BIOLOGICAL PROPERTIES OF STREPTONIGRIN DERIVATIVES

III. *IN VITRO* AND *IN VIVO* ANTIVIRAL AND ANTITUMOR ACTIVITIES

YUKINORI TAKE, TAE KUBO, ERIKO TAKEMORI, YOSHIO INOUYE and SHOSHIRO NAKAMURA

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan

TOSHIO NISHIMURA, HIDEO SUZUKI and HIDEYO YAMAGUCHI

Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received for publication December 19, 1988)

Antitumor antibiotic streptonigrin (STN-COOH) is a potent inhibitor of avian myeloblastosis virus (AMV) and human immunodeficiency virus reverse transcriptases. The carboxyl group at 2′-position of STN-COOH was modified to give esters, hydrazide, amides and amino acid derivatives for biological studies.

Against AMV reverse transcriptase, the hydrazide, amides and amino acid derivatives showed inhibitory activity, which compared favorably to that of STN-COOH, with the ID_{50} values ranging 2~8 μg/ml. In contrast, the esters lacked this activity except for those having a dimethylamino group in the substituent. Splenomegaly caused by Friend leukemia virus infection was significantly inhibited by STN-COOH and STN-$COO(CH_2)_3N(CH_3)_2$, but not STN-$CONH(CH_2)_3N(CH_3)_2$. Doxorubicin-resistant murine lymphoblastoma L5178Y cells showed collateral sensitivity to both STN-COOH and STN-$COO(CH_2)_3N(CH_3)_2$ not only *in vitro* but also *in vivo*.

Streptonigrin (STN-COOH, **1**) was first isolated as an antitumor antibiotic, especially active against osteosarcomas, from *Streptomyces flocculus* in 1960[1~3]. However, the severe side effects of **1**, mainly due to bone marrow depression, resulted in the discontinuation of its clinical application. It has been reported that STN-$COOCH_3$ (**2**) is clinically superior to **1** as an antitumor agent in terms of chemotherapeutic coefficient[4,5].

Besides antitumor activity, the strong inhibition of avian myeloblastosis virus (AMV) and human immunodeficiency virus (HIV) reverse transcriptases by STN-COOH (**1**) has been reported[6,7]. Since reverse transcription is a pivotal step in the replication of retroviruses, inhibitors of this enzyme have been a target of the search for chemotherapeutics against retrovirus-related diseases, including acquired immune deficiency syndrome (AIDS). The synthesis of novel hydrazides, a hydroxamic acid derivative, amides and amino acid derivatives of streptonigrin such as STN-$CONH(CH_2)_3N(CH_3)_2$ (**12**) and STN-$CONHCH_2COOH$ (**13**), and the comparison of their biological activities with those of **1** and STN-$COOCH_3$ (**2**) have been described previously[8]. The inhibition of AMV reverse transcriptase by the individual hydrazides, amides and amino acid derivatives compared favorably to that of **1**, while **2** was essentially devoid of this activity. In contrast, the prominent inhibition of the growth of murine lymphoblastoma L5178Y cells was not observed with the amides and amino acid derivatives except for **12** and STN-$CONH(CH_2)_3NH(CH_2)_4NHCO$-STN, suggesting the role of the *N*-alkylamino group in the substituent.

For more comprehensive understanding of the marked difference between the biological properties of the methyl ester and those of the hydrazides, amides and amino acid derivatives as well as the role of the *N*-alkylamino group, the alkyl esters (**3**~**7**), STN-COO$(CH_2)_nN(CH_3)_2$ (**8** (n=2) and **9** (n=3)), STN-CONHN$(CH_3)_2$ (**10**), STN-CONH$(CH_2)_2N(CH_3)_2$ (**11**) and the amino acid derivatives (STN-CONHCHRCOOH, **14**~**23**) were newly synthesized.

In this paper, the biological properties of these compounds were compared with a special emphasis on their *in vivo* antiviral and antitumor activities.

Materials and Methods

Materials

STN-COOH (**1**) was prepared as previously reported[9]. The synthesis of STN-$COOCH_3$ (**2**), STN-CONH$(CH_2)_3N(CH_3)_2$ (**12**) and STN-$CONHCH_2COOH$ (**13**) was described previously[10]. The alkyl esters (**3**~**7**), STN-COO$(CH_2)_nN(CH_3)_2$ (**8** and **9**), hydrazide (**10**), amide (**11**) and the amino acid derivatives (**13**~**23**) were newly synthesized according to the previous method[10]. The structures of the streptonigrin derivatives were confirmed by ^1H NMR and fast atom bombardment (FAB)-MS (data not shown). All other materials were commercial products of analytical grade.

In Vitro Antiviral and Antitumor Activities

The details of the assay method for reverse transcriptase, HIV replication and the culture conditions for L5178Y cells were described in previous papers[8,9,11].

In Vivo Antiviral Activity against Friend Leukemia Virus (FLV)

Male *dd*Y mice were sacrificed 12 days after viral infection. The 10% homogenate of the enlarged spleen was used as a viral stock. The viral stock was diluted with 9 volumes of saline at usage to prepare a viral inoculum. Male *dd*Y mice, 4 weeks old and weighing about 20 g, were ip injected with 0.2 ml of the viral inoculum. STN-COOH (**1**), STN-COO$(CH_2)_3N(CH_3)_2$ (**9**) and STN-CONH$(CH_2)_3$-$N(CH_3)_2$ (**12**) were dissolved in 5% glucose, and daily ip administration was started 5 hours after viral injection and continued for 5 days. Control groups included mice infected with FLV which were not treated, and uninfected mice with or without drug-treatment. Each experimental group consisted of 5 mice. On day-12, all mice were killed and spleens were taken out to be weighed.

In Vivo Antitumor Activity against L5178Y Cells

Parental and doxorubicin (adriamycin: ADM)-resistant cell lines of murine lymphoblastoma L5178Y cells (L5178Y/S and L5178Y/ADM[12], respectively) were maintained in an ascites form in CDF_1 mice. Female CDF_1 mice, 6 weeks old and weighing about 25 g, were ip inoculated with 1×10^6 cells on day-0. The daily ip administration of STN-COOH (**1**), STN-COO$(CH_2)_3N(CH_3)_2$ (**9**) or STN-CONH$(CH_2)_3$-$N(CH_3)_2$ (**12**) was started on day-1 and continued for 10 days. Drugs were dissolved in dimethyl sulfoxide at a concentration of 5 mg/ml and diluted with 5% glucose. Each group consisted of 6 mice except for the control group of 12 mice.

Stability of STN-COO$(CH_2)_3N(CH_3)_2$ (**9**) and STN-CONH$(CH_2)_3N(CH_3)_2$ (**12**) in Cell Culture

The 6.6×10^6 L5178Y/ADM cells suspended in 1 ml of Fischer's medium were incubated in the presence of 50 μg/ml STN-COO$(CH_2)_3N(CH_3)_2$ (**9**) or STN-CONH$(CH_2)_3N(CH_3)_2$ (**12**) at 37°C for 1 and 3 hours. The whole culture was extracted with 1 ml of EtOAc at alkaline and acidic pH's. The combined EtOAc-extract was evaporated to dryness, dissolved in 50 μl of MeOH and analyzed by TLC on pre-coated Kieselgel 60 F_{254} plate (0.25 mm thickness, E. Merck, Darmstadt) developed with $CHCl_3$ - MeOH (4:1).

Physico-chemical Properties

FAB-MS were determined on a Jeol JMS-HX-100 spectrometer. The mp's were measured with a Yanagimoto micro melting point apparatus. IR spectra were run on a Shimadzu IR-408 spectrometer in $CHCl_3$. UV spectra were obtained on a Hitachi 124 spectrometer in CH_3CN. CD spectra were recorded on a Jasco J-600 spectrometer in $CHCl_3$.

Results and Discussion

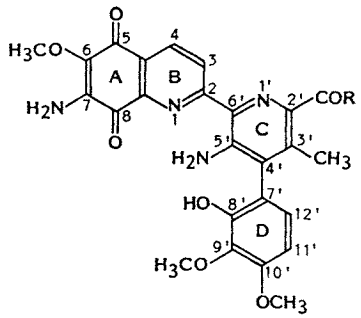
Synthesis of Esters, Hydrazide, Amides and Amino Acid Derivatives

The esters (**3**~**9**) were synthesized by the reaction of streptonigrin acid chloride, readily prepared with thionyl chloride, and an appropriate alcohol. In the case of the hydrazide, amides and amino acid derivatives, the direct condensation of the carboxyl group of STN-COOH (**1**) with 1,1-dimethylhydrazine, 3-dimethylaminopropylamine or an amino acid using 3,3′-(phenylphosphoryl)bis(1,3-thiazolidine-2-thione) was adopted. The products were purified by silica gel column chromatography. The structures of semisynthetic streptonigrins are shown in Fig. 1.

Stereochemistry of α-Amino Acid Derivatives (**18**~**23**)

In further purification by preparative TLC on silica gel, the α-amino acid derivatives (**18**~**23**) always gave two spots with different Rf values. However, the components with higher Rf values (**18U**~**23U**) were not distinguished from their counterparts (**18L**~**23L**) in terms of biological activities and physico-chemical properties such as ^{1}H NMR, FAB-MS, UV and IR spectra (data for STN-CO-L-Ala (**18**) and STN-CO-D-Ala (**19**) shown in Table 1). However, the Rf values of **18U** and **18L** were identical with those of **19U** and **19L**, respectively. Further stereochemical studies were carried out by comparing CD spectra of these compounds (Fig. 2). The nature of stereoisomerism between **18U** and **18L**, and between **19U** and **19L** was established by their optical rotations of similar magnitude and opposite sign, and nearly mirror image relationship in their CD curves. According to the X-ray analysis of STN-COOH (**1**), the rings A, B and C (see Fig. 1) are nearly coplanar with a short hydrogen bond (2.65 Å) between the NH_2 group on the ring C and the nuclear nitrogen atom in ring B, and the hydrogen bond appears to be bent[13]. Taking all these data into consideration, the diastereomer-type isomerism between **18U**~**23U** and **18L**~**23L** could be accounted for by the co-

Fig. 1. Structures of streptonigrin derivatives (STN-COR).



1	R=OH	**13**	R=NHCH$_2$COOH (Gly)
2	R=OCH$_3$	**14**	R=NH(CH$_2$)$_3$COOH
3	R=OCH$_2$H$_5$	**15**	R=NH(CH$_2$)$_4$COOH
4	R=O(CH$_2$)$_2$CH$_3$	**16**	R=NH(CH$_2$)$_5$COOH
5	R=OCH(CH$_3$)$_2$	**17**	R=NH(CH$_2$)$_7$COOH
6	R=O(CH$_2$)$_3$CH$_3$	**18**	R=L-Ala
7	R=OC(CH$_3$)$_3$	**19**	R=D-Ala
8	R=O(CH$_2$)$_2$N(CH$_3$)$_2$	**20**	R=L-Leu
9	R=O(CH$_2$)$_3$N(CH$_3$)$_2$	**21**	R=L-Val
10	R=NHN(CH$_3$)$_2$	**22**	R=L-Phe
11	R=NH(CH$_2$)$_2$N(CH$_3$)$_2$	**23**	R=L-Tyr
12	R=NH(CH$_2$)$_3$N(CH$_3$)$_2$		

Table 1. Biological and physico-chemical properties of L- and D-alanine derivatives of streptonigrin.

	STN-CO-L-Ala-U (18U)	STN-CO-L-Ala-L (18L)	STN-CO-D-Ala-U (19U)	STN-CO-D-Ala-L (19U)
MP (°C)	199~201	196~198	198~200	196~199
TLC Rf[a]	0.27	0.21	0.28	0.21
FAB-MS (*m/z*)	578 $(M+H)^+$	578 $(M+H)^+$	578 $(M+H)^+$	578 $(M+H)^+$
UV $\lambda_{max}^{CH_3CN}$ nm	210, 246, 290, 378	210, 247, 290, 378	210, 247, 290, 378	210, 247, 290, 378
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3460, 3370, 1640, 1610	3460, 3370, 1640, 1610	3460, 3370, 1640, 1610	3460, 3370, 1640, 1610
ID_{50} (μg/ml)				
RT(AMV)[b]	3	2	2	2
L5178Y/S	>8.0	>8.0	0.04	0.13

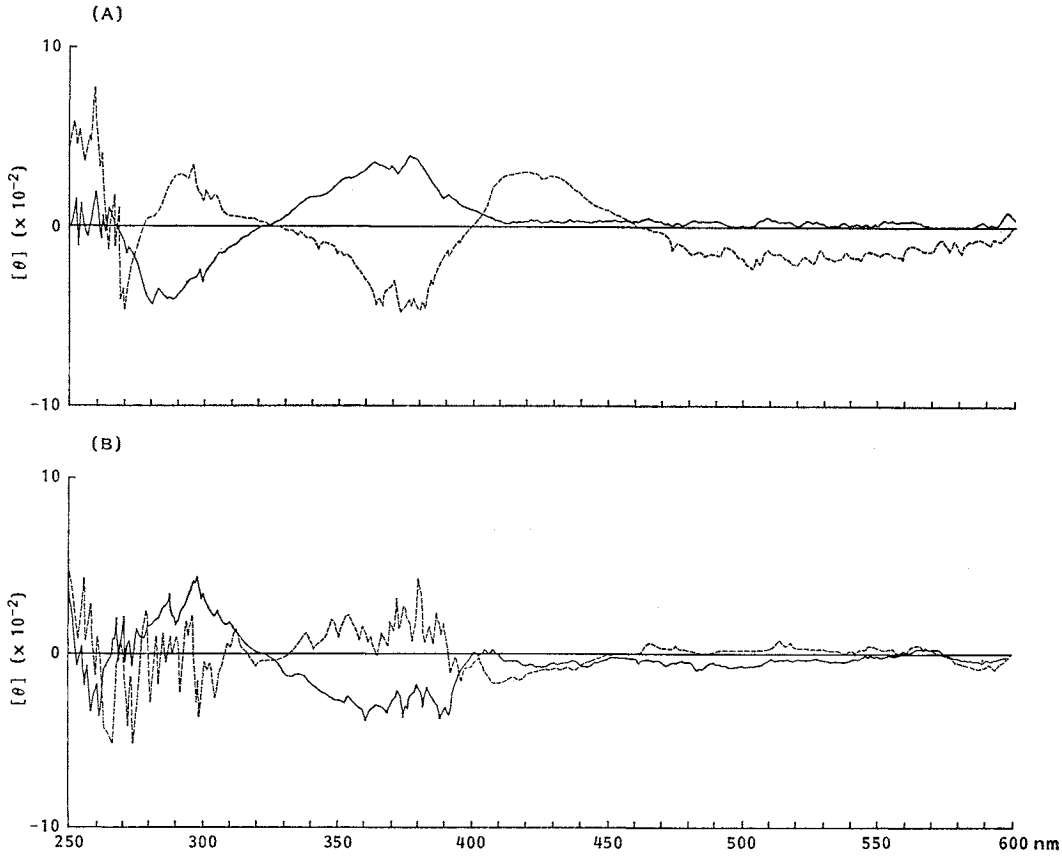
[a] Pre-coated Kieselgel 60 F_{254} plate; 0.25 mm thickness, E. Merck, Darmstadt; solvent: $CHCl_3$ - MeOH (4:1).

[b] AMV reverse transcriptase.

Fig. 2. CD spectra of L- and D-alanine derivatives of streptonigrin.

(A) —— STN-CO-L-Ala-U (**18U**), - - - STN-CO-L-Ala-L (**18L**).

(B) —— STN-CO-D-Ala-U (**19U**), - - - STN-CO-D-Ala-L (**19L**).



existence of the asymmetric carbon in the α-amino acid moiety and planar chirality in the core portion caused by the short hydrogen bond between the rings B and C. This hypothesis was further supported by the findings that a mixture of **18U** and **18L** was obtained when either **18U** or **18L** was refluxed in

CH_3CN and only the streptonigrin derivatives with an asymmetric carbon in the side chain gave twin spots by silica gel TLC.

Biological Properties

The ID_{50} values against AMV reverse transcriptase and the growth of L5178Y/S and L5178Y/ADM are shown in Table 2. A mixture of the upper and lower components of the α-amino acid derivatives was employed for the measurement of their biological activities since both diastereoisomers were identical to each other in terms of the biological properties as can be seen in Table 1. The hydrazide, amides and amino acid derivatives inhibited AMV reverse transcriptase to the same extent as STN-COOH (**1**); the ID_{50} values were in the range of 2~8 μg/ml. Up to 4 μg/ml, the highest concentration tested, the inhibition of cell growth was not significant with the amino acid derivatives. The lower cytocidal activity of the amino acid derivatives might be due to their poor membrane transport as was proposed for STN-CONHCH$_2$-COOH (**13**) in the previous paper[14]. Furthermore, the other amino acid derivatives could not suppress HIV replication as well as **13**[15]. In contrast, the inhibition of HIV replication by the STN-CONHN(CH$_3$)$_2$ (**10**) and STN-CONH(CH$_2$)$_n$N(CH$_3$)$_2$ (**11** (n=2) and **12** (n=3)) is secondary to the decrease in cell viability.

Table 2. Biological properties of streptonigrin derivatives.

Compound	ID_{50} (μg/ml)		
	RT(AMV)[a]	L5178Y/S	L5178Y/ADM
1	3	0.004	0.00025
2	>40	0.001	
3	>40	0.001	
4	>40	0.004	
5	>40	0.04	
6	>40	0.004	
7	>40	0.01	
8	5	0.003	
9	4	0.004	0.0003
10	2	0.4	
11	7	0.2	
12	3	0.5	0.06
13	2	>4.0	
14	5	>4.0	
15	5	>4.0	
16	4	>4.0	
17	8	>4.0	
18	3	>4.0	
19	2	0.1	
20	3	>4.0	
21	4	>4.0	
22	6	>4.0	
23	2	>4.0	
Doxorubicin		0.1	1.5

The purity of all streptonigrin derivatives was confirmed by TLC on silica gel, FAB-MS and ^{1}H NMR, though the α-amino acid derivatives (**18**~**23**) gave twin spots on TLC due to the diastereomer-type isomerism.

[a] AMV reverse transcriptase.

Like STN-COOCH$_3$ (**2**), the alkyl esters (**3**~**7**) did not show any marked inhibition of AMV reverse transcriptase even at a concentration of 40 μg/ml. The dimethylamino group endowed the ester derivatives with inhibitory activity against AMV reverse transcriptase as shown by the results for STN-COO(CH$_2$)$_n$N(CH$_3$)$_2$ (**8** and **9**). As for cytocidal activity, the newly synthesized esters (**3**~**9**) proved to be as potent as STN-COOCH$_3$ (**2**). Due to their significant cytocidal activity, **8** and **9** could not be applied to the anti-HIV test.

In conclusion, the previous findings concerning the difference in the biological properties among the methyl ester, hydrazides, amides and amino acid derivatives were in good coincidence with those obtained with the newly synthesized streptonigrin derivatives. Owing to the presence of the dimethylamino group in the substituents, STN-COO(CH$_2$)$_n$N(CH$_3$)$_2$ (**8** and **9**) fully recovered the inhibitory activity against AMV reverse transcriptase and, in contrast, STN-CONH(CH$_2$)$_n$N(CH$_3$)$_2$ (**11** and **12**) were able to inhibit the growth of L5178Y cells.

To test whether the potent cytocidal activity of the esters results from their susceptibility to hydrolysis in cell culture or not, STN-COO(CH$_2$)$_3$N(CH$_3$)$_2$ (**9**) was kept with L5178Y/ADM cells at

Table 3. Therapeutic effect of streptonigrin and its derivatives on FLV-infected mice.

Compound	Dose (mg/kg/day)	Weight of spleen (mg) mean±SD		Inhibition (%)
		V^+D^+	V^-D^+	
STN-COOH (**1**)	0.8	No survivor	No survivor	Toxic
	0.2	220±130	139±46	94
	0.05	303±181	213±42	93
	0.0125	951±447	164±1	37
	Control	V^+D^- 1,363±517		
		V^-D^- 120±6		
$STN\text{-}COO(CH_2)_3N(CH_3)_2$	2	24±6	36±12	100
(**9**)	0.5	247±78	140±33	92
	0.125	1,207±952	156±24	16
	Control	V^+D^- 1,406±686		
		V^-D^- 149±19		
$STN\text{-}CONH(CH_2)_3N(CH_3)_2$	5	968±541	183±36	15
(**12**)	1.25	967±546	185±40	15
	0.31	1,367±517	142±17	−33
	Control	V^+D^- 1,080±321		
		V^-D^- 160±39		
Doxorubicin	10	607±537	120±48	29
	2.5	1,231±260	175±40	−54
	0.625	936±258	143±14	16
	Control	V^+D^- 854±466		
		V^-D^- 172±28		

V^+: Infected with FLV, V^-: not infected with virus, D^+: drug administered, D^-: no drug administered.
Inhibition (%) $=\{1-(V^+D^+-V^-D^+)/(V^+D^--V^-D^-)\}\times 100$.
Schedule:

37°C for 1 or 3 hours; $STN\text{-}CONH(CH_2)_3N(CH_3)_2$ (**12**) was also treated in the same way as a control. The products were extracted with an equal volume of EtOAc at acidic and alkaline pH's, and analyzed by TLC on silica gel. In both cases, the compounds incubated with the cell culture were recovered without being accompanied by STN-COOH (**1**) (data not shown).

Splenomegaly induced by FLV infection was significantly suppressed by the treatment with $STN\text{-}COO(CH_2)_3N(CH_3)_2$ (**9**) as well as STN-COOH (**1**), whereas no effect was observed with $STN\text{-}CONH(CH_2)_3N(CH_3)_2$ (**12**) even at a daily dose as high as 5 mg/kg/day (Table 3). Doxorubicin could not protect mice from FLV infection up to the highest dose, 10 mg/kg/day. The protective dose of **1** is about 1/10 that of **9**.

Doxorubicin-resistant cells (L5178Y/ADM) showed collateral sensitivity to STN-COOH (**1**) and its derivatives including $STN\text{-}COO(CH_2)_3N(CH_3)_2$ (**9**) and $STN\text{-}CONH(CH_2)_3N(CH_3)_2$ (**12**)[16], in spite of the resistance of the cells to various antitumor drugs *in vitro*[12]. Therefore, the antitumor activity of **1**, **9** and **12** *in vivo* was determined against L5178Y/S- and L5178Y/ADM-implanted CDF_1 mice (Tables 4 and 5). As previously reported[17], the mice implanted ip with L5178Y/ADM survived about 2-fold longer than those with the parental cells (L5178Y/S). The daily ip administration of **1**

Table 4. Antitumor effect of streptonigrin and its derivatives on L5178Y/S-implanted mice.

Compound	Dose (mg/kg/day)	Treatment schedule (days)	Survival (days) mean±SD	Survivor (50 days)	T/C	*P*
Control			16.6±1.0	0/12	100	
STN-COOH (**1**)	0.2	1~10	15.7±1.6	0/6	94	
	0.05	1~10	16.7±1.0	0/6	101	
	0.0125	1~10	17.3±0.8	0/6	105	
STN-COO$(CH_2)_3N(CH_3)_2$ (**9**)	0.2	1~10	16.3±0.8	0/6	98	
	0.05	1~10	16.7±0.8	0/6	101	
	0.0125	1~10	18.2±1.2	0/6	110	
STN-CONH$(CH_2)_3N(CH_3)_2$ (**12**)	1.0	1~10	17.3±0.8	0/6	105	
	0.25	1~10	17.2±1.3	0/6	104	
Doxorubicin	1.8	1~10	33.8±12.7	2/6	204	<0.05
	0.45	1~10	22.7±3.1	0/6	137	<0.01
Mitomycin C	1.0	1~10	27.2±10.3	0/6	164	
	0.25	1~10	19.0±2.1	0/6	115	<0.05

Table 5. Antitumor effect of streptonigrin and its derivatives on L5178Y/ADM-implanted mice.

Compound	Dose (mg/kg/day)	Treatment schedule (days)	Survival (days) mean±SD	Survivor (50 days)	T/C	*P*
Control			31.3±2.9	0/12	100	
STN-COOH (**1**)	0.2	1~10	13.0±1.1	0/6	42	
	0.05	1~10	53.3±8.0	0/6	171	<0.002
	0.0125	1~10	64.2±9.5	4/6	205	<0.001
STN-COO$(CH_2)_3N(CH_3)_2$ (**9**)	0.2	1~10	66.7±8.2	5/6	213	<0.001
	0.05	1~10	40.5±15.1	1/6	130	
	0.0125	1~10	31.0±3.2	0/6	99	
STN-CONH$(CH_2)_3N(CH_3)_2$ (**12**)	1.0	1~10	32.3±1.8	0/6	103	
	0.25	1~10	34.5±3.7	0/6	110	
Doxorubicin	1.8	1~10	48.7±12.2	1/6	156	<0.02
	0.45	1~10	42.0±16.4	1/6	134	
Mitomycin C	1.0	1~10	43.5±9.2	0/6	139	<0.05
	0.25	1~10	46.3±18.2	1/6	148	

from days 1 to 10 significantly prolonged the lifespan of L5178Y/ADM-implanted mice at 0.05 and 0.0125 mg/kg/day, whereas **1** failed to exhibit therapeutic activity against L5178Y/S-implanted mice at the same doses. In contrast, doxorubicin was more effective to L5178Y/S- than L5178Y/ADM-implanted mice. The highest therapeutic activity of **9** against L5178Y/ADM-implanted mice was observed at 0.2 mg/kg/day; five mice out of 6 survived more than 70 days with T/C of 213, though **9** also failed to show the effectiveness to L5178Y/S-implanted mice at any doses employed. These results suggest that the collateral sensitivity of L5178Y/ADM to **1** and its derivatives observed *in vitro* influences the therapeutic activity of these drugs *in vivo*. The reason why **12** does not exhibit therapeutic activity against either L5178Y/S- or L5178Y/ADM-implanted mice remains to be solved.

In tissue such as liver the reduction of STN-COOH (**1**) may lead to its conjugation and detoxication, whereas in other tissues and cells **1** may be extremely cytotoxic compound. It has been reported that both intra- and extra-mitochondrial NAD(P)H is oxidized by DT-diaphorase (E.C.1.6.99.2) in the presence of catalytic amount of **1** in a non-stoichiometric fashion[18]. In couple with the oxidation of NAD(P)H, **1** was reduced to the hydroquinone. The rapid autoxidation of the hydroquinone

results in the formation of hydrogen peroxide and the regeneration of the quinone. Glutathione peroxidase represents a major pathway for hydrogen peroxide detoxication even in catalase-rich cells[19,20]. **1** may undergo extensive damage under conditions in which reduced glutathione is depleted, *e.g.* in the absence of NADH or NADPH required for glutathione reductase activity. The cellular concentration of reduced glutathione is also regulated by the glutathione synthetase. Therefore, the enhanced sensitivity of L5178Y/ADM when compared with L5178Y/S may result from the decreased DT-diaphorase activity and/or the metabolic change of reduced glutathione.

Acknowledgment

This work was supported in part by Grant-in-Aid for General Scientific Research and Cancer Research from the Ministry of Education, Science and Culture, Japan. The authors are grateful to Dr. T. HIRATA of Department of Science, Hiroshima University for his kind help in the measurement of CD spectra. We also thank Drs. M. ARAI and T. HANEISHI of Fermentation Research Laboratories, Sankyo Co., Ltd. for the measurement of FAB-MS and ^{1}H NMR spectra.

References

1) RAO, K. V. & W. P. CULLEN: Streptonigrin, an antitumor substance. I. Isolation and characterization. *In* Antibiotics Annual 1959–1960. *Eds.*, H. WELCH & F. MARTI-IBAÑEZ, pp. 950~953, Antibiotica, Inc., New York, 1960
2) OLESON, J. J.; L. A. CALDERELLA, K. J. MJOS, A. R. REITH, R. S. THIE & I. TOPLIN: The effects of streptonigrin on experimental tumors. Antibiot. Chemother. 11: 158~164, 1961
3) HACKETHAL, C. A.; R. B. GOLBEY, C. T. C. TAN, D. A. KARNOFSKY & J. H. BURCHENAL: Clinical observation on the effects of streptonigrin in patients with neoplastic disease. Antibiot. Chemother. 11: 178~183, 1961
4) HUMPHEREY, E. W. & F. S. DIETRICH: Clinical experience with methyl ester of streptonigrin (NSC-45384). Cancer Chemother. Rep. 33: 21~26, 1963
5) RIVERS, S. L.; R. M. WHITTINGTON & T. J. MEDREK: Methyl ester of streptonigrin (NSC-45384) in treatment of malignant lymphoma. Cancer Chemother. Rep. 46: 17~21, 1965
6) CHIRIGOS, M. A.; J. W. PEARSON, T. S. PAPAS, M. A. WOODS, H. B. WOOD, Jr. & G. SPAHN: Effect of streptonigrin (NSC-45383) and analogs on oncornavirus replication and DNA polymerase activity. Cancer Chemother. Rep. 57: 305~309, 1973
7) TAKE, Y.; Y. INOUYE, S. NAKAMURA, H. S. ALLAUDEEN & A. KUBO: Comparative studies of the inhibitory properties of antibiotics on human immunodeficiency virus and avian myeloblastosis virus reverse transcriptases and cellular DNA polymerases. J. Antibiotics 42: 107~115, 1989
8) OKADA, H.; H. MUKAI, Y. INOUYE & S. NAKAMURA: Biological properties of streptonigrin derivatives. II. Inhibition of reverse transcriptase activity. J. Antibiotics 39: 306~308, 1986
9) HANAJIMA, S.; K. ISHIMARU, K. SAKANO, S. K. ROY, Y. INOUYE & S. NAKAMURA: Inhibition of reverse transcriptase by limocrocin. J. Antibiotics 38: 803~805, 1985
10) MIYASAKA, T.; S. HIBINO, Y. INOUYE & S. NAKAMURA: Synthesis of novel streptongrin 2-amide derivatives with 3,3'(phenylphosphonyl)bis(1,3-thiazolidine-2-thione). J. Chem. Soc. Perkin Trans. I 1986: 479~482, 1986
11) HARADA, S.; Y. KOYANAGI & N. YAMAMOTO: Infection of human T-lymphotropic virus type-I (HTLV-I)-bearing MT-4 cells with HTLV-III (AIDS virus). Virology 14: 272~281, 1985
12) NISHIMURA, T.; K. MUTO & N. TANAKA: Drug sensitivity of an adriamycin-resistant mutant subline of mouse lymphoblastoma L5178Y cells. J. Antibiotics 31: 493~495, 1978
13) CHIU, Y.-Y. H. & W. N. LIPSCOMB: Molecular and crystal structure of streptonigrin. J. Am. Chem. Soc. 97: 2525~2530, 1975
14) INOUYE, Y.; H. OKADA, J. UNO, T. ARAI & S. NAKAMURA: Effects of streptonigrin derivatives and sakyomicin A on the respiration of isolated rat liver mitochondria. J. Antibiotics 39: 550~556, 1986
15) INOUYE, Y.; Y. TAKE, S. NAKAMURA, H. NAKASHIMA, N. YAMAMOTO & H. KAWAGUCHI: Screening for inhibitors of avian myeloblastosis virus reverse transcriptase and effect on the replication of AIDS-virus. J. Antibiotics 40: 100~104, 1987
16) INOUYE, Y.; H. OKADA, S. K. ROY, T. MIYASAKA, S. HIBINO, N. TANAKA & S. NAKAMURA: Biological

properties of streptonigrin derivatives. I. Antimicrobial and cytocidal activities. J. Antibiotics 38: 1429~1432, 1985

17) SUGIMOTO, Y.; Y. HIRAKAWA, N. TANAKA, M. TAHARA, I. SATO, T. NISHIMURA, H. SUZUKI & N. TANAKA: Transplantability and sensitivity to natural killer cells of aclarubicin-resistant murine lymphoma. Cancer Res. 46: 5646~5648, 1986

18) HOCHSTEIN, P.; J. LASZLO & D. MILLER: A unique, dicoumarol-sensitive, non-phosphorylating oxidation of DPNH and TPNH catalyzed by streptonigrin. Biochem. Biophys. Res. Commun. 19: 289~295, 1965

19) COHEN, G. & P. HOCHSTEIN: Glutathione peroxidase: The primary agent for the elimination of hydrogen peroxide in Erythrocytes. Biochemistry 2: 1420~1428, 1963

20) COHEN, G. & P. HOCHSTEIN: Generation of hydrogen peroxide in erythrocytes by hemolytic agents. Biochemistry 3: 895~900, 1964