplantation.

DISCUSSION

Liver disease in humans results in a variety of pathophysiologic disturbances that include extra- or intrahepatic shunting of blood, hepatocyte dysfunction, qualitative and quantitative changes in serum proteins, and changes in hepatic blood flow and biliary secretion.

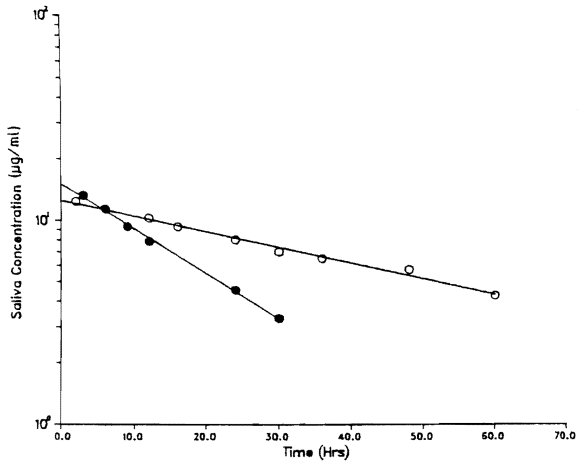

Fig. 1. Antipyrine salivary levels before (○) and after (●) liver transplantation in patient 8.

Table III. Percentage of dose administered excreted as unchanged antipyrine in urine

	Control subjects	*Patients with liver disease*		*Patients after liver transplantation*
		Actual values	*Predicted values*	
	4.4	5.5	7.8	2.9
	2.2	11.7	15.8	4.3
	4.4	19.9	28.6	7.3
	5.0	7.6	9.9	3.3
	3.2	4.3	6.6	2.8
	2.4	—	—	—
	3.9	—	—	—
$\bar{X}$	3.6*	9.8	13.7	4.1*
±SD	1.1	6.3	9.0	1.9

*P < 0.05 compared with patients with liver disease.

One of the many end results of such disturbances is the altered disposition of a variety of drugs in patients with liver disease. Recently, liver transplantation has become a promising therapeutic option for the treatment of certain liver diseases.[3,4] We have used antipyrine as a model drug to study the drug metabolizing status of the liver in patients with advanced liver disease and in patients after liver transplantation.

Antipyrine has been used extensively as a model drug for the assessment of the oxidative metabolizing capacity of the liver.[7] It is an ideal drug for such studies because it is completely and rapidly absorbed from the gastrointestinal tract, is distributed in total body water with negligible binding to tissue or plasma proteins, is almost quantitatively metabolized in the liver,[7] and is a low-clearance drug with single-compartment kinetics.[9] Moreover, the clearance of antipyrine increases significantly in patients who receive enzyme-inducing agents such as phenytoin[10,11] and is reduced significantly in patients with liver disease as well as in patients receiving drugs that inhibit microsomal enzyme systems such as cimetidine.[12,13]

The reported antipyrine arithmetic $\bar{X}$ (±SD) CL and $t_{1/2}$ values in normal subjects are 38.4 ± 4.4 ml/min and 10.3 ± 0.6 hours.[12] The reported antipyrine CL values in patients with liver disease range from 22.8 ± 3.2 to 10.3 ± 1.6 ml/min and the $t_{1/2}$ values range from 14.5 ± 1.1 to 53.1 hours.[12] Our results in normal subjects and in patients with liver disease are in agreement with reported values. Whereas antipyrine CL was significantly lower in patients with liver disease as compared with normal subjects, there was no significant difference in V_{area} between these two groups. Drug metabolizing activity is therefore significantly lower in patients with liver disease than in normal subjects. A comparison of the patients who received liver transplant and the control subjects reveals that there were no significant differences in the $t_{1/2}$, CL, and V_{area} values for these two groups. The oxidative metabolizing capacity of the patients after liver transplantation, as indicated by antipyrine kinetics, appears to be similar to that of normal subjects. Therefore, drugs that undergo in vivo metabolism by the same pathways as antipyrine[14,15] should behave similarly in normal subjects and in stable patients after liver transplantation. Most importantly, after successful liver transplantation patients who previously had liver disease eliminate antipyrine in a normal manner. This is apparent from the marked increase in antipyrine CL after transplantation in these patients.

Observations based on the salivary data are substantiated by the urinary data. Table III shows that there are no significant differences in the amount of antipyrine (as percentage of the dose) excreted unchanged between the control and the transplant groups. Because antipyrine appears to be metabolized to the same extent in these two groups, urinary data suggest that antipyrine is being absorbed to the same extent in the transplant and the control groups.

Four of the patients studied were taking drugs that could potentially alter antipyrine metabolism. However, data analysis that omitted these patients yielded the same conclusions as discussed earlier.

It has recently been reported that steroids induce[16] while cyclosporine inhibits[17] drug metabolism. In our transplant group the oral cyclosporine dosage used

ranged from 200 to 800 mg b.i.d., while the cyclosporine trough blood levels as measured by RIA ranged from 261 to 1731 ng/ml. The prednisone dosage ranged from 10 to 20 mg/day. Neither of these two variables correlated significantly with antipyrine $t_{1/2}$ or CL. It is possible that the steroids and cyclosporine received by these patients might have opposite effects on the CL and $t_{1/2}$ of antipyrine, resulting in the lack of correlation observed between these variables and the antipyrine CL or $t_{1/2}$. Our results should therefore be interpreted with some caution.

Nonetheless, the patient who has received a liver transplant provides a unique model for the examination of the effect of liver disease on drug disposition, by allowing the study of drug kinetics before and after transplantation in the same patient. Our results indicate that oxidative drug metabolism in clinically stable patients after liver transplantation is comparable to that found in normal subjects as determined by antipyrine pharmacokinetics. We have also shown that liver transplantation improves oxidative metabolism in patients with liver disease.

References

1. Pesayre D, Lebrec D, Descatorie V, Peignoux M, Benhamon JP. Mechanisms for reduced drug clearance in patients with cirrhosis. Gastroenterology 1978;74:566-71.
2. Williams RL, Mamelok RD. Hepatic disease and drug pharmacokinetics. Clin Pharmacokinet 1980;5:528-647.
3. Malatack JJ, Zitelli BJ, Gartner JC, Shaw BW, Iwatsuki S, Starzl TE. Pediatric liver transplantation under therapy with cyclosporin A and steroids. Transplant Proc 1983; 15:1292-6.
4. Starzl TE, Iwatsuki S, Van Thiel DH, et al. Report of Colorado-Pittsburgh liver transplantation studies. Transplant Proc 1983;15:2582-5.
5. Campbell TM, Murdaugh EW, Killenberg PG. Determination of antipyrine in plasma by reversed-phase high-performance liquid chromatography. J Chromatogr 1979; 163:236-8.
6. Metzler CM. NONLIN a computer program for parameter estimations in nonlinear situations. Users manual. Kalamazoo, Mich: The Upjohn Company, 1974.
7. Vessel ES. The antipyrine test in clinical pharmacology: conceptions and misconceptions. CLIN PHARMACOL THER 1979;26:275-86.
8. Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, Inc, 1982:10.
9. Huffman DH, Shoeman DW, Azarnoff DL. Correlation of the plasma elimination of antipyrine and the appearance of 4-hydroxy antipyrine in the urine of man. Biochem Pharmacol 1974;23:197-201.
10. Welch RM, DeAngelis RL, Wingfield M, Farmer TW. Elimination of antipyrine from saliva as a measure of metabolism in man. CLIN PHARMACOL THER 1975;18: 249-58.
11. Perucca E, Hedges A, Makki KA, Richens A. A comparative study of antipyrine and lignocaine disposition in normal subjects and in patients treated with enzyme-inducing drugs. Br J Clin Pharmacol 1980;10:491-7.
12. Branch RA, James J, Read AE. The clearance of antipyrine and indocyanine green in normal subjects and in patients with chronic liver disease. CLIN PHARMACOL THER 1976;20:81-9.
13. Dossing M, Pilsgaard H, Rasmussen B, Enghusen Poulsen H. Time course of phenobarbital and cimetidine mediated changes in hepatic drug metabolism. Br J Clin Pharmacol 1983;25:215-22.
14. Kapitulnik J, Poppers PJ, Conney AH. Comparative metabolism of benzo[a]pyrine and drugs in human liver. CLIN PHARMACOL THER 1977;21:166-76.
15. Hepner GW, Vessel ES, Lipton A, Harvey HA, Wilkinson GR, Schenker S. Disposition of aminopyrine, antipyrine, diazepam and indocyanine green in patients with liver disease or on anticonvulsant drug therapy: diazepam breath test and correlations in drug elimination. J Lab Clin Med 1977;90:440-56.
16. Shukla VK, Garg SK, Mathur VS. Influence of prednisolone on antipyrine and chloramphenicol disposition in rabbits. Pharmacology 1984;29:117-20.
17. Ost L, Kilntmalm G, Ringden O. Mutual interaction between prednisone and cyclosporine in renal transplant patients. Transplant Proc 1985;17:1252-5.