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Comparative Effects of Yoshi-864 and Busulfan on Certain Transplantable Murine Tumors[1] (38751)

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In an investigation of antineoplastic activity in a series of methane sulfonic acid esters of aminoglycols, 1-propanol, 3,3′-iminodi-, dimethanesulfonate (ester) hydrochloride (Yoshi-864; NSC-102627) was found to be highly effective against certain experimental tumors which were naturally resistant to alkylating agents (1); activity against the L1210 leukemia was demonstrated subsequently (2). More recently, Imamura *et al.* (3) detected a synergism between Yoshi-864 and certain conventional antitumor agents against the Yoshida sarcoma. Their most impressive data were obtained using Yoshi-864 in combination with 6-mercaptopurine. The latter, when given alone, had an ED_{50} of 36 mg/kg. When given in combination with one half of the ED_{50} of Yoshi-864, this value decreased to 6 mg/kg. Tashiro and Sakurai (4) invoked the elevation of glycogen and oligosaccharide contents which they observed in treated tumors as underlying the mechanism of action of this compound. Since clinical experiences with Yoshi-864 in Japan were encouraging, it is now available in the United States for clinical trials (5).

The following is a report of our studies of Yoshi-864 and the chemically similar alkylator, busulfan, on two transplantable murine tumors. Structural formulas of the two drugs are shown in Fig. 1.

Materials and Methods. Yoshi-864 was obtained from Dr. Milan Slavik, National Cancer Institute; busulfan was from Nutritional Biochemicals Corp. The Ehrlich ascites tumor was propagated in SCH:ARS(ICR)f mice (Sprague–Dawley). The L1210 leukemia was propagated in DBA/2 mice; testing was done in BDF_1 (C57BL/6 × DBA/2) mice. Both of these latter strains were from various sources in accordance with availability. In assessing survival times of mice, BDF_1 mice received 10^6 L1210 cells and SCH:ARS(ICR)f mice received 10^7 Ehrlich ascites tumor cells ip. Cell viability *in vitro* was determined by the Trypan blue exclusion method. Measurement of macromolecular synthesis *in vitro* was by the incorporation of thymidine-methyl-^{3}H (DNA), uridine-5-^{3}H (RNA), or L-leucine-^{3}H (protein) into the acid-insoluble fraction of standardized cell suspensions after incubation in Eagle's minimum essential medium with Hanks' balanced salt solution (MEM; Microbiological Associates). Radioactivity was measured with a liquid scintillation spectrometer (Nuclear Chicago, Mark I).

Results and Discussion. Results of a typical experiment to assess the effects of equimolar doses of Yoshi-864 and busulfan on survival times of mice bearing each tumor are shown in Table I. The highly predictive L1210 leukemia was quite susceptible to the action of Yoshi-864, as indicated by a doubling of the control survival time. It should be noted that this 103% increase in life span is fourfold the 25% increase established by the Drug Evaluation Branch, National Cancer Institute as indicative of an active compound "... worthy of further study" (6). By interpolation of the graph compiled by DeVita and Schein (7) relating survival time to size of cell inoculum, it can be estimated that a doubling of survival time resulting from treatment following administration of 10^6 L1210 cells corresponds to reduction of the tumor burden to approximately 30–50 cells, or >99.99% (>4-log) cell kill. Busulfan conferred a significant but only slight increase in survival time. Similar effects were noted using the less predictable Ehrlich ascites tumor; the increased survival following Yoshi-864 treatment was greater than in the L1210 model,

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and the increase following busulfan treatment was not significant.

Viability of Ehrlich ascites tumor cells incubated at 37° *in vitro* with 10^{-4} *M* Yoshi-864 or busulfan was unaffected during the 7-hr period of observation (Table II). A positive control drug, *cis*-dichlorodiammineplatinum(II) (NSC-119875) yielded data altogether compatible with that reported earlier (8).

To determine if either Yoshi-864 or busulfan affected the synthesis of DNA, RNA, or protein *in vitro*, a 1% (vol/vol) Ehrlich ascites tumor cell suspension in MEM was incubated with each drug at 10^{-4} *M* for up to 3 hr prior to addition of the radioactively-labeled precursor for each macromolecule. After a further 20-min incubation, measurement of radioactivity incorporated into the acid-insoluble fraction of cells from each flask showed a slight depression (26–28%) of the rate of DNA synthesis by both drugs, but no detectable effect on synthesis of RNA or protein.

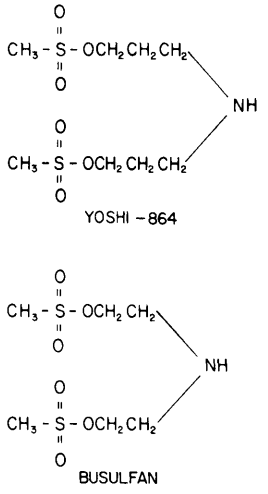

FIG. 1. Structural formulas of Yoshi-864 and busulfan.

Consequently, mice bearing well developed Ehrlich ascites tumors were given a single ip injection of equimolar doses of Yoshi-864 and busulfan (75 mg/kg and 57 mg/kg, respectively). At 24 hr, 48 hr, and 72 hr, two mice from each group, along with two tumor-bearing, nontreated mice were sacrificed, the tumor cells from each two mice were pooled, and a standardized cell suspension (10^6 cells/ml) in MEM was prepared from each. Microscopic examination of cells in the presence of Trypan blue showed >95% viability in all cases. Four-ml aliquots of each of the three cell suspensions were added to individual reaction vessels, which subsequently received 4.0 μCi of thymidine–methyl-^{3}H, uridine-5-^{3}H, or L-leucine-^{3}H. After 20 min incubation at 37° the reactions were terminated by addition of 4 ml of cold 10% trichloroacetic acid, the insoluble cell fraction from each vessel was washed thrice with cold 5% acid by sedimentation and resuspension, and the incorporated radioactivity was measured. Figure 2 shows that cells from mice which

TABLE I. EFFECTS OF EQUIMOLAR DOSES OF YOSHI-864 AND BUSULFAN ON MEAN SURVIVAL TIMES OF BDF_1 MICE BEARING THE L1210 LEUKEMIA AND OF SCH:ARS (ICR)f MICE BEARING THE EHRLICH ASCITES (EA) TUMOR.

Tumor[a]	Drug[b]	Mean survival time; L1210: hr ± SD, EA: days ± SD	% Increase	*P*[c]
L1210	None	146 ± 4		
	Yoshi-864[d]	296 ± 24	103	<0.001
	Busulfan[e]	163 ± 13	11	<0.005
EA	None	8.6 ± 3.6		
	Yoshi-864	21.2 ± 6.5	147	<0.001
	Busulfan	9.3 ± 0.7	9	N.S.

[a] Mice received 10^6 L1210 cells or 10^7 EA cells on day 0; each group contained 10 mice.
[b] Treatment was one ip injection on day 1 only: Yoshi-864, 75 mg/kg; busulfan, 57 mg/kg.
[c] Level of significance of difference between each treated group and its control group.
[d] Mol wt = 325.83 as the hydrochloride salt.
[e] Mol wt = 246.30.

TABLE II. EFFECTS OF YOSHI-864, BUSULFAN, AND NSC-119875 ON VIABILITY OF EHRLICH ASCITES TUMOR CELLS *in vitro*.[a]

Period of incubation, hr	% Viable cells when incubated with 10^{-4} *M*		
	Yoshi-864	Busulfan	NSC-119875
1	100	99	100
2	99	99	96
3	99	100	98
4	100	100	86
5	96	97	27
6	100	96	1
7	100	98	0

[a] Experimental conditions: 0.2% (vol/vol) cell suspension in MEM (8 parts), 0.1 *M* Na-K phosphate buffer, pH 7.4 (1 part), and autochthonous ascitic fluid (1 part); 37°. After mixing a small aliquot with an equal part of Trypan blue, 100 cells were counted at each interval and viable cells were considered to be those which excluded the dye. Data given are mean values from three separate experiments.

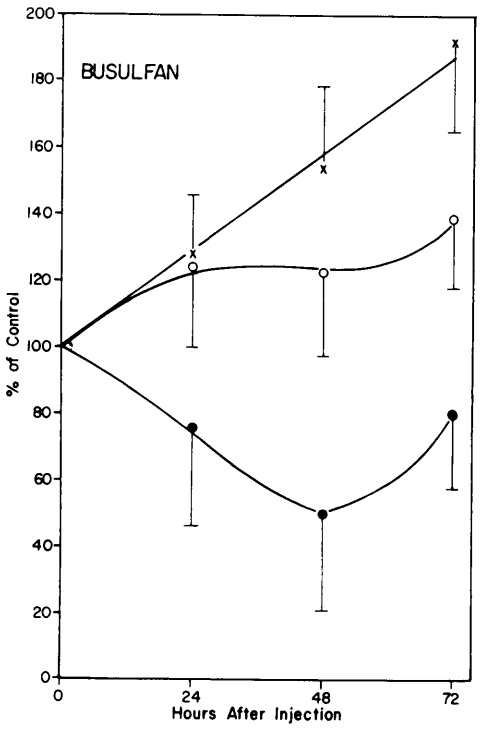

FIG. 2. Synthesis of DNA (●), RNA (○), and protein (×) in Ehrlich ascites tumor cells from mice which received one injection of busulfan, 57 mg/kg. Data are mean values of four separate experiments, and vertical bars represent 1 SD.

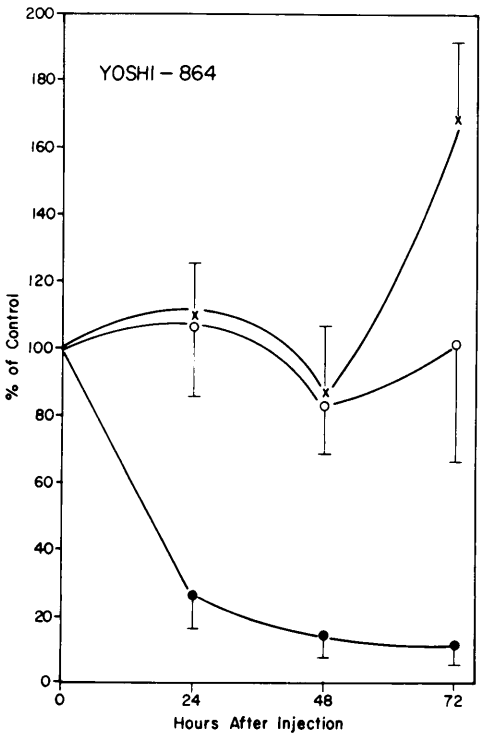

FIG. 3. Same as Fig. 2, except mice received Yoshi-864, 75 mg/kg.

received busulfan were moderately but reversibly compromised in the ability to incorporate thymidine in DNA. The rate of synthesis of RNA was virtually unchanged (±1 SD), while the synthesis of protein ultimately reached a rate approaching twice that of control cells. Cells from mice which received Yoshi-864 (Fig. 3) displayed a similar pattern as regards synthesis of RNA and protein; however, the rate of DNA synthesis in these cells was markedly suppressed, and remained so during the 3-day period of observation (the upper limit of 3 days was imposed by deaths of tumor-bearing, nontreated control mice).

It is thus demonstrated that a very subtle change in the busulfan molecule, consisting solely of lengthening the bis alkylating moieties of the amine by one additional carbon atom, enhances markedly the antineoplastic properties of the molecule as well as its inhibitory action on DNA synthesis. Studies to define the basis of this enhanced activity at the molecular level should perhaps be directed toward definition of possible dif-

ferences in modes of cross-linking by the two drugs.

Summary. Yoshi-864 extends markedly the survival times of mice bearing L1210 leukemia or Ehrlich ascites carcinoma. Busulfan, with methanesulfonate leaving groups identical with those of Yoshi-864, is without effect. Tumor cells from mice bearing the Ehrlich tumor and treated with Yoshi-864 have a persistent reduction in ability to synthesize DNA. Synthesis of DNA in cells from mice treated with busulfan is moderately suppressed at 48 hr after treatment, but returns virtually to the control value at 72 hr.

1. El-Merzabani, M. M., and Sakurai, Y., Gann **56,** 589 (1965).
2. El-Merzabani, M. M., and Sakurai, Y., Gann **58,** 199 (1967).
3. Imamura, H., Ikegami, K., and Okumoto, T., Gann **64,** 427 (1973).
4. Tashiro, T., and Sakurai, Y., Gann **60,** 465 (1969).
5. Broder, L. E., and Carter, S. K., 1-Propanol, 3,3′-iminodi-, dimethanesulfonate (ester), hydrochloride, NSC-102627. Clinical Brochure. National Cancer Institute, Chemotherapy, November, 1970.
6. Geran, R. I., Greenberg, N. H., Macdonald, M. M., Schumacher, A. M., and Abbott, B. J., Cancer Chemother. Rep. **3**(Part 3), 7 (1972).
7. DeVita, V. T., and Schein, P. S., New Engl. J. Med. **288,** 998 (1973).
8. Gale, G. R., Walker, E. M., Jr., Atkins, L. M., Smith, A. B., and Meischen, S. J., Res. Commun. Chem. Pathol. Pharmacol. **7,** 529 (1974).

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