ANTITUMOR ACTIVITY AND PHARMACOKINETICS OF 7-*N*-(*p*-HYDROXYPHENYL)MITOMYCIN C IN HUMAN TUMOR XENOGRAFTS TRANSPLANTED INTO NUDE MICE

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The effects of 7-*N*-(*p*-hydroxyphenyl)mitomycin C (M-83), a derivative of mitomycin C, against eight human tumor xenografts serially transplanted into male BALB/c nude mice were evaluated. M-83 showed positive antitumor effect against six out of eight tumor strains. The antitumor spectrum of this agent was similar to that of mitomycin C except for two strains.

The serum level of M-83 detected by bioassay was biphasic, elimination half-life $T_{1/2\beta}$ was 10.9 minutes and the area under curve AUC_0^{60} was 112.4 μg·minute/ml when 15 mg/kg of the agent was administered. In the tumor, the peak concentration and AUC_0^{60} detected by radioassay correlated well with the value of drug efficacy T_{RW}/C_{RW}. The ratio of the concentrations detected by bioassay to radioassay in the tumor was approximately 10%, which was thought to be affected by inactivation of the agent in the tumor.

7-*N*-(*p*-Hydroxyphenyl)mitomycin C (M-83) was synthesized as one of the 7-*N*-alkyl derivatives of mitomycin C and was reported in 1980 to have more potent antitumor activity against the ascitic form of P-388 leukemia than mitomycin C[1,2]. Mitomycin C is an antibiotic that shows a broad spectrum of antitumor activities against human solid tumors transplanted into nude mice[3~5]. Mitomycin C is reported to disappear rapidly in the body[6] suggesting rapid inactivation of the drug *in vivo*. It appears that the pharmacokinetics of M-83 is similar to that of mitomycin C and the mechanism of action of this drug might involve alkylating effects on the synthesis of tumor cell DNA[7,8]. It was already reported that mitomycin C was not detected in the reaction mixture of M-83 and liver tissue[9,10]; therefore, the effect of M-83 was thought to be independent of the activity of mitomycin C. The present paper reports the antitumor effects of M-83 against eight human tumors in nude mice and concentrations of the drug in blood, liver, kidney and three strains of tumor by both radioassay and bioassay. The pharmacokinetic parameters of M-83 in mice were studied in terms of the antitumor effects of the drug.

Materials and Methods

Animals

Male nude mice with a genetic background of BALB/c, weighing 20~22 g and aged 4~6 weeks, were purchased from the Central Institute for Experimental Animals (Kawasaki). The mice were bred and maintained in specific pathogen-free conditions using laminar air-flow racks in the Experimental Animal Center of our university.

Tumors

Eight human tumor xenografts were used for the experiments. They included three gastric adeno-

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carcinomas (St-4, poorly differentiated; St-10, well differentiated; St-40, well differentiated), two colon adenocarcinomas (Co-3, well differentiated; Co-4, poorly differentiated), one breast carcinoma (MX-1, undifferentiated), one bile duct carcinoma (Ch-1, well differentiated) and one hemangiosarcoma (LC-27). Tumor fragments 2~3 mm in size were inoculated subcutaneously into the backs of mice. The width (W) and the length (L) of the tumors were measured by the same person three times weekly. Estimated tumor weight was calculated according to the formula of GERAN *et al.*[11]: tumor weight (mg)=$(W^2 \times L)/2$.

Drugs

7-*N*-(*p*-Hydroxyphenyl)mitomycin C (M-83), provided by Kyowa Hakko Kogyo Co. (Tokyo), was dissolved in physiological saline by the following procedures; after 10 mg of the compound was dissolved in ethanol, hydrogenated castor oil (HCO-60) and polyethylene glycol were added in weight ratios of 1:15:40, and the solution was concentrated by evaporation at room temperature under reduced pressure. Labeled compound of M-83, 7-*N*-(*p*-hydroxyphenyl)-[3,5-^{14}C]mitomycin C (^{14}C-M-83, activity 3.42 μCi/mg, purity 97.6%), was provided by Kyowa Hakko Kogyo Co. (Shizuoka). A commercially available preparation of mitomycin C (Kyowa Hakko Kogyo Co.) was used for the experiments to compare the antitumor activity.

Treatment Schedule

Drug administration was started when the estimated tumor weight reached 100~300 mg. M-83 in a dose of 10 mg/kg was administered intraperitoneally with a schedule of q4d(every four days)×3. The agent was also intravenously administered to the other mice in a dose of 7.5, 10, 15 mg/kg once or q4d×3, respectively. Mitomycin C was administered intraperitoneally at a dosage of 3 mg/kg with a schedule of q4d×3. Controls of each experimental group received comparable injections of physiological saline on the same schedule.

Evaluation of the Effect of the Agents

Observation of tumor growth was made for 2~3 weeks after initial treatment. The relative mean tumor weight (RW) for each test group and the control group was calculated according to the method of GERAN *et al.*[11], using the formula RW=Wi/Wo, where Wi is the mean tumor weight of a group at any given time and Wo is the mean tumor weight of that group at the initiation of treatment. In the formula T_{RW}/C_{RW}, T_{RW} is the relative mean tumor weight of a treated group and C_{RW} is the relative mean tumor weight of the control group. Antitumor activity was evaluated as follows; ++, regression of tumor [RW <1.0]; +, retardation of tumor growth [$T_{RW}/C_{RW} \leq 42\%$]; −, inactive [$T_{RW}/C_{RW} > 42\%$]. To estimate the minimum effective dose (MED) of M-83 and mitomycin C, the dose response effect against the MX-1 tumor was investigated. One-half, 1/4 and 1/8 of the maximum tolerable dose (MTD) of these drugs were respectively administered once intravenously and the lowest T_{RW}/C_{RW} was used for the assessment. The coefficient of correlation (r) between the doses of drugs and the natural logarithm of T_{RW}/C_{RW} was calculated. After the statistical significances of the r values were confirmed, the MED value was obtained from the regression equation where T_{RW}/C_{RW} was 42%.

Whole-body Autoradiogram

Nude mice bearing Co-3 or Co-4 were injected intravenously with 15 mg (51.3 μCi) of ^{14}C-M-83 per kg. Then the mice were sacrificed by freezing in acetone chilled by dry ice 15 minutes and 3 hours after the injection, and specimens were cut into 40 μm slices with a cryotome. After drying the specimens, X-ray films were exposed to the sections in a dark-room for 5 months, and the films were developed.

Radioassay

The samples including the blood, liver, kidney and tumor were obtained from mice bearing one of the MX-1, Co-3 and Co-4 at the time of 1, 5, 15, 30, 60 and 180 minutes, respectively, after intravenous injection of ^{14}C-M-83 in a dose of 15 mg/kg. After weighing out the specimens, they were dried in a draft for several days and oxidized with sample-oxidizer (Aloka ASC-111). The radioactivity of each sample was measured by liquid scintillation counter (Aloka LSC-653) and the concentration of the drug was calculated.

Bioassay

M-83 was also injected at a dosage of 15 mg/kg intravenously into mice bearing one of the MX-1, Co-3 and Co-4, respectively. The mice were sacrificed by exsanguination at the same intervals after injection as in the radioassay. After tissue samples were immediately excised, rinsed and weighed, 10% homogenates were made with 1/15 M phosphate-buffered solution (PBS) (pH 7.2). Serum or these homogenates were extracted with 3 ml of chloroform. After shaking for 10 minutes, the chloroform layer was dried in reduced pressure and the residue was dissolved in 50 μl of methanol and 1 ml of 1/15 M PBS before analysis. The antimicrobial activity of the residue was determined by the thin-layer cup method using *Escherichia coli* B and the concentration of M-83 was obtained from the standard curve.

Chemical Assay

To assess the value obtained by bioassay, the same serum sample was analyzed by high pressure liquid chromatography (HPLC) using Hitachi 638-50 chromatograph. Serum was extracted with ethyl acetate and after drying under reduced pressure the residue was dissolved in chloroform and methanol (94: 6). Detection was performed with Hitachi 638-41 UV detector at a fixed wavelength of 360~365 nm.

Analysis of Pharmacokinetics

Since the semilogarithmic plots of serum levels of M-83 detected by chemical and bioassay *versus* time following intravenous injection were biphasic, the pharmacokinetic parameters were calculated according to a two-compartment model using the following equations[12]:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad (\alpha > \beta)$$

$$T_{1/2(r)} = 0.693/r \qquad (r = \alpha, \beta)$$

The estimation of areas under the curves (AUC) in serum and tumor was made employing the linear trapezoidal method[13]:

$$AUC\Big|_{t_0}^{t_n} = \int_{t_0}^{t_n} \phi(t)dt = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i + C_{i+1})$$

C_i: drug concentration at time t_i

The AUC for each tumor and antitumor effects of M-83 were compared by the coefficients of correlation.

Inactivation of M-83 in Tumor Homogenates[9,10]

Tumor homogenates (20%) of MX-1, Co-3 and Co-4 were prepared in 1/15 M PBS (pH 7.2) by homogenization with a Polytron (Kinematica, Luzern) for 10~15 seconds. M-83 was dissolved in PBS (20 μg/ml) and was mixed with the same volume of tumor homogenate (the final concentration of M-83 was 10 μg/ml in 10% tumor homogenates in PBS). Immediately after mixing and 5, 15, 30 and 60 minutes after incubation at 37°C, 1 ml of the reaction mixture was extracted with 3 ml of chloroform. The residual antimicrobial activity was measured by the same bioassay procedure as described above.

Results

Antitumor Effect of the Agents

The effect of M-83 and mitomycin C on eight human tumor xenografts are summarized in Table 1. Both M-83 and mitomycin C were effective against six out of eight tumor strains. The spectra of the antitumor activity of both drugs were similar except for St-10 and Co-3. As shown in Table 2, the antitumor effects of M-83 and mitomycin C revealed a statistically significant dose responsiveness against MX-1. The minimal effective dose (MED) obtained from the regression equation was 2.7 mg/kg for M-83 and 0.8 mg/kg for mitomycin C. The chemotherapeutic indices (MTD/MED) of M-83 and mitomycin C were 5.6 and 7.5 respectively.

Table 1. Effect of M-83 and mitomycin C against human tumor xenografts serially transplanted into nude mice.

Tumor	M-83					Mitomycin C				
	Dose (mg/kg)	Schedule	RW[a]	T_{RW}/C_{RW}[b] (%)	Effects[c]	Dose (mg/kg)	Schedule	RW	T_{RW}/C_{RW} (%)	Effect
St-4	7.5	q4d×3 iv	1.20	49.2	−	3	q4d×3 ip	1.90	65.0	−
St-10	10	q4d×3 ip	1.65	48.2	−	3	q4d×3 ip	0.57	13.0	++
St-40	7.5	q4d×3 iv	0.77	12.3	++	3	q4d×3 ip	0.31	7.3	++
Co-3	10	q4d×3 ip	1.23	32.7	+	3	q4d×3 ip	1.14	58.1	−
	15	qd×1 iv	1.00	29.8	+					
	7.5	qd×1 iv	1.16	54.9	−					
Co-4	10	q4d×3 ip	0.68	22.4	++	3	q4d×3 ip	0.60	13.8	++
	5	q4d×3 ip	0.82	28.2	++					
MX-1	15	qd×1 iv	0.08	0.8	++	6	qd×1 iv	0.04	0.4	++
						3	q4d×3 ip	0.52	7.9	++
Ch-1	10	qd×1 iv	0.32	4.1	++	3	q4d×3 ip	0.01	0.2	++
	7.5	qd×1 iv	0.81	16.1	++					
LC-27	7.5	q4d×3 iv	1.91	31.4	+	3	q4d×3 ip	1.15	9.3	+
Response rate			75% (6/8)					75% (6/8)		

[a] RW: Relative mean tumor weight (RW=Wi/Wo) where Wi is the mean tumor weight of a group at any given time and Wo is the mean tumor weight of that group at the initiation of treatment.

[b] T_{RW}/C_{RW}: T_{RW} is the relative mean tumor weight of a treatment group and C_{RW} is the relative mean tumor weight of the control group, and the lowest T/C during the observation was picked.

[c] Effect: Evaluation was made as follows;
++: Regression of tumor RW <1.0, +: retardation of tumor growth $T_{RW}/C_{RW} \leq 42\%$, −: inactive $T_{RW}/C_{RW} > 42\%$.

Table 2. Chemotherapeutic index of M-83 and mitomycin C against MX-1.

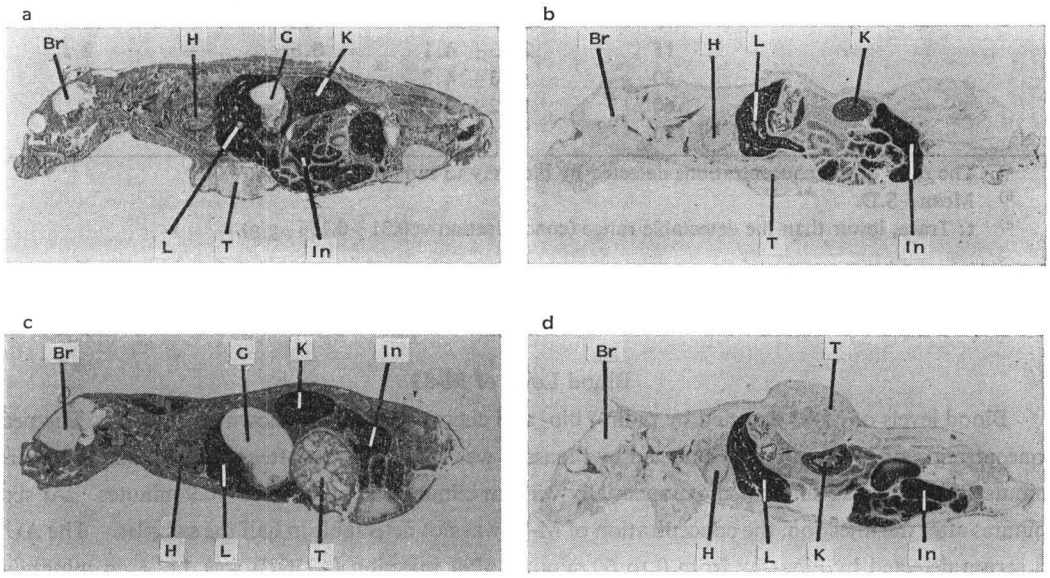
Drug	M-83		Mitomycin C	
	Dose (mg/kg)	T_{RW}/C_{RW} (%)	Dose (mg/kg)	T_{RW}/C_{RW} (%)
Effect on MX-1[a]	7.5	2.0	3	0.5
	3.75	18.8	1.5	7.9
	1.875	76.7	0.75	54.5
r (P)[b]	−0.998 ($P<0.05$)		−0.996 ($P<0.10$)	
MED[c] (mg/kg)	2.7		0.8	
LD_{50} (mg/kg)	23		9	
MTD[d] (mg/kg)	15		6	
C.I.[e]	5.6		7.5	

a) The agent was administered once intravenously when the estimated tumor weight reached 100~300 mg.
b) Coefficient of correlation between the dose of the drug and natural logarithm of T_{RW}/C_{RW} (%).
c) MED: Minimum effective dose, obtained from the regression equation where T_{RW}/C_{RW} value was 42%.
d) MTD: Maximum tolerable dose, two-thirds of the value of LD_{50} (mg/kg).
e) C.I.: Chemotherapeutic index=MTD/MED.

Fig. 1. Whole-body autoradiograms of nude mice after intravenous injection of ^{14}C-M-83.

Dark areas correspond to high concentration of labeled substance. a) A mouse bearing the Co-3 tumor, 15 minutes after the injection, b) 180 minutes. c) A mouse bearing the Co-4 tumor, 15 minutes after the injection, d) 180 minutes. Accumulations can be seen in the liver, kidney and intestinal tract. No accumulation in cerebrospinal space nor in the gastric cavity. Note accumulation in the peripheral area of the Co-4 tumor where necrotic tissue was thought to be minimal.

Br: Brain, H: heart, G: gastric cavity, In: intestinal tract, L: liver, K: kidney, T: tumor.



Whole-body Autoradiogram

Autoradiograms of mice bearing Co-3 or Co-4 are shown in Fig. 1. ^{14}C-M-83 was distributed mainly in the liver, kidney and intestinal tract and was not found either in the cerebrospinal space or in the gastric cavity. Although the blood level decreased markedly after 3 hours, the radioactivity in the liver, kidney and intestinal tract was preserved. Marked differences of distribution of radioactivity were not

Table 3. Time lapse change of M-83 in blood.

Time (minutes)	Radioassay (μg/ml)	Bioassay (μg/ml)	Chemical assay (μg/ml)
1	25.0±2.9[a]	13.8±1.9	16.3±3.9
5	12.5±2.4	3.9±0.6	3.9±1.2
15	6.0±1.0	1.8±0.5	1.4±0.0
30	4.7±1.2	0.7±0.2	0.5±0.1
60	3.2±0.6	0.4±0.2	0.1±0.0
180	2.3±0.4	t[b]	—
$T_{1/2\alpha}$ (minutes)	—	1.0	1.2
$T_{1/2\beta}$ (minutes)	31.8[c]	10.9	10.4
AUC_0^{60} (μg·minute/ml)	391.3	112.4	112.0

a) Mean±S.D.
b) t: Trace, lower than the detectable range (concentration <0.31~0.156 μg/ml).
c) Elimination half-life from 5 to 60 minutes after the injection.

Table 4. Concentration of M-83 in liver and kidney.

Organ	Time (minutes)	Radioassay (μg/g)	Bioassay (μg/g)	Ratio[a] (%)
Liver	1	79.7± 6.5[b]	2.7±0.7	3.3
	5	83.1±13.1	1.8±1.1	2.1
	15	44.9±11.8	0.6±0.4	1.3
	30	37.8± 3.4	t[c]	—
	60	23.0± 0.6	t	—
	180	17.4± 2.5	t	—
Kidney	1	92.9± 6.2	—	—
	5	57.9±20.3	7.2	12.4
	15	24.3± 4.1	0.6	2.5
	30	18.3± 4.2	0.3	1.6
	60	16.2± 4.2	t	—
	180	10.5± 1.2	t	—

a) The ratio of the concentrations detected by bioassay to radioassay (%).
b) Mean±S.D.
c) t: Trace, lower than the detectable range (concentration <0.31~0.156 μg/g).

found between the mice bearing Co-3 and Co-4. In the tumor of Co-4, radioactivity was present mainly in the peripheral area where necrotic tissue was thought to be minimal.

Blood Level of M-83

Blood levels of M-83 detected by radio-, bio- and chemical assay are shown in Table 3. The peak concentration of M-83 in serum detected by bioassay was seen 1 minute after the injection, and after 5 minutes the drug was eliminated exponentially with an elimination half-life of 10.9 minutes. At sixty minutes after the injection, the concentration of M-83 was not detectable in half the samples. The AUC in serum detected by bioassay from 0 to 60 minutes after injection (AUC_0^{60}) was 112.4 μg·minute/ml when calculated in terms of the linear trapezoidal method[13]. The concentrations of M-83 detected by chemical assay were similar to those detected by bioassay. $T_{1/2\beta}$ was 10.4 minutes and AUC_0^{60} was 112.4 μg·minute/ml. The peak concentration of ^{14}C-M-83 in blood detected by radioassay was observed 1 minute after the injection. The concentration decreased exponentially in the early phase but the rate of decrease diminished in the late phase. The elimination half-life from 5 to 60 minutes after the injection was 31.8 minutes, and AUC_0^{60} was 391.3 μg·minute/ml.

Table 5. Concentration of M-83 in tumors.

Tumor	Time (minutes)	Radioassay (μg/g)	Bioassay (μg/g)	Ratio[a] (%)
MX-1	1	4.3±0.3[b]	0.47±0.06	10.9
	5	4.5±1.4	0.43±0.06	9.6
	15	3.3±1.0	0.43±0.06	13.0
	30	3.0±0.1	0.40	13.3
	60	2.7±0.3	t[c]	—
	180	2.1	—	—
	AUC_0^{60} (μg·minute/g)	191.5	18.6	9.7
Co-3	1	1.7±0.2	0.40	23.5
	5	2.9±1.3	0.20	6.9
	15	2.2±0.4	0.19	8.6
	30	1.8±0.5	t	—
	60	1.7±0.3	t	—
	180	1.3	t	—
	AUC_0^{60} (μg·minute/g)	118.1	4.8	4.1
Co-4	1	2.6±0.8	0.72±0.48	27.7
	5	3.2±0.6	0.56±0.28	17.5
	15	2.5±0.2	t	—
	30	2.0±0.2	t	—
	60	1.7	t	—
	180	1.7	—	—
	AUC_0^{60} (μg·minute/g)	130.7	5.7	4.4

a) The ratio of the concentrations or AUC detected by bioassay to radioassay (%).
b) Mean±S.D.
c) t: Trace, lower than the detectable range (concentration $<0.31\sim0.156$ μg/g).

Concentration of M-83 in Liver and Kidney

The concentrations of M-83 in the liver and kidney detected by radioassay and bioassay are shown in Table 4. The radioactivity in the liver and kidney was 3~8 times higher than that in the blood. The antimicrobial activities of the extracts from these organs were low. In the liver no bioactivity was detected in 30 minutes or more after the injection and in the kidney no bioactivity was found in 60 minutes or more after the injection. The ratio of the concentrations of M-83 detected by bioassay to those by radioassay in the liver varied from 3.3 to 1.3%, which decreased with time. The ratio in the kidney ranged from 12.4 to 1.6%, the decrease of which was also time-dependent. In the liver the ratios were lower than in the kidney.

Table 6. *In vitro* inactivation of M-83 by tumor homogenate[a].

Time (minutes)	MX-1	Co-3	Co-4
5	100[b]	80.0	95.8
15	94.8	77.8	93.8
30	86.8	63.8	93.8
60	82.5	60.0	87.5

a) The mixture of M-83 (10 μg/ml) and tumor homogenate (10%) was incubated at 37°C aerobically.
b) Residual antimicrobial activity of M-83 is shown as percentage of the concentration of the agent in the mixture at each time to that immediately after mixing.

Concentration of M-83 in the Tumors

The concentrations of M-83 in the tumors are shown in Table 5. The peak concentration of ^{14}C-M-83 detected by radioassay was seen at 5 minutes after the injection and the radioactivity decreased gradually. The radioactivity in the tumors was lower than that in the blood at any time. AUC_0^{60} detected by radioassay for each of the tumors was 118.1~191.5 μg·minute/g, which correlated well

with the peak concentration detected by radioassay. The peak concentration of M-83 detected by bioassay was found 1 minute after the injection. The rate of elimination of bioactivity in the tumors was relatively slow in comparison with that in the liver or kidney. The antimicrobial activity of M-83 in the extracts of tumor was variable and occasionally not detected several minutes after the injection. On 60 minutes or more after the injection, M-83 was not detected in any tumor by bioassay. The ratio of the concentrations of M-83 detected by bioassay to those by radioassay in the tumor of MX-1 was approximately 10%. AUC_0^{60} detected by bioassay in the MX-1 tumor was 18.6 μg·minute/g, which was approximately corresponding to 10% of the AUC obtained by radioassay.

Inactivation of M-83 by Tumor Homogenate

As shown in Table 6, M-83 was inactivated a little by tumor homogenate *in vitro*. The antimicrobial activity of M-83 was reduced to 60% by tumor homogenate of Co-3 when the mixture was incubated at 37°C for 60 minutes. The inactivation ability of tumor homogenate of Co-3 against M-83 was more remarkable than those of MX-1 and Co-4.

Discussion

The experiments described above demonstrated the antitumor activity of M-83, a new derivative of mitomycin C, in a human tumor xenograft - nude mice system. M-83 was selected among several 7-*N*-phenyl derivatives of mitomycin C tested in rodent tumor systems (P-388 leukemia, ip-ip). It was reported that M-83 was more effective than mitomycin C in the ip-ip system but only as effective as mitomycin C in the solid tumor of Lewis lung carcinoma[2,10]. In our human tumor xenograft - nude mice system using MX-1, M-83 caused regression and disappearance of the tumor but the chemotherapeutic index (MTD/MED) of M-83 was 5.6, which was less than the mitomycin C value of 7.5. HOUCHENS *et al.*[14] reported that three strains of L1210 leukemia and B16 melanoma murine models and MX-1 (breast) xenograft selected for 94% of antitumor agents which had been assessed by more complicated and expensive protocols. Human tumor xenograft models such as MX-1 in a nude mice system are thought to be significant in predicting antitumor activity in clinical studies of newly developed agents[15~17].

The antitumor spectra of M-83 and mitomycin C against eight human tumor xenografts were similar except for St-10 and Co-3. It was reported that mitomycin C was not detected in the metabolites of M-83[9,10], suggesting that the *p*-hydroxyphenyl residue is not separated from the quinone structure of M-83 in its metabolic pathway. From the fact that M-83 is not masked compound of mitomycin C, the antitumor spectrum of M-83 is suspected to be different a little from that of mitomycin C. In clinical trials, phase I-II studies were already carried out in patients with gastrointestinal carcinoma or lung cancer[18,19]. It is recommended that M-83 should be used also for various carcinomas which are insensitive to mitomycin C.

In whole-body autoradiogram, ^{14}C-M-83 was distributed mainly in the liver and kidney. From the fact that radioactivity was strongly detected in the intestinal tract but not in the gastric cavity, the drug in part was thought to be excreted as metabolites into the bile. The drug did not pass the blood brain barrier. Radioactivity was found mainly in the peripheral area of the tumor of Co-4 where necrotic tissue was thought to be minimal[20].

The serum level of M-83 detected by bioassay was biphasic and the drug was eliminated exponentially 5 minutes or more after injection with an elimination half-life of 10.9 minutes. IMAI *et al.*[10] have reported that in normal mice the half-lives of M-83 in β phase were 17.9 and 7.5 minutes at a dosage of 20 and 10.2 mg/kg respectively. There were no significant differences in time lapse change of M-83 in serum between tumor-bearing nude mice and normal mice. AUC_0^{60} of 112.4 μg·minute/ml in serum detected by bioassay was important to compare the effect of the agent with those in clinical trials or *in vitro* clonogenic assay using tumor cell cultures.

Table 7. Pharmacokinetic parameters of M-83 in tumors and drug efficacy.

Tumor	Radioassay		Bioassay		Ratio[a] (%)	*In vitro*[b] (%)	T_{RW}/C_{RW} (%)	Effect
	PC* (μg/g)	AUC_0^{60} (μg·minute/g)	PC (μg/g)	AUC_0^{60} (μg·minute/g)				
MX-1	4.5	191.5	0.47	18.6	9.6	82.5	0.8	++
Co-3	2.9	118.1	0.40	4.8	6.9	60.0	29.8	+
Co-4	3.2	130.7	0.72	5.7	17.5	87.5	22.4	++
r[c]	−0.994	−0.996	0.241	−0.999	0.204	−0.411		
P	<0.10	<0.10	NS[d]	<0.01	NS	NS		

* Peak concentration.

[a] The ratio of the concentrations of M-83 detected by bioassay to those by radioassay in the tumor 5 minutes after the injection.

[b] *In vitro* residual antimicrobial activity of M-83 when the mixture of the drug and tumor homogenate was incubated at 37°C for 60 minutes.

[c] Coefficient of correlation between natural logarithm of T_{RW}/C_{RW} (%) and one of the peak concentration (μg/g), AUC_0^{60} (μg·minute/g), ratio (%) and *in vitro* residual antimicrobial activity (%).

[d] NS: Not significant.

The HPLC values of M-83 in serum were similar to those detected by bioassay. Both the bioassay and chemical assay showed the quantity of the drug molecule which was unchanged in the sample. The chemical assay provides more precise values and has more wide detectable range than bioassay but the assay procedure is complicated and many samples can not be tested at the same time.

In the radioassay the blood level of M-83 is expressed in terms of total radioactivity in blood and was stabilized at a level equivalent to 2~3 μg/ml 60 minutes or more after the injection. Since no bioactivities of M-83 were detected in the late phase, the radioactivity of the sample in the late phase was assumed to be caused by ^{14}C in the metabolites of M-83. The radioassay was considered to be valuable in analyzing the pharmacokinetics in the early phase after administration of the isotope-labeled drug.

In the liver and kidney, the bioactivity of M-83 decreased rapidly below the detectable range after 15~30 minutes. Because M-83 detected by radioassay persisted during those times, the drug molecule was considered to be metabolized to inactivated form or covalently bound to protein in the cell substance. The ratio of the concentrations of M-83 detected by bioassay to radioassay in the liver decreased more rapidly than those in the kidney, suggesting more potent inactivation in the liver.

Concerning the concentration of M-83 in the tumors, in Table 7 the peak concentration and AUC_0^{60} detected by radioassay and bioassay, the ratio of the concentrations detected by bioassay to those by radioassay and inactivation ability of tumor homogenate *in vitro* were compared with T_{RW}/C_{RW} value as the drug efficacy. The peak concentration and AUC_0^{60} detected by radioassay correlated well with T_{RW}/C_{RW} value. AUC_0^{60} detected by bioassay was incomplete for evaluation. The concentration of M-83 in the tumor detected by radioassay shows the quantity of the drug after its transport between blood and tumor cells, which consists of both the drug molecule and its metabolites. Bioassay shows the quantity of unchanged molecule after the drug is metabolized to inactivated form by tumor cell substance. Therefore, the ratio of the concentrations of M-83 detected by bioassay to radioassay in the tumor is determined by the inactivation ability of each tumor *in vivo*. The ratio of the concentrations in the tumor of MX-1 detected by bioassay to radioassay was approximately 10% and did not clearly decreased with time as in the liver or kidney. From *in vitro* inactivation test of M-83, tumor homogenates failed to show so strong inactivation ability under aerobic condition without any enhancing agents such as NADPH. It was suggested that part of unchanged molecule in the tumor is bound to receptor in cell membrane or cell substance, not extracted by chloroform or ethyl acetate even after homogenization and perhaps excreted out from the tumor. These factors such as receptors, inactivation and quantity of NADPH were thought to affect the drug efficacy of tumor cells by inhibiting the arrival of the drug molecule at the nucleus.

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