UDC 615.771.7 : 577.158.04 : 616-006-085

98. Yosio Sakurai, Hiroshi Imamura, and Ayako Moriwaki: The Effect of Anti-tumor Agents on the Dehydrogenase Activity of Tumors.

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An experimental method for selecting the most effective among anti-tumor drugs of the present clinical application had been reported by Nishioka, *et al.* in 1957.[1] The principle of the procedure (INK-method) was based on the estimation *in vitro* of the potency of a drug in inhibiting dehydrogenase activity of the clinical specimen by biopsy.

This paper deals with the question of whether there is actually any parallelism between the two effects of a drug, inhibition of this enzymic process and of growth of tumor cells.

Experiments and Results

The Yoshida sarcoma, and rat ascitic hepatomas AH-130 and AH-7974, were used in this experiment. Both the Yoshida sarcoma and AH-130 are known to be sensitive against nitrogen mustard and its N-oxide, but, on the contrary, AH-7974 was found to be 10 times resistant to the former agent and 50 times resistant to the latter.

For estimation of the inhibitive potency of the test compounds on dehydrogenase activity of the tumors, CAP-method was applied which was reported in 1956 by Yamamoto, *et al.*[2] Three experiments using the three kinds of tumors were carried out for each compound, always at a time and with the same lot of Hank's agar medium. Number of tumor cells in agar medium was adjusted every time to about 5,000,000/cc. The compound to be tested, except 6-mercaptopurine and Carzinophilin, was serially diluted with a phosphate buffer solution (pH 7.4). 6-Mercaptopurine was dissolved with Na_2CO_3 (0.9%) and Carzinophilin with $NaHCO_3$ (2%) to make the initial solutions, which were again serially diluted with a phosphate buffer (pH 7.4) to the necessary concentrations. The series of agar plates, added with the serially-diluted solution of the compound, were incubated at 37°, usually for 17 hrs., but in the case of 6-mercaptopurine, incubation should be prolonged for 48 hrs. Each plate was then added with 8 cc. of a mixture of equal parts of horse serum and of phosphate buffer solution of indophenol of 2, 6-dichlorophenol (0.3%). The inhibitive potency of the compound on dehydrogenase of the cell was assayed by measuring the diameter (in mm.) of the blue circle formed around the cylinder on the agar after further incubation for 1 hr. at 37°. The minimum concentration, at which the inhibition circle of 15-mm. diameter could be seen, was defined in this paper as the minimum effective concentration. The results are shown in Tables I and II.

Discussion

The clinical application of INK-method had been based on an assumption that there was, at least to a certain extent, a parallel relation between anti-tumor effectiveness of the drug and its inhibitive activity on dehydrogenase of the same tumor. The present experiments on rat tumors, however, resulted in some unexpected phenomena.

As shown in Tables I and II, the difference between the degree of inhibition of the compound on dehydrogenase activity of the three kinds of tumors was found to be far less than that expected from the results of animal experiments on growth retardation of the tumors.

As an index of sensitivity of the tumor against the compound *in vivo*, minimum effective dose** of each compound was compared and are shown in Table III for reference.

Although the minimum effective dose of methyl-bis(2-chloroethyl)amine N-oxide (HN_2-N-oxide) or triethylenemelamine (TEM) *in vivo* on AH-7974 was determined by Yoshida *et al.* to be 50 times larger than that on AH-130, the difference was only slight between the concentrations which exhibited the same diameter of the inhibiting circle on agar plate of different tumors. Furthermore, the inhibiting circle also appeared by diethyl-2-chloroethyl-

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1) K. Nishioka *et al.*: Nihon Rinsho, **15**, 1937(1957).

2) T. Yamamoto *et al.*: Gann, **47**, 424(1956).

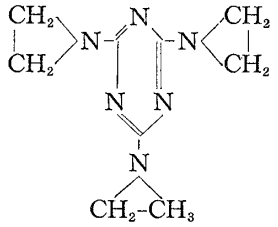
** cf. M. Ishidate, *et al.*: Gann, **45**, 484(1954).

TABLE I.

Compound	Concn. in mM / Tumor	50	20	10	5	2	1	0.5	0.2	0.1	0.05	0.02	0.01
Methyl-bis(2-chloroethyl)-amine	Yoshida sarcoma						40	34	19	16	0*		
	AH-130					38	32	28	23	18	16	12	0
	AH-7974					30	23	21	14	10	0	0	0
Methyl-bis(2-chloroethyl)-amine N-oxide	Yoshida sarcoma					32	30	18	15	0	0	0	0
	AH-130					30	25	20	17	13	10	0	0
	AH-7974					32	25	20	15	0	0	0	0
Diethyl-2-chloroethylamine	Yoshida sarcoma				30	24	20	16	11	0	0	0	0
	AH-130				30	25	20	15	0	0	0	0	0
	AH-7974				30	20	17	15	0	0	0	0	0
A	Yoshida sarcoma					17	11	0	0	0	0	0	0
	AH-130					16	11	0	0	0	0	0	0
	AH-7974					16	12	0	0	0	0	0	0
B	AH-130	20	14	10	0	0	0	0	0				
	AH-7974	17	11	0	0	0	0	0	0				
6-Mercaptopurine	AH-130		34	21	15								
	AH-7974		20	12	0								

* Figure 0 signifies no inhibition circle on agar outside of the cylinder.

A:



B:

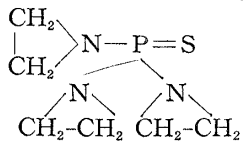


TABLE II.

Compound	Concn. in mg. equiv./cc. / Tumor	20	10	5	2	1	0.5	0.2	0.1
Sarkomycin	Yoshida sarcoma	36	30	25	20	15	11	0*	0
	AH-130		15	11	0	0	0	0	0
	AH-7974	27	22	16	11	0	0	0	0

Compound	Concn. in unit/cc. / Tumor	2000	1000	500	200	100	50	20	10
Carzinophilin	Yoshida sarcoma	19	16	16	12	0	0	0	0
	AH-130	0	0	0	0	0	0	0	0
	AH-7974	0	0	0	0	0	0	0	0

* Figure 0 signifies no inhibition circle on agar outside of the cylinder.

amine at nearly the same concentration level, in spite of its complete ineffectiveness against tumor animals.

On the contrary, Carzinophilin was found to be active *in vivo* both on these hepatomas and on the Yoshida sarcoma, but inhibiting circle in CAP-test was observed only with the

TABLE III.

Compound	Tumor	MED in rat	Resistance index of tumors
Methyl-bis(2-chloroethyl)-amine	Yoshida sarcoma	0.05 mg./kg.[a)]	5
	AH-130	0.01	1
	AH-7974	0.1	10
Methyl-bis(2-chroloethyl)-amine N-oxide	Yoshida sarcoma	1.00[b)]	1
	AH-130	1.00[b)]	1
	AH-7974	50.00[b)]	50
Diethyl-2-chloroethylamine	Yoshida sarcoma	no effect[c)]	
	AH-130	no effect	
	AH-7974	no effect	
A	Yoshida sarcoma	0.05[c)]	5
	AH-130	0.01	1
	AH-7974	0.5	50
B	AH-130	1.0	
	AH-7974	no effect	
6-Mercaptopurine	AH-130	100	
	AH-7974	no effect	
Sarkomycin	Yoshida sarcoma	10 mg. equiv./kg.	1
	AH-130	50	5
	AH-7974	100	10
Carzinophilin	Yoshida sarcoma	50 unit/kg.	1
	AH-130	100	2
	AH-7974	500	10

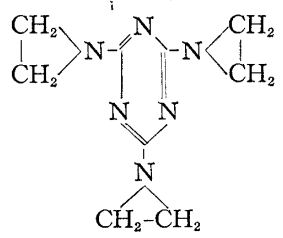
Unless otherwise noted, all data were taken from unpublished experimental data of Dr. H. Satoh.

a) cf. H. Satoh, *et al.*: Gann, **45,** 516(1954).

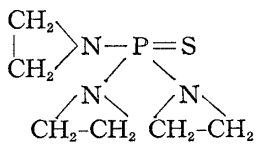
b) cf. M. Ishidate, *et al.*: *Ibid.* **47,** 334(1956).

c) cf. M. Ishidate, *et al.*: *Ibid.*, **43,** 171(1952).

(A)



(B)



Yoshida sarcoma alone, and no inhibition with the hepatomas up to the concentration of 2000 unit/cc.

In place of tumor cells on agar plate, similar experiment was carried out with suspended tumor cells in a phosphate buffer (pH 7.0). The solution (5 cc.) in each test tube, containing HN_2-N-oxide and suspended tumor cells (5,000,000 cells/cc.), was incubated with shaking at 37° for 30 mins. and then 0.5 cc. each of developer and horse serum were added. The developer was made by dissolving 4 mg. of indophenol of 2, 6-dichlorophenol in 30 cc. of a phosphate buffer solution. After 30 mins. of further incubation, the minimum concentration of HN_2-N-oxide was determined by observing the limit concentration at which the blue

color of the solution remained. The result from this experiment was completely the same as that from agar plate method, as shown in Table IV.

TABLE IV.

Compound	Tumor	MED in rat mg./kg.	Resistance index of tumors	Concn. in m*M*					
				100	50	20	10	5	0
Methyl-bis(2-chloro-ethyl)amine N-oxide	AH-130	1	1	+	+	−	−	−	−
	AH-7974	50	50	+	+	−	−	−	−

Conclusion

The non-parallelism between two biological activities of a compound, the tumor growth retardation and inhibition on its enzymic action, is worth a careful consideration when selecting the most effective among the anti-tumor agents for clinical application by experiments based on the estimation of dehydrogenase activity of the tumor specimen from a patient.

The authors are much indebted to Dr. H. Satoh for his collaboration in animal experiments and also wish to thank Dr. D. Mizuno of National Institute of Health, Tokyo, for his kind advice concerning the CAP-method.

Summary

The fact was presented in this paper that there was no quantitative parallelism between the tumor-growth retarding effect of a compound on animal tumors and its inhibiting effect on dehydrogenase activity *in vitro* of the living cells of the same kind of tumor. An attention should therefore be paid to this fact when a drug is to be selected for clinical purpose from the anti-cancer agents by comparing their inhibiting activity on this enzyme of tumor piece taken from a patient.

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99. Masao Uchibayashi: Studies on Steroids. XII.*
Isolation of Gitorin from *Digitalis lanata*.

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The cardiac glycoside, gitorin, was isolated from the leaves of *Digitalis lanata* by Tschesche and his co-workers[1] in 1952 and was formulated as gitoxigenin glucoside on the ground that it gives dianhydrogitoxigenin and glucose on acid hydrolysis and gitoxigenin on enzymatic hydrolysis. They found that the material obtained through the usual procedures of isolation was a mixture of gitorin and digitalinum verum, and that separation of the two was difficult, the isolation of gitorin being achieved only through its acetyl derivative. Hasegawa and his collaborators[2] obtained gitorin, without deriving it to its acetate, by repeated chromatography on charcoal and through counter-current distribution method. It is the purpose of this paper to report an efficient method for obtaining gitorin from the leaves of *Digitalis lanata* and to present the results of the Mannich hydrolysis of gitorin.

Dried leaves of *Digitalis lanata* were submitted to extraction referring to the known method.[3] Somewhat differing from description in the literature, however, concentration of

* The paper which appeared in this Bulletin, **6**, 255(1958), constituted the last in a series entitled "Studies on the Components of *Rhodea japonica* Roth.", and the title is changed with this report to "Studies on Steroids" by Hayao Nawa.

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1) R. Tschesche, G. Grimmer, F. Neuwald: Chem. Ber., **85**, 1103(1952).
2) H. Hasegawa, K. Inoue, J. Ishii, H. Iijima: This Bulletin, **4**, 319(1956).
3) A. Stoll, W. Kreis: Helv. Chim. Acta, **16**, 1049(1933).