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Metabolism and Pharmacokinetics of S-(N,N-Diethyldithiocarbamoyl)-N-acetyl-L-cysteine in Rats

Byung-Hoon Lee, Yun-Seon Song, Jongsei Park and Jae-Chun Ryu

Doping Control Center, Korea Institute of Science and Technology P.O. BOX 131, Cheongryang, Seoul 130-650, Korea

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The metabolism and pharmacokinetics of a mixed disulfide S-(N,N-diethyldithiocarbamoyl)-N-acetyl-L-cysteine (AC-DDTC) were studied in rats. Two metabolites of AC-DDTC following iv and po administration were identified in plasma and liver by HPLC and GC, namely N,N-diethyldithiocarbamate (DDTC) and the methyl ester of DDTC (Me-DDTC). AC-DDTC was very unstable *in vivo* and could not be detected neither in plasma nor in urine. Pharmacokinetic parameters of DDTC following intravenous administration of AC-DDTC (20 mg/kg) were calculated. DDTC has a low affinity to rat tissue and the total body clearance was 9.0± 3.4 ml/min/kg. The mean residence time (MRT) was 111.5± 16.3 min. After oral administration of 20 mg/kg AC-DDTC, maximal plasma concentration (C_{max}) was 3.8± 0.2 nmol/ml and the bioavailability was 7.04%. C_{max} for DDTC at a dose of 120 mg/kg. AC-DDTC was 40.1± 2.2 nmol/ml. MRT was 47.1± 2.8 min at a dose of 20 mg/kg and 110.5± 6.0 min at 120 mg/kg.

Key words: S-(N,N-diethyldithiocarbamoyl)-N-acetyl-L-cysteine, Mixed Disulfide, Disulfiram, Metabolism, Pharmacokinetics, High Performance Liquid Chromatography, Gas Chromatography.

INTRODUCTION

Disulfiram (tetraethylthiuram disulfide; DSF, see Fig. 1), a drug used in the treatment of alcoholism in man, is known to possess cancer chemopreventive activity. A partial or complete blocking of chemically induced carcinogenesis has been reported when DSF was concomitantly administered with carcinogens (Wattenberg, 1975; Malejka-Giganti *et al.*, 1980). DSF is rapidly metabolized *in vivo* to its reduced form, N,N-diethyldithiocarbamate (DDTC) (Strömme, 1963). DDTC is conjugated with glucuronic acid (Kaslander, 1963) or methylated to its methyl ester S-methyl-N,N-diethyldithiocarbamate (Me-DDTC) (Gessner and Jakubowski, 19 72). Me-DDTC is oxidized to its monothio-analogue by cytochrome P450 monooxygenase (Hart *et al.*, 1990) or further metabolized to sulfate ion and formaldehyde. An alternative metabolic pathway is the cleavage of DDTC to diethylamine and carbon disulfide (Faiman *et al.*, 1978).

Correspondence to: Jae-Chun Ryu, Doping Control Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea

DSF is known to react with sulfhydryl groups containing amino acids or proteins to form mixed disulfides via thiol-disulfide exchange reaction (Strömme, 1965a, 1965b). In the case of some thiol containing compounds, such as captopril and meso-2,3-dimercaptosuccinic acid, mixed disulfides with cysteine, N-acetylcysteine and glutathione are identified as urinary metabolites in rats, dogs and humans (Ikeda *et al.*, 1981; Maiorino *et al.*, 1989). Vallari and Pietruszko (1982) suggested that the inhibition of human aldehyde dehydrogenase by DSF is attributed to this type of reaction. Sany and Weiner (1987) also proposed the possible role of mixed disulfide formation of DSF on the inhibition of horse liver mitochondrial aldehyde dehydrogenase.

A series of mixed disulfides of DSF have been synthesized as model compounds in order to elucidate whether they may play a decisive role in the anticarcinogenic activities of the parent compound (Rajca *et al.*, 1990a). It was also of interest to investigate the possibility that mixed disulfides may serve as a new chemical class of chemopreventive agents like sugar-linked DSF derivatives (Lee *et al.*, 1994a). Lee *et al.* (1994b) found some positive effects on the metabo-

lism and genotoxicity of N-nitrosodiethylamine by mixed disulfides of DSF with N-acetyl-L-cysteine and glutathione. Recently, our laboratory reported on the kinetic behavior of stanozolol, an anabolic steroid, in rats using coupled gas chromatography-mass spectrometry (Ryu *et al.*, 1992). The present study was undertaken to investigate the metabolism and pharmacokinetic properties of a mixed disulfide S-(N,N-diethyldithiocarbamoyl)-N-acetyl-L-cysteine (AC-DDTC) in rats.

MATERIALS AND METHODS

Reagents and Chemicals

DSF, DDTC sodium salt (Na-DDTC), N-acetyl-L-cysteine were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Organic solvents for high performance liquid chromatography (HPLC) and for extraction were either of HPLC grade or distilled before use. All other chemicals and reagents used were of the highest purity available. The synthesis of AC-DDTC was performed in our laboratory according to the method previously reported (Rajca *et al.*, 1990a). Three alkyl esters of DDTC (methyl, ethyl and n-propyl) were prepared by reacting alkyl halide with an equimolar quantity of Na-DDTC in EtOH. The yellow oily layer in the lower phase was dried and distilled as described by Masso and Kramer (1981). NMR spectroscopy was used to confirm the identity of the products and the purity was assessed by HPLC and gas chromatography (GC).

Animals

Specific pathogen free male Sprague-Dawley rats weighing 150-200 g were obtained from Genetic Engineering Research Institute, Korea Institute of Science and Technology. The animals were housed in an air-conditioned room having a 12 h light/dark cycle and were acclimatized to the animal facility and environment for at least 1 week before use. All animals were maintained ad libitum on a standard laboratory chow and tap water. They were randomly allocated to the experimental groups.

Apparatus and Chromatographic Conditions

HPLC analysis was performed with a system composed of a Waters 590 Pump, Waters 712 WISP Automatic Sample Injection System, Waters 745 Data Module (Waters Associates ,Milford, MA, USA) and M720 Absorbance Detector (Young-In Scientific Co., Korea). A μBondapak phenyl column (300×3.9 mm; Waters Associates, Milford, MA, USA) was used throughout the study. The mobile phase (flow rate 1 ml/min) employed was a mixture of MeOH and 7.9 mM Na_2HPO_4+5 mM NaH_2PO_4 in H_2O (pH 7.4) (20 : 80) for AC-DDTC and acetonitrile and H_2O (35 : 65) for the alkyl ester of DDTC. The eluent of AC-DDTC and alkyl esters of DDTC were detected at 254 nm and 280 nm, respectively.

GC was carried out with Hewlett Packard Model 5890, equipped with a DB 5 fused-silica capillary column (30 m×0.32 mm ID; 1 mm film thickness; J&W Scientific, USA) and a nitrogen phosphorus detector. Helium was used as a carrier gas with a total flow of 15.7 ml/min. Injector port and detector temperature were set at 240℃ and 250℃, respectively. Oven temperature was raised from 90℃ to 150℃ at a rate of 15℃/min and to 300℃ at a rate of 30℃/min. Final temperature of 300℃ was maintained for 10 min.

Derivatization and Extraction Procedures

For the determination of AC-DDTC, a plasma sample was deproteinized with 15% $ZnSO_4$+saturated Ba $(OH)_2$ and subsequently with MeOH and directly analyzed using HPLC. For the analysis of metabolites, an aliquot of plasma (100-200 μl) was mixed with the same volume of 0.05 M Tris buffer (pH 8.5) with 0.01 M EDTA which contains Prop-DDTC as the internal standard. Ethyl iodide (5-10 μl) was added and incubated at 40℃ for 30 min. Zinc sulfate powder and diethyl ether were added, shaken for 10 min and then centrifuged for 10 min at 1200 g. The Ether layer was removed, dried with sodium sulfate, 1 ml of MeOH was added and the mixture was conc. concentrated to a final volume of about 100 μl. Aliquots were injected onto the column for chromatographic analysis.

Analysis of Metabolites

AC-DDTC (120 mg/kg) was administered to overnight fasted rat by gavage. Thirty minutes after the administration, the rat was sacrificed by decapitation and the liver was removed. The liver was washed with 0.05 M Tris-EDTA buffer (pH 8.5) and homogenized in the same buffer with Ultra-Turrax tissuemizer (Janke & Kunkel GmbH, Staufen i. Br., Germany). The liver homogenate was centrifuged (1200 g; 10 min at 4℃) and the supernatant was analyzed for metabolites. The derivatization and extraction process was the same as the method used for the plasma sample except that the ether extract was washed extensively with 0.1 M carbonate buffer (pH 11).

Pharmacokinetics

Three rats in each group were anesthetized with Entobar (Hanlim Pharm. Co., Korea) and surgically fitted with catheters in the femoral vein and artery for drug administration and blood sampling. AC-DDTC (20 mg/kg, iv; 120 mg/kg, po) was administered and blood samples were collected in an heparinized tube at the times indicated in Fig. 6, Fig. 7 and Fig. 8. The blood

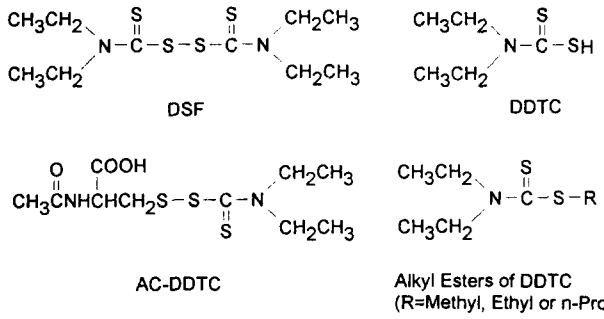

Fig. 1. Structural formulae of disulfiram (DSF), N,N-diethyldithiocarbamate (DDTC), mixed disulfide of DDTC with N-acetyl-L-cysteine (AC-DDTC) and alkyl esters of DDTC.

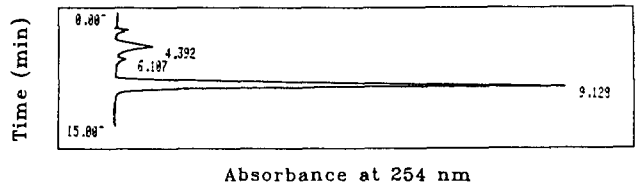

Fig. 2. HPLC chromatogram of AC-DDTC standard solution. Conditions: μBondapak phenyl column; mobile phase MeOH-7.9 mM Na_2HPO_4+5 mM NaH_2PO_4 (pH 7.4) in ratio of 20:80; Flow rate 1.0 ml/min; UV 254 nm.

was centrifuged (1200 g, 5 min at 4℃) and the plasma was stored at −20℃ until analysis.

Pharmacokinetic Analysis

For the intravenous data, the pharmacokinetic analysis used the PKCALC program (Shumaker, 1987). The area under the curve (AUC) was calculated according to the trapezoidal rule. The area under the first moment of the curve (AUMC), defined as the area under the curve of the product of time and plasma concentration from zero to infinity was also calculated with the aid of the trapezoidal rule. Total body clearance (CL) was calculated as dose divided by AUC. Volume of distribution (V_{ss}) was estimated using the equation dose×AUMC/AUC^2. Mean residence time (MRT) was calculated as AUMC/AUC. For oral data, maximal plasma concentration (C_{max}) was defined as the highest experimental plasma concentration obtained, and t_{max} was the time for C_{max}. Bioavailability (F) was calculated as AUC(oral)/AUC(iv) (Gibaldi and Perrier, 1985).

RESULTS AND DISCUSSION

HPLC and GC chromatograms of AC-DDTC, DDTC (as Et-DDTC), Me-DDTC and Prop-DDTC (internal standard) are illustrated in Fig. 2 and Fig. 3. Calibration of AC-DDTC was linear over the concentrations of 1-100 μg/ml (r=0.998) and the detection limit was 2.5

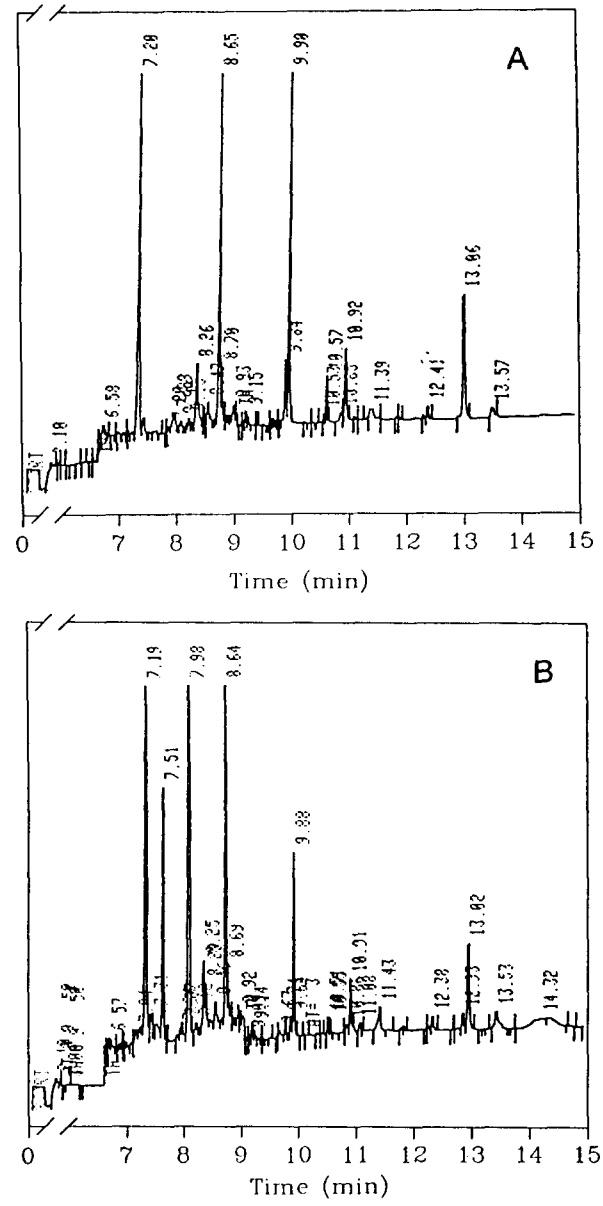

Fig. 3. GC separation of the metabolites of AC-DDTC on a DB 5 fused-silica capillary column using nitrogen phosphorus detector. Components were derivatized and extracted from blank (A) and with AC-DDTC (120 mg/kg; po) treated rat plasma (B). Peaks at the retention times of 7.51 min, 7.98 min and 8.64 min represent Me-DDTC, DDTC (as Et-DDTC) and Prop-DDTC (ISTD), respectively.

μg/ml. The standard curves obtained for DDTC and Me-DDTC in plasma are shown in Fig. 4. Na-DDTC and Me-DDTC were added to plasma in a concentration of 0.01-1 μg/ml and the samples derivatized and extracted as described. The concentration of drug added was plotted against the ratio of the peak height obtained for the drug to that for the internal standard. Linearity was observed throughout the concentration range tested (0.01-1 μg/ml) with a correlation coeffi-

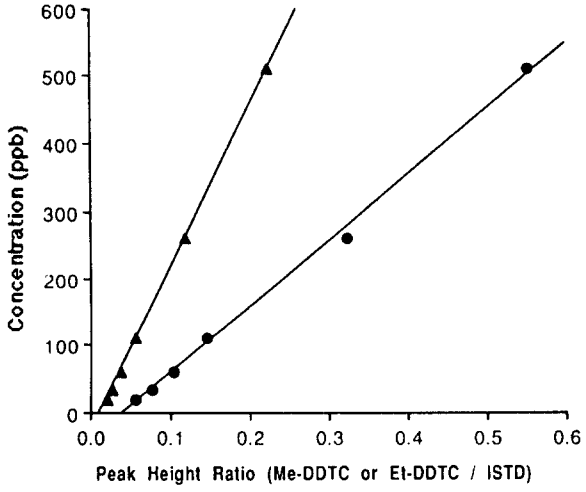

Fig. 4. Standard curves prepared by plotting concentrations of Me-DDTC (●) and DDTC (▲) extracted from plasma against the peak height ratio of Me-DDTC or DDTC/ISTD.

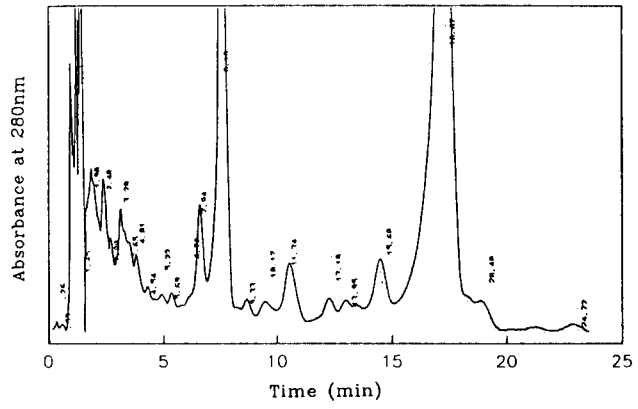

Fig. 5. HPLC separation of metabolites of AC-DDTC from the rat liver homogenate administered with AC-DDTC (120 mg/kg; po). Conditions: μBondapak phenyl column; mobile phase acetonitrile-H_2O in ratio of 35:65; Flow rate 1.0 ml/min; UV 280 nm. Peaks of the retention times of 7.04 min, 11.34 min and 18.67 min represent Me-DDTC, DDTC (as Et-DDTC) and Prop-DDTC (ISTD), respectively.

cient of 0.998 and 0.999 for DDTC and Me-DDTC, respectively. The limit of detection for both compounds by GC was 5 ng/ml. Percentage conversion of DDTC to its ethyl ester was 75± 2.5% (mean SD; n=3) under the derivatization conditions used. This result is comparable to the reported value by Masso and Kramer (1981). The recoveries of the Et-DDTC formed and Me-DDTC from plasma were 75± 3.7% (n=3) and 73± 5.2% (n=3), respectively.

A remarkable difference between the metabolic profile in the liver homogenate and that in plasma was observed, i.e. Me-DDTC is found to be the major metabolite in the liver (Fig. 5), while DDTC is predominant in plasma (Fig. 6). It is therefore reasonable

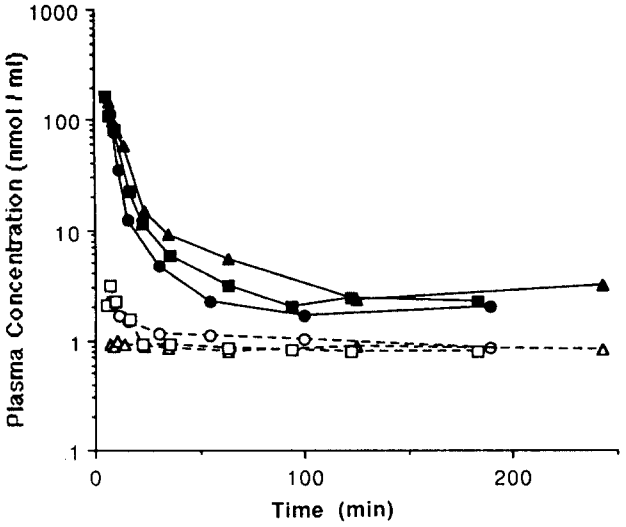

Fig. 6. Plasma concentrations of DDTC and Me-DDTC following single iv dose of AC-DDTC (20 mg/kg) to rats. The data of individual rats are presented with ○, □ and △, either closed or open. Closed symbols with solid line and open symbols with dotted line represent DDTC and Me-DDTC, respectively.

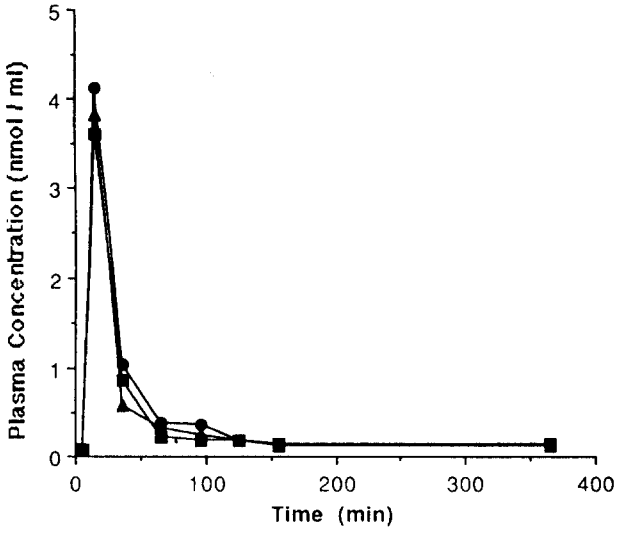

Fig. 7. Plasma concentrations of DDTC following single oral dose of AC-DDTC (20 mg/kg) to rats. The data of individual rats are presented with ●, ■ and ▲.

to assume that the disulfide bond of AC-DDTC is cleaved mainly by plasma protein via thiol-disulfide exchange reaction to produce DDTC which is then methylated in the liver. The methylation is catalyzed by a highly active enzyme S-adenosylmethionine transmethylase associated with the microsomal fraction of liver and kidney (Eneanya *et al.*, 1981). Similarly, it is reported that S-methylation is involved in the metabolism of thiopurines and thiopyrimidines in liver and kidney tissues (Remy, 1963). The parent compound

Table I. Pharmacokinetic Parameters of DDTC After Intravenous Administration of 20 mg/kg AC-DDTC to Rats

Rat	1	2	3	Mean± SD
AUC(nmol/min/ml)	982.5	1883.2	1393.6	1419.8± 368.2
$t_{1/2}(\alpha)$ (min)	2.5	5.2	4.5	4.1± 1.1
$t_{1/2}(\beta)$ (min)	137.6	114.6	119.0	123.7± 9.9
CL (ml/min/kg)	13.8	6.5	6.8	9.0± 3.4
V_{ss} (ml/kg)	1841.0	687.5	647.5	1058.7± 553.4
MRT (min)	133.7	105.8	94.9	111.5± 16.3

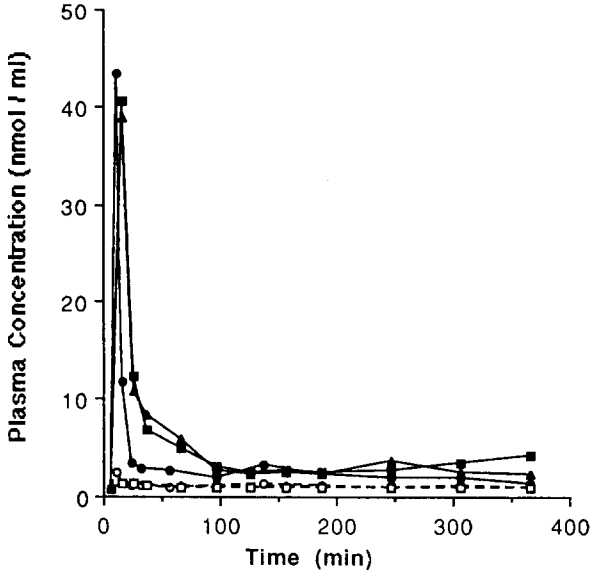

Fig. 8. Plasma concentrations of DDTC and Me-DDTC following single oral dose of AC-DDTC (120 mg/kg) to rats. The data of individual rats are presented with ○, □ and △, either closed or open. Closed symbols with solid line and open symbols with dotted line represent DDTC and Me-DDTC, respectively.

AC-DDTC was very unstable in vivo and could not be detected neither in plasma nor in liver homogenate. The possibility of the acid degradation after oral administration can be excluded as this compound is known to be stable under acidic conditions (Rajca *et al.*, 1990b).

The plasma concentration-time profiles of DDTC and Me-DDTC following iv (20 mg/kg) administration of AC-DDTC to rats are shown in Fig. 6. The elimination of DDTC from plasma was best approximated by a two compartment model and the pharmacokinetic parameters are listed in Table I. Me-DDTC was detected only in trace amounts throughout the experiment. The plasma concentration of DDTC appeared to decline in a biexponential fashion with mean half-lives of 4.1± 1.1 min (α-phase) and 123.7± 9.9 min (β-phase). The V_{ss} of DDTC was considerably low suggesting that DDTC has a low affinity to rat tissue. Since DDTC is a hydrophilic compound and to some extent is probably bound to other components in plasma, this value is in good accordance with the distribution in extracellular water. The tissue distribution study using [^{14}C] labeled AC-DDTC confirms this result (Lee *et al.*, submitted for publication). The total body clearance of DDTC was 9.0± 3.4 ml/min/kg.

Observed plasma concentrations of DDTC and Me-DDTC after oral administration of 20 mg/kg and 120 mg/kg AC-DDTC to rats are shown in Fig. 7 and Fig. 8. Data on the C_{max} and bioavailability, F, of DDTC are presented in Table II. Mean C_{max} value for DDTC after 20 mg/kg AC-DDTC treatment in rats was 3.8± 0.2 nmol/ml. t_{max} value (not presented in the table) for DDTC was 10 min and the MRT of DDTC was 47.1± 2.8 min. At the oral dose of 120 mg/kg AC-DDTC, C_{max} of DDTC was 40.1± 2.2 nmol/ml with the t_{max} value of 10 min and the MRT was 110.5± 6.0 min. F of DDTC calculated from areas under the curves of the two dose levels tested (20 and 120 mg/kg) was found to be 7.0% and 13.7%, respectively.

In conclusion, two metabolites of AC-DDTC have been identified in rat plasma and in rat liver homogenate. One is a cleavage product of the disulfide bond of AC-DDTC and the other is a methyl derivative of the former. The *in vivo* studies described herein show that AC-DDTC undergoes significant metabolic transformation, with parent drug and metabolites being less distributed in tissues. The bioavailability calculated from the plasma concentration-time curve was relatively low.

To confirm these results and to obtain more information with regard to the fate and distribution of this

Table II. Pharmacokinetic Parameters of DDTC After Oral Administration of AC-DDTC to Rats

Rat	4	5	6	Mean± SD	7	8	9	Mean± SD
	20 mg/kg				120 mg/kg			
C_{max} (nmol/ml)	3.5	3.8	4.0	3.8± 0.2	38.1	39.7	42.5	40.1± 1.8
AUC (nmol/min/ml)	93.5	95.4	111.1	100.0± 7.9	1332.9	1381.5	795.4	170.0± 265.6
MRT (min)	50.9	46.3	44.2	47.1± 2.8	104.5	116.5	106.7	109.2± 5.2
F				7.0				13.7

compound, a whole body absorption, distribution, metabolism and excretion study using radiolabeled compound is now in progress in our laboratory.

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