PHARMACOKINETIC STUDIES ON 1-(2-CHLOROETHYL)-3-ISOBUTYL-3-(β -MALTOSYL)-1-NITROSOUREA (TA-077). I. BLOOD AND TISSUE CONCENTRATIONS OF A NEW NITROSOUREA ANTI-TUMOR AGENT TA-077 AND ITS METABOLITE TA-G AFTER INTRAVENOUS INJECTION OF TA-077 IN VARIOUS EXPERIMENTAL ANIMALS

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1-(2-Chloroethyl)-3-isobutyl-3-(β -maltosyl)-1-nitrosoarea (TA-077) is a new masked antitumor agent, which is hydrolyzed by maltase to give rise to a more cell-permeable and, hence, more cytotoxic metabolite, TA-G.

TA-077 was intravenously administered to mice, rats, guinea pigs, rabbits and dogs and the time-courses of blood concentrations of TA-077 and TA-G were followed. The time-course patterns of blood TA-077 in these animals were markedly different from one another depending on the level of plasma maltase, while those of blood TA-G were all similar except in the early stage after the injection; *i.e.*, the blood concentration of TA-G reached a peak shortly after the injection and decreased rapidly with a half-life of 6 to 15 min, and the higher the level of plasma maltase, the earlier the time of peak appearance.

Tissue concentrations of TA-077 and TA-G in major organs were also measured at various times after intravenous injection of TA-077 to guinea pigs and VX-2 tumor-bearing rabbits. In both species, tissue concentrations of TA-077 did not exceed the blood concentration and rapidly decreased to insignificant levels by 30 to 45 min. The tissue level of TA-G in the kidney, a maltase-rich organ, was always the highest among the organs examined in both species. The concentration of TA-G in the VX-2 tumor was relatively high.

Possible significance of tissue maltase to the cellular uptake and antitumor effect of TA-077 is also discussed.

Keywords — antitumor agent; 1-(2-chloroethyl)-3-isobutyl-3-(β -maltosyl)-1-nitrosoarea; TA-077; active metabolite; TA-G; pharmacokinetics; maltase activity; laboratory animal; VX-2 carcinoma

INTRODUCTION

TA-077, 1-(2-chloroethyl)-3-isobutyl-3-(β -maltosyl)-1-nitrosoarea (Fig. 1), is one of many 3-alkyl-3-disaccharide-substituted analogs synthesized by Tsujihara *et al.* in an attempt to develop water-soluble nitrosoarea antitumor agents with minimal myelotoxicity and improved chemotherapeutic ratios.¹⁾ TA-077 exhibits antitumor activity against a broad spectrum of experimental tumors and its chemotherapeutic ratios are superior to other existing nitrosoarea drugs, especially when it is administered to tumor-bearing animals in daily repeated dosage for 5 to 7 d.^{2,3)} In acute and sub-acute toxicity tests with mice, rats and beagle dogs, recovery from its myelotoxic effects such as leukocytopenia and thrombocytopenia was prompt and its toxic effects on liver, kidney and other major organs were only slight in comparison

with other known nitrosoarea agents (Y. Arai *et al.*, unpublished data).

The molecule of TA-077 possesses a maltosyl group on 3-N, which is expected to prevent the molecule from readily entering the cell. Therefore, TA-077 is only weakly cytotoxic against cultured cells of various tumor cell lines.⁴⁾ However, once administered *in vivo*, TA-077 is hydrolyzed by tissue maltase to give rise to a 3-N-glucosyl metabolite, TA-G (Fig. 1), which can more freely pass through the cell membrane and is therefore much more cytotoxic than the parent drug.⁴⁾

This property of TA-077 as a prodrug may be responsible for its chemotherapeutic superiority to other nitrosoareas. The present investigation was undertaken to study its pharmacokinetics by following the time course of blood and tissue concentrations of both TA-077 and TA-G after

FIG. 1. Chemical Structures of TA-077 and TA-G

intravenous administration in various experimental animals. It was found that its pharmacokinetics is greatly influenced by plasma and tissue maltase activity.

MATERIALS AND METHODS

1. Animal Experiments

Mice — Groups of about 25 male BDF₁ mice (Shizuoka Laboratory Animal Center, Hamamatsu), weighing about 25 g, were injected with 10, 30 or 100 mg TA-077/8 ml saline/kg into the tail vein. Three to 4 mice from each group were sacrificed by cervical dislocation at 2, 5, 10, 20, 30, 40 and 60 min after the injection, and bled from the femoral artery. Half a ml of the blood was mixed with 0.4 ml of 0.1 M citric acid in a test tube, into which was added 0.1 ml of an internal standard solution (see below). The mixture was instantly frozen in dry ice-acetone and kept at -20°C until analysis.

Rats — Female Donryu rats (Shizuoka Laboratory Animal Center, Hamamatsu), weighing about 250 g, were injected with 50 mg TA-077/ml/kg. Three rats each were sacrificed by bleeding from the carotid artery at 5, 10, 20, 30 and 45 min under ether anesthesia and 0.5 ml of the blood was used for analysis as described above.

Guinea Pigs — For determination of serial blood concentrations of TA-077 and TA-G, 3 male Hartley guinea pigs (Japan Laboratory Animal Inc., Tokyo), weighing about 300 g, were anesthetized by intraperitoneal injection of 4 ml/kg of 25% urethane. A plastic cannula (2 mm in diameter) was inserted into the carotid and a 3-way stopcock was attached to the other end of the cannula. To prevent blood clotting, the pathway of blood was filled with a heparin solution (5 units/ml saline), each time after blood sampling, through a syringe connected to the remaining opening of the stopcock. At 2, 5,

10, 20, 30, 40 and 60 min after injection of TA-077 (50 mg/0.5 ml/kg) into the femoral vein, blood was withdrawn into a syringe, from which 0.5 ml was used for analysis.

For determination of tissue concentrations, 2 groups of 5 guinea pigs, weighing 250 to 300 g, were injected with 50 mg TA-077/ml/kg into the ear vein, and sacrificed by a blow on the head at 10 and 30 min, respectively, after the injection. The animals were bled to death from the femoral artery and, immediately, main organs were excised, washed with saline, frozen in liquid nitrogen and stored at -20°C until analysis.

Rabbits — For determination of serial blood concentrations, 3 female Japanese white rabbits (Funabashi Farm Inc., Funabashi), weighing about 2.5 kg, were injected with 50 mg TA-077/0.5 ml/kg into the right-ear vein and blood (*ca.* 1 ml) was sampled from the left-ear vein at 2, 5, 10, 20, 30, 40 and 60 min after the injection.

For determination of tissue concentrations, 5 groups of 3 rabbits, weighing 1.5 to 2.0 kg and bearing a palpable tumor (2 to 3 cm in diameter) 3 to 4 weeks after inoculation of 1.0×10^7 VX-2 carcinoma cells into the dorsal muscle of a hind limb, were injected with 50 mg TA-077/0.5 ml/kg into the ear vein and sacrificed by bleeding to death from the femoral artery 10, 30, 45, 60 and 90 min, respectively, after the injection. The liver, kidney, lung, heart and the tumor were quickly excised, washed with saline, frozen in liquid nitrogen and stored at -20°C until analysis.

Dogs — Three male beagle dogs (Yoshiki Yakuhin, Furukawa, Gifu), weighing about 10 kg, were injected with 3 mg TA-077/ml/kg from the medial saphenous vein and blood (*ca.* 1 ml) was sampled from the cephalic vein at 2, 5, 10, 20, 30, 40 and 60 min after the injection.

2. Quantitation Methods

Internal Standards — In experiments with mice, rats, guinea pigs and rabbits, 1-(2-chloroethyl)-3-*n*-amyl-3-(β -maltosyl)-1-nitrosourea was used as the internal standard. The maltase activity of dog blood was so high that the above internal standard was substantially (*ca.* 70%) hydrolyzed during the short contact (*ca.* 10 s) to ice-cold blood before extraction. Therefore, the above internal standard was replaced by a 3-*n*-amyl-3- β -D-glucopyranosyl

analog. The internal standard, 5 to 50 μg per sample depending on the expected concentration, was added as a methanol solution to an ice-cold citrate-buffered blood sample immediately after blood sampling.

Extraction — A mixture of 0.5 ml of blood and 0.4 ml of 0.1 N citric acid gave a pH of 4.2, where TA-077 is known to be most stable.

The stored blood sample, already containing the internal standard, was mixed with 3.5 ml of ice-cold chloroform-methanol (2:1) under vigorous agitation for 1 min with a Vortex mixer. Half a gram of organ tissue was mixed with 0.4 ml of 0.1 N citric acid, 3.5 ml of chloroform-methanol (2:1) and 0.1 ml of internal standard solution in a 10 ml-polyethylene test tube and homogenized for 30 s with a Polytron® (Kinematika). The extraction mixture was centrifuged at $12000 \times g$ for 10 min in a refrigerated centrifuge and the supernatant aqueous layer was subjected to high performance liquid chromatographic (HPLC) analysis.

The recovery ratios of 2.5 μg TA-077 and 2.5 μg TA-G from triplicate 0.5 ml samples of guinea pig blood were 80.1 ± 4.1 and $47.8 \pm 2.6\%$, respectively.

HPLC — The HPLC system consisted of Waters' 6000A and M45J pumps, an M710B sample processor (WISP), an M440 UV detector (254 nm), an RCM-100 radial compression separation system, an M720 system controller and a Shimadzu's C-R1B integrator. The column system consisted of a Brownlee's RP-18 guard cartridge (3 cm $\times$ 4.6 mm i.d., particle size 10 μm) and a Waters' Radial-PAK cartridge C₁₈ (10 cm $\times$ 8 mm, particle size 10 μm).

Separation of TA-077, TA-G and both internal standards was accomplished by a linear gradient elution with 25 to 55% acetonitrile in 0.05 M acetate buffer (pH 4.2) for 11 min at a flow rate of 2 ml/min (Fig. 2). Since the internal standard used for blood samples of mice, rats, guinea pigs and rabbits did not give an appreciable peak ascribable to the hydrolyzed product (Fig. 2), it was assumed that conversion of TA-077 to TA-G during the short sample-handling time was negligible.

Calibration Curves — Regression lines for calibration were obtained by least squares analysis of ratios of the peak heights of 0.05, 0.1, 0.2, 1.0, 2.5 and 5.0 μg drug to the peak height of 1.0 μg internal standard: $y = 0.392x + 0.070$

FIG. 2. HPLC Separation of TA-077, TA-G and an Internal Standard 1-(2-Chloroethyl)-3-n-amyl-3-(β -maltosyl)-1-nitrosourea

(A) Standard solutions. (B) Blood from an untreated mouse. (C) Blood taken from a mouse 2 min after intravenous injection of 100 mg TA-077/kg. a, TA-077; b, TA-G; c, internal standard. See the text for the extraction and HPLC conditions.

($r = 0.996$) for TA-077, and $y = 0.264x + 0.082$ ($r = 0.998$) for TA-G except for determination of dog blood concentrations. For the latter, $y = 0.118x + 0.098$ ($r = 0.999$) was obtained for TA-G.

3. Pharmacokinetic Analysis

The time-course curve for blood TA-077 concentrations in all the animal species except the dog was drawn to fit the following equation for a two-compartment open model:

$$C_{(t)} = A e^{-\alpha t} + B e^{-\beta t}$$

The curve for blood TA-G concentrations in the guinea pig and rabbit was constructed according to the equation:

$$C_{(t)} = A \times k_a / (k_a - k_e) \times (e^{-k_a t} - e^{-k_e t})$$

assuming that the conversion of TA-077 to TA-G follows first-order kinetics with a subsequent disappearance of TA-G in a one-compartment model. Blood TA-G concentrations of the dog, mouse and rat were best described by the following equation:

$$C_{(t)} = AA e^{-k_a t} + A e^{-\alpha t} + B e^{-\beta t}$$

FIG. 3. Blood Concentrations of TA-077 and TA-G after Intravenous Injection of TA-077 in Various Experimental Animals

TA-077 was administered at the dose of 3 mg/kg in dogs (A, $n = 3$), 100 mg/kg in mice (B, $n = 4$), 50 mg/kg in rats (C, $n = 3$), 50 mg/kg in guinea pigs (D, $n = 6$) and rabbits (E, $n = 3$). $\circ$, TA-077; $\bullet$, TA-G.

which is a combination of TA-G formation from TA-077 in a first-order kinetics and disappearance of TA-G in a two-compartment model. In the above equations, $C_{(t)}$ is drug concentration at t min after injection, AA , A and B are constants, k_a is the velocity constant of TA-G formation and α , β and k_e are disappearance velocity constants. The pharmacokinetic parameters, *i.e.*, $t_{1/2}$, C_{max} , t_{max} and AUC_0^∞ were calculated from the concentrations of TA-077 and TA-G measured at time t with a computer program MULTI⁵⁾ of the non-linear, least-square method combined with C^{-2} weighting.

RESULTS

1. Blood Concentrations of TA-077 and TA-G after Intravenous Injection of TA-077 into Various Experimental Animals

Time-courses of blood concentrations of TA-077 and TA-G after intravenous injection of TA-077 into the mouse, rat, guinea pig, rabbit

and dog are shown in Fig. 3. The disappearance of TA-077 from the circulation was biphasic in all species except in the dog. The conversion of TA-077 to TA-G in the dog was so fast that TA-077 was undetectable as early as 2 min after the injection and concentration of TA-G gave a biphasic decline. Hydrolysis of TA-077 in the guinea pig was slowest among the five species and the blood concentration of TA-G never exceeded that of TA-077 up to 60 min after the injection. The rates of TA-G formation, judged from Fig. 3, were thus in the decreasing order of dog, mouse, rat, rabbit and guinea pig. The last two animals have been reported to be devoid of significant plasma maltase activity.^{6,7)} Therefore, blood TA-G is attributable to tissue maltase activity in these species. Blood concentrations of TA-G in the guinea pig and rabbit gave a peak between 5 and 10 min and declined thereafter monophasically with half-lives of 15.3 and 6.7 min, respectively. Plasma maltase activity in the

TABLE I. *Pharmacokinetic Parameters of Blood TA-G in Various Animals after Intravenous Injection of TA-077*

Animal Dose (mg/kg)	$t_{1/2}$ (min)	C_{max} (nmol/ml)	AUC (Infinite) (nmol · min/ml)
Mouse			
10	7.0	20.5	146.9
30	5.4	62.1	411.5
100	6.4	233.0	1420.0
Rat			
50	7.1	67.8	760.3
Guinea pig			
50	15.3	23.3	761.4
Rabbit			
50	6.7	27.7	371.4
Dog			
3	8.3	21.4	180.7

Values of $t_{1/2}$, C_{max} and AUC were calculated by the use of a two-compartment model for mouse, rat and dog or one-compartment model for guinea pig and rabbit.

TABLE II. *Blood and Tissue Levels of TA-077 and TA-G in Guinea Pig after Intravenous Injection of TA-077 (50 mg/kg)*

	Concentration (nmol/ml or g) mean $\pm$ S.D.					
	10 min			30 min		
	TA-077	TA-G	Mol ratio TA-G/ TA-077	TA-077	TA-G	Mol ratio TA-G/ TA-077
Blood	61.0 $\pm$ 5.0	15.4 $\pm$ 1.1	0.3	3.5 $\pm$ 0.4	4.2 $\pm$ 0.5	1.3
Kidney	30.8 $\pm$ 7.2	190.8 $\pm$ 47.7	5.1	2.7 $\pm$ 0.4	12.0 $\pm$ 2.0	5.3
Liver	10.9 $\pm$ 0.9	12.4 $\pm$ 1.1	1.2	1.2 $\pm$ 0.3	1.7 $\pm$ 0.4	1.2
Pancreas	18.0 $\pm$ 1.6	12.4 $\pm$ 1.3	0.7	0.6 $\pm$ 0.1	5.1 $\pm$ 0.2	8.8
Lung	39.6 $\pm$ 3.5	18.1 $\pm$ 3.7	0.5	3.7 $\pm$ 0.7	5.0 $\pm$ 0.6	1.5
Spleen	12.8 $\pm$ 0.6	14.7 $\pm$ 0.6	1.2	ND	2.8 $\pm$ 0.5	Infinite
Whole brain	Trace	ND	—	ND	ND	—
Duodenum	17.5 $\pm$ 6.0	21.5 $\pm$ 3.2	1.8	ND	3.9 $\pm$ 0.4	Infinite
Stomach	41.3 $\pm$ 16.0	15.8 $\pm$ 3.5	0.5	2.2 $\pm$ 0.7	3.1 $\pm$ 0.2	1.4

ND, levels lower than the quantitation limit (0.4 nmol/ml or g). $n = 5$.

mouse and rat appeared to be high enough to allow TA-G concentrations to surpass TA-077 concentrations within 5 min, but, unlike the dog, both rodent species retained measurable blood concentrations of TA-077. The β phase of TA-077 clearance in rat blood was a relatively slow process ($t_{1/2}$ 18.2 min).

The $t_{1/2}$, C_{max} and AUC values of the circulating cytotoxic metabolite TA-G in various animals were computed and listed in Table I. The AUC values obtained at three doses in the

mouse were proportional to the dose. When corrected for the dose, the AUC values were of similar magnitudes in all species except in the dog. The exceptionally high AUC value in the dog is probably ascribable to instantaneous hydrolysis of TA-077 and the relatively slow clearance of TA-G.

2. Blood and Tissue Concentrations of TA-077 and TA-G after Intravenous Injection of TA-077 in Guinea Pigs

Tissue distribution of TA-077 and TA-G was

examined at 10 and 30 min after intravenous injection of TA-077 in guinea pigs (Table II). This species was chosen because, like man, it lacks plasma maltase activity.⁶⁾ At 10 min after the injection, *i.e.*, towards the end of the α phase of TA-077 clearance, the blood showed the highest concentration of TA-077, followed by those in the stomach, lung and kidney. The TA-077 concentrations in the pancreas, duodenum, spleen and liver, decreasing in this order, were of similar magnitudes. TA-077 scarcely enters the central nervous system (CNS), because only a trace of TA-077 was detectable in the brain. The tissue concentrations of TA-G at 10 min after the injection were all of similar magnitudes and lower than those of TA-077 except in the kidney, where the concentration of TA-G was by far the highest and about 5 times the concentration of TA-077. Excepting the kidney, the duodenum showed the highest molar ratio (1.80) of TA-G to TA-077, followed by those (*ca.* 1) in the liver and spleen. The ratio was lowest in the blood (0.31). At 30 min after the injection, the tissue concentrations of TA-077 mostly dropped to less than one tenth the corresponding concentrations at 10 min and those in the spleen and duodenum were undetectable. On the other hand, the decrease in the concentration of TA-G was much slower than that of TA-077, giving higher TA-G/TA-077 ratios at 30 min than at 10 min except in the kidney where the ratio remained to be constant. The ratios in the duodenum, spleen and pancreas were higher than that in the kidney.

3. Blood and Tissue Concentrations of TA-077 and TA-G after Intravenous Injection of TA-077 in VX-2 Tumor-Bearing Rabbits

In order to study the distribution of TA-G in

tumoral tissues in an animal possessing little plasma maltase, tissue distribution of TA-077 and TA-G was studied in VX-2 tumor-bearing rabbits (Table III). At 10 min, as in the guinea pig, the concentration of TA-077 was highest in the blood and lowest in the liver. TA-077 concentration in the tissues including the tumor rapidly decreased and became undetectable after 45 min but there were some slowly declining fractions in the kidney and liver. The rate of clearance of TA-G was somewhat slower and more variable depending on the organ than that of TA-077, although TA-G was no longer detectable in any organs at 90 min. The concentration of TA-G in the kidney was always highest among the organs examined, as in the guinea pig. The tumor retained relatively high concentrations of TA-G until 60 min after the injection, although the blood levels of both TA-077 and TA-G were already nil at 45 min.

DISCUSSION

Since the hydrolytic cleavage of one glucose molecule off the maltosyl residue of TA-077, *i.e.*, the maltase reaction, is the initial major reaction of TA-077 metabolism, it is not surprising that the pharmacokinetic behavior of TA-077 and, hence, that of TA-G differ greatly depending on the tissue distribution of maltase activity. Thus, the dog, which has a very high activity of plasma maltase,⁷⁾ has completely hydrolyzed intravenously injected TA-077 within 2 min, giving no trace of circulating TA-077 under the present experimental conditions (Fig. 3). The guinea pig, on the other hand, has no activity of plasma maltase and therefore showed a slowly declining curve of blood TA-077. The

TABLE III. Blood and Tissue Levels of TA-077 and TA-G in Rabbits Bearing VX-2 Carcinoma after Intravenous Injection of TA-077 (50 mg/kg)

	Concentration (nmol/ml or g) mean $\pm$ S.D.									
	10 min		30 min		45 min		60 min		90 min	
	TA-077	TA-G	TA-077	TA-G	TA-077	TA-G	TA-077	TA-G	TA-077	TA-G
Blood	49.9 $\pm$ 9.8	28.1 $\pm$ 8.9	2.1 $\pm$ 1.1	3.3 $\pm$ 0.8	ND	ND	ND	ND	ND	ND
Kidney	10.7 $\pm$ 2.1	852.8 $\pm$ 12.6	1.0 $\pm$ 1.2	32.8 $\pm$ 10.0	0.8 $\pm$ 0.7	5.0 $\pm$ 2.8	0.6 $\pm$ 0.3	2.1 $\pm$ 1.0	ND	ND
Liver	3.5 $\pm$ 0.9	3.8 $\pm$ 0.8	ND	0.3 $\pm$ 0.4	0.5 $\pm$ 0.5	ND	0.7 $\pm$ 0.3	ND	ND	ND
Heart	11.6 $\pm$ 0.7	22.1 $\pm$ 2.3	0.4 $\pm$ 0.3	3.5 $\pm$ 0.6	ND	0.5 $\pm$ 0.4	ND	ND	ND	ND
Lung	16.9 $\pm$ 4.3	24.0 $\pm$ 2.1	0.5 $\pm$ 0.5	2.4 $\pm$ 2.0	ND	0.3 $\pm$ 0.5	ND	0.2 $\pm$ 0.2	ND	ND
Tumor	20.9 $\pm$ 1.0	22.8 $\pm$ 1.7	1.1 $\pm$ 0.7	5.9 $\pm$ 1.1	ND	1.8 $\pm$ 0.8	ND	1.3 $\pm$ 0.6	ND	ND

ND, levels lower than the quantitation limit (0.4 nmol/ml or g). *n* = 3.

other animal species, possessing slight or intermediate levels of plasma maltase activity, exhibited more rapidly declining curves of blood TA-077.

Plasma maltase activity also affected the shape of the time-course curve of blood TA-G: *i.e.*, the higher the activity, the sooner the rise of the curve. The fact that the levels of blood TA-G were much lower than that accounted for by the disappearance rates of TA-077 could be ascribable to other non-hydrolytic routes of transformation of TA-077 and possibly to easier uptake of TA-G by the tissues.

The half-lives of blood TA-G in various animals are similar to one another in spite of the large differences in the pattern of TA-077 clearance. The specific *AUC* values of TA-G (corrected for the dose) are also similar except in the dog, which showed an exceptionally high value probably due to near instantaneous hydrolysis of TA-077 to TA-G by plasma maltase. The above similarities in pharmacokinetic parameters of circulating TA-G also hold true even if man is included.⁸⁾ These similarities and the linear relationships between the dose and *AUC* observed in the mouse (Table I) and man⁸⁾ would justify extrapolation of therapeutic and toxic doses in clinical trials from efficacy and toxicity data in animal experiments, since TA-G appears to be the main and single active species of TA-077 metabolites.

Since guinea pig blood totally lacks maltase activity, the site of production of circulating TA-G was sought among other tissues by measuring tissue concentrations of TA-G after intravenous injection of TA-077 (Table II). Available literature indicates that, in rat and rabbit, maltase activity is by far the highest in the kidney and intestine⁹⁾ and that the enzyme is localized in the intestinal and renal brush borders.¹⁰⁾ In other organs, part of maltase activity may also exist on the cell membrane as an ectoenzyme so that the cell may be able to hydrolyze available maltosyl substrates to glucose, which is then taken up by the cell. This type of maltase localization is understandable in view of the role of maltase in intestinal and renal absorption of maltose *via* glucose. The most active production of TA-G from TA-077 by the kidney in our experiment (Table II) agrees well with the above distribution pattern of tissue maltase in the body, but the TA-G concentrations in the

duodenum were much lower than those in the kidney and not much different from those in other major organs. The intravenously administered TA-077 may not have reached the lumen side of the intestinal mucosal cells, or the TA-G, if produced, may have been lost in the intestinal contents. On the other hand, the high concentrations of TA-G in the kidney may mostly represent the amounts present in the glomerular filtrate. Thus, the organ which contributed most to the circulating level of TA-G has not been identified. The findings that the TA-007 concentration was highest, and the TA-G/TA-077 ratio was lowest, in the blood and that the tissue ratio of TA-G/TA-077 increased with time are in agreement with the notion that TA-077 is much less readily taken up by the tissues than TA-G and TA-G is produced from TA-077 by peripheral organs.

Since the tumor is the target tissue for the antitumor agent, the distribution of TA-077 or TA-G in the tumor is of utmost concern. In order to examine the affinity of the drug to tumoral tissues in comparison with other organs, we used VX-2 tumor-bearing rabbits because rabbits have virtually no maltase activity in plasma.⁹⁾ The organ distribution patterns of both TA-077 and TA-G (Table III) were similar to those in the guinea pig. This similarity, in addition to the similarities of pharmacokinetics of blood TA-G among various animals (Fig. 3), suggests that TA-077 and TA-G would not exhibit large species differences in drug disposition except those in the early period after injection due to the differences in the plasma level of maltase.

The concentration of TA-G in VX-2 tumors declined less rapidly compared to the other organs (Table III). Whether this relatively high affinity is due to a high rate of drug uptake or to a low rate of drug disposition in this tissue is unknown. Since the blood levels of both TA-077 and TA-G were nil at 45 min, the latter possibility is more likely. However, the high level of TA-G in the tumor in the earlier stage may have resulted from a rapid conversion of TA-077 to TA-G in the tumor because the blood level of TA-077 was higher than those in the other tissues and there were relatively high uptakes of TA-077 by the tumor at 10 and 30 min. Maltase activities of various rat tumors were reported to be comparable to those in the liver and lung.⁹⁾ If

there is a tumor relatively rich in a particular ectoenzyme that catalyzes, on the cell surface, the removal of a bulky hydrophilic group from a cytotoxic agent and thus renders the agent more permeable into the cell, this agent would be a selective antitumor agent. The possibility that TA-077 and maltase would represent such a combination in certain tumors seems to be worth exploring.

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