TRIMETHADIONE TOLERANCE TEST FOR EVALUATION OF FUNCTIONAL RESERVE OF THE LIVER IN PATIENTS WITH LIVER CIRRHOSIS AND ESOPHAGEAL VARICES

EINOSUKE TANAKA,* AKIO ISHIKAWA,** ATSUSHI ONO, TAKAO OKAMURA,*** SHINICHI KOBAYASHI, HAJIME YASUHARA⁴* AND SHOGO MISAWA*

Department of Legal Medicine, Institute of Community Medicine, and Department of Surgery, Institute of Clinical Medicine,** The University of Tsukuba and Tsukuba Gakuen Hospital,*** Ibaraki-ken, 305, Japan and Department of Pharmacology, School of Medicine, Showa University,⁴ * Tokyo, 142, Japan*

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Dimethadione (DMO)/trimethadione (TMO) ratios in serum after oral administration of TMO were investigated in 15 normal subjects and in 20 patients with cirrhosis and esophageal varices. Severe impairment of liver function was associated with a decrease in total cholesterol, total protein and plasma albumin, or an increase in total bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum γ -glutamyl transpeptidase and indocyanine green retention rate (ICG R_{15}). Serum concentration ratios of DMO to TMO at 2 or 4 h after oral administration of TMO in patients were significantly decreased by 67 or 66%, respectively, compared to normal subjects. DMO/TMO ratios at 2 or 4 h following oral administration of TMO were well correlated with the liver function parameters (plasma albumin $r=0.758$ at 2 h, $r=0.776$ at 4 h; total protein $r=0.613$ at 2 h, $r=0.619$ at 4 h; ICG R_{15} $r=-0.683$ at 2 h, $r=-0.746$ at 4 h, in patients only; cholinesterase $r=0.873$ at 2 h, $r=0.908$ at 4 h) as well as with pharmacokinetic parameters (total body clearance $r=0.794$ at 2 h, $r=0.786$ at 4 h) in both the normal subjects and the patients. It suggests that the DMO/TMO ratio obtained in a single blood sample collected after oral administration of TMO might provide a useful measure for function hepatic reserve.

Keywords — liver functional reserve; cirrhosis varix; esophageal varix; trimethadione; dimethadione; drug metabolizing capacity

INTRODUCTION

Recently, a number of papers in the field of surgical and internal medicine have dealt with the methodology for measuring the complicated function of the human liver and estimating the functional reserve of the liver. The methods most frequently used in those investigations were routine laboratory liver function tests, and various tolerance tests for galactose,^{1–3)} indocyanine green (ICG),^{4–7)} antipyrine^{8–11)} and aminopyrine.^{12,13)} These different tolerance tests do not always give equal results. Even in the intraindividual clinical evaluation, divergent results are often obtained from different methods of testing. Liver disease is usually diagnosed by evaluating the results of several tolerance tests and other laboratory tests together. Establishment of more precise method has been sought. In the present study, we have estimated the functional reserve of the liver by using trimethadione (TMO) as an indicator. Previously, we determined blood levels of TMO and its only metabolite dimethadione (DMO) 1 and 2 h after admin-

istration of TMO, and demonstrated good correlation between the ratio of DMO to TMO and the hepatic drug-oxidizing capacity of the animals.^{14–21)} We suggested that the human liver function might also be estimated by analyzing a single blood sample collected at an appropriate interval after administration of TMO. With this background, we carried out a TMO tolerance test in patients with cirrhosis and esophageal varices to evaluate the adequacy of this method for estimation of the functional reserve of the liver.

MATERIALS AND METHODS

Materials — The TMO used was commercially available as a 66.7%-pure powder (Mino-Aleviatin®: Dainippon Pharmaceutical Co., Ltd. Osaka, Japan).

Clinical Study — The study was performed at the Tsukuba Gakuen Hospital, Ibaraki, Japan. Twenty patients with cirrhosis and esophageal varices, who gave their informed consent, participated in this study. Diagnosis was established

by esophagofiberscope, ultrasonography and laboratory tests including total cholesterol, total protein, plasma albumin, total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum γ -glutamyl transpeptidase (SGGTP), and cholinesterase. Most of the patients were treated with one or more (up to 5) of the following drugs: sucralfate, aceglutamide aluminium, glutathione, spironolactone, aluminium hydroxide, magnesium hydroxide and vitamins.

Twelve healthy volunteers were collected from the university staff served as controls. The mean laboratory data and liver function data are listed in Table I.

The study was performed in the morning after a 12 h fast. TMO 4 mg/kg was given orally with 100 ml water, followed by breakfast 2 h later. Blood samples (0.5 ml) for estimation of TMO and DMO were collected at 0.5, 1, 2 and 4 h after administration of TMO, and centrifuged. The serum was separated and frozen at -20°C until assays were performed.

TMO and DMO Assay — Serum TMO and DMO levels were determined by a gas-liquid chromatographic (GLC) method using paramethadione as an internal standard, as reported previously.²²⁾

Pharmacokinetic Studies — Concentration-time curves for TMO and DMO levels were drawn on semilogarithmic scales. The half-life ($t_{1/2}$) and elimination rate constant (K_e) were calculated by a linear regression analysis. The ap-

parent volume of distribution (V_d) was calculated from the ratio of the administered dose to the concentration extrapolated to time zero.

Total body clearance (CL) was calculated according to the equation $\text{CL} = 0.693 \cdot V_d / t_{1/2}$.

Indocyanine Green (ICG) Retention Rate — ICG retention rate (R_{15}) was measured following intravenous administration of ICG 0.5 mg/kg and the collection of venous blood samples from the cubital vein of the opposite arm was performed at 15 min after administration of ICG. R_{15} was calculated according to the equation:

$$R_{15} = \frac{C}{1.00} \times 100 (\%)$$

C_{15} : plasma ICG concentration (mg/dl) at 15 min.

1.00 : mean plasma ICG concentration (mg/dl) at 0 min.

Statistical Analysis — Results are expressed as means $\pm$ S.E.M. The statistical comparison between the different groups was made by Cochran-Cox's t -test or Student's t -test.

RESULTS

Laboratory data including liver function data in normal subjects and patients with cirrhosis and esophageal varices are shown in Table I. The liver function parameters showed significant difference between the normal subjects and the patients except for SGGTP.

The results of pharmacokinetic parameters

TABLE I. *Clinical and Liver Function Characteristics in Normal Subjects and in Patients with Cirrhosis and Esophageal Varices*

<i>n</i> (Male/female)	Controls 15 (13/2)	Patients 20 (16/4)	Normal Laboratory values
Age (years)	46 $\pm$ 5	45 $\pm$ 3	—
Body weight (kg)	61.1 $\pm$ 1.9	57.5 $\pm$ 2.3	—
Total cholesterol (mg/dl)	166 $\pm$ 9.7	130 $\pm$ 9.2 ^{a)}	130–230
Total protein (g/dl)	7.6 $\pm$ 0.1	6.7 $\pm$ 0.1 ^{a)}	6.5–8.2
Plasma albumin (g/dl)	4.7 $\pm$ 0.1	3.4 $\pm$ 0.1 ^{a)}	3.7–5.2
Total bilirubin (mg/dl)	0.6 $\pm$ 0.1	1.4 $\pm$ 0.2 ^{a)}	0.2–1.0
SGOT (U/l)	17.7 $\pm$ 1.7	56.3 $\pm$ 9.9 ^{a)}	7–38
SGPT (U/l)	13.6 $\pm$ 2.2	54.3 $\pm$ 9.6 ^{a)}	8–40
SGGTP (mU/ml)	18.9 $\pm$ 3.1	25.8 $\pm$ 5.8	2–29
Cholinesterase (Δ pH)	0.79 $\pm$ 0.03	0.38 $\pm$ 0.03 ^{a)}	0.6–1.1
ICG R_{15} (%)	ND	31.1 $\pm$ 3.9	<10

Mean values $\pm$ S.E.M. ND: not detected.

a) Significantly different from control at $p < 0.01$.

TABLE II. Pharmacokinetic Parameters Determined Following Oral Administration of Trimethadione in Normal Subjects and in Patients with Cirrhosis and Esophageal Varices

Parameter	Controls (<i>n</i> = 15)	Patients (<i>n</i> = 20)
$t_{1/2}$ (h)	11.1 $\pm$ 1.1	25.3 $\pm$ 4.4 ^{a)}
V_d (l/kg)	0.89 $\pm$ 0.03	0.66 $\pm$ 0.04 ^{b)}
CL (ml/kg/h)	60.2 $\pm$ 5.2	26.2 $\pm$ 3.3 ^{b)}

$t_{1/2}$, half-life; V_d , apparent volume of distribution; CL, total body clearance. Mean values $\pm$ S.E.M. a) Significantly different from controls at $p < 0.05$ b) Significantly different from controls at $p < 0.01$.

FIG. 1. The DMO/TMO Ratios at 2 and 4 h after Oral Administration of TMO 4 mg/kg in Normal Subjects and Patients

a) $p < 0.01$. Mean values $\pm$ S.E.M. $\square$, control subjects; $\square$ (stippled), patients.

determined following oral administration of TMO in normal subjects and patients are shown in Table II. TMO $t_{1/2}$ was 2.3 times longer in the patients than in the normal subjects (25.3 $\pm$ 4.4 vs. 11.1 $\pm$ 1.1, mean $\pm$ S.E.M., $p < 0.05$). In the patients, V_d or CL was significantly decreased by 25% (0.89 $\pm$ 0.03 vs. 0.66 $\pm$ 0.04, $p < 0.01$) or by 56% (60.2 $\pm$ 5.2 vs. 26.2 $\pm$ 3.3, $p < 0.01$), respectively. As shown in Fig. 1, the DMO/TMO ratios at 2 or 4 h after oral administration of TMO 4 mg/kg in patients were significantly decreased by 67% (0.513 $\pm$ 0.049 vs. 0.171 $\pm$ 0.016, $p < 0.01$) or 66% (0.772 $\pm$ 0.072 vs. 0.265 $\pm$ 0.027, $p < 0.01$) respectively, compared to the normal subjects. Correlation coefficients between DMO/TMO ratios at 2 or 4 h and laboratory data in the normal subjects and patients are shown in Table III and Fig. 2. DMO/TMO ratios were well correlated with CL, total protein, plasma albumin, cholinesterase (Fig. 2) or ICG R_{15} , whereas the correlation was

TABLE III. Correlation between DMO/TMO Ratios at 2 or 4 h Determined Following Oral Administration of Trimethadione and Liver Function Parameters in Normal Subjects and in Patients with Cirrhosis and Esophageal Varices

	2 h	4 h
Pharmacokinetic parameters		
$t_{1/2}$	$r = -0.461^b)$	$r = -0.488^c)$
CL	$r = 0.794^c)$	$r = 0.786^c)$
Liver function characteristics		
Total cholesterol	$r = 0.420^b)$	$r = 0.387^b)$
Total protein	$r = 0.613^c)$	$r = 0.619^c)$
Plasma albumin	$r = 0.758^c)$	$r = 0.776^c)$
Total bilirubin	$r = -0.459^b)$	$r = -0.476^c)$
Cholinesterase	$r = 0.873^c)$	$r = 0.908^c)$
SGOT	$r = -0.506^c)$	$r = -0.509^c)$
SGPT	$r = -0.460^b)$	$r = -0.471^c)$
ICG $R_{15}^a)$	$r = -0.683^c)$	$r = -0.746^c)$

a) Only patients.

b) $p < 0.05$.

c) $p < 0.01$.

FIG. 2. The Correlations between DMO/TMO Ratios at 2 h (A) or 4 h (B) Determined Following Oral Administration of Trimethadione and Cholinesterase in Normal Subject and in Patients with Cirrhosis Esophageal Varices
○, control; ●, patient.

poor for total cholesterol, total bilirubin, SGOT, SGPT and $t_{1/2}$.

DISCUSSION

In the present study, we evaluated the adequacy of the TMO tolerance test for estimation of the functional reserve of the liver in patients with cirrhosis and esophageal varices. It is advantageous to know the size of the functional reserve of the liver before starting internal or surgical treatment. Routine liver function tests which contribute to judgement of the severity of liver cirrhosis include tolerance tests for galactose,¹⁻³⁾ ICG,⁴⁻⁷⁾ antipyrine⁸⁻¹¹⁾ and aminopyrine.^{12,13)} Total protein, serum albumin, cholinesterase, transaminase (glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), and prothrombin time are also used for this purpose. None of these methods, however, can be used to precisely estimate the functional reserve of the liver because of the complicated nature of the procedure. In recent years, the antipyrine half-life or its clearance is most widely used as an index of the hepatic drug-metabolizing capacity *in vivo*.²³⁾ It is questionable, however, whether antipyrine can offer a reliable index of oxidative drug metabolizing

capacity in a given individual subject. Many investigators have attempted to use antipyrine elimination rates to accurately predict the rates of oxidative metabolism of other drugs.¹⁰⁻¹³⁾ However, the correlations between antipyrine elimination rates and the rates of oxidative metabolism of other drugs were generally poor, not statistically significant and consequently of no value for the predictions. Thus, the overall elimination rate of antipyrine appears to have a rather disappointing value as a reliable index of an individual's capacity for oxidizing drugs. There are several factors which may explain this, including those related to the choice of the optimal pharmacokinetic parameters to be used for analyzing these correlations. However, the most important factor is probably related to the heterogeneity of the mixed function oxidase system which is the enzyme system that catalyzes the oxidation of drugs.²⁴⁾ Galactose is utilized as an energy source in the body. The results of the tolerance test using these substrates is rather sensitive to endogenous intervention, for example, by pancreatic hormones. In some instances, the results may not reflect the functional reserve of the liver but reflect the energy metabolism which proceeds in response to physiological

demands. These tolerance tests are excellent in their reproducibility. The ICG tolerance test has been widely used in recent years, but there are some problems with this test. One problem is the effect of hepatic blood flow on the result of the ICG tolerance test.⁵⁾ Another is the infrequent occurrence of abnormally deviated ICG $R_{\max}$ values.⁹⁾

In the present study, we have chosen TMO as an indicator compound for estimating the functional reserve of the liver. This compound is chemically neutral, exclusively metabolized to DMO by cytochrome P-450-dependent monooxygenase in liver microsomes and is almost completely absorbed from the gastrointestinal tract when given orally.²⁵⁾ Neither TMO nor DMO is bound to plasma protein. Referring to these properties of TMO which meet the requirements proposed for a hepatic function indicator by Branch²⁶⁾ or Park,²⁷⁾ we used this compound as a model drug, and previously investigated the serum concentration ratios of DMO/TMO as an index of hepatic drug-oxidizing capacity in rats¹⁴⁻²¹⁾ and humans.²⁸⁾ In our previous studies using rats with the liver experimentally damaged by allyl alcohol,²¹⁾ brombenzen,²¹⁾ carbon tetrachloride,¹⁶⁾ galactosamine,¹⁸⁾ or α -naphthylisothiocyanate,¹⁹⁾ the DMO/TMO concentration ratios in blood or saliva collected at an interval after administration of TMO were determined, and it was demonstrated that these ratios reflected the changes in the metabolic capacity or the functional reserve of the damaged liver. In the present study, we found significant differences in laboratory data except for SGGTP and ICG R_{15} between the patients and the normal subjects. DMO/TMO ratios determined at 2 or 4 h showed good correlation with the following laboratory and pharmacokinetic data: CL, total protein, plasma albumin, cholinesterase, or ICG R_{15} . Thus, the results of our TMO tolerance test proved to be well correlated with other current methods in most frequent use. It suggests that the DMO/TMO ratio obtained in a single blood sample collected after oral administration of TMO might provide a useful measure for functional hepatic reserve. Our method has some advantages over other methods used for the same purpose. It requires only one occasion of blood sampling at either 2 or 4 h after administration of TMO, and gives the result within a short

period of time (within 30 min). Estimation of TMO and DMO by means of GLC²²⁾ or high-performance liquid chromatography (HPLC)²⁹⁾ augments the quality of the method. Thus, the method reported herein is considered to be a qualified method for estimating the functional reserve of the liver. What advantages or disadvantages this method has and what types of liver damage can be exactly predicted by this method should further be investigated.

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