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Increased Lifespan by the Sequential Treatment with Improsulfan and Cyclophosphamide in Rats bearing Yoshida Ascites Sarcoma[1)]

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Improsulfan had the topical antitumor activity against Yoshida sarcoma cells, in comparison with cyclophosphamide. The mean survival time of rats bearing the tumor treated intraperitoneally with each optimal dose of improsulfan (10 mg/kg/day) or cyclophosphamide (5 mg/kg/day) from day 3 for 2 weeks was 32.2 or 31.7 days, respectively. In the sequential treatment of the two drugs, the mean survival time of the group administered with improsulfan for the first week and with cyclophosphamide for the second week was 46.3 days, which was the longest day among all of the groups tested. The therapeutic effect may be improved even by combinations of alkylating agents.

Various combinations of antitumor drugs have been tested clinically and preclinically to enhance drug effects. Antitumor drugs, having different fundamental mechanisms of action, have been combined in many studies. The enhancement on rodent tumor systems was reported by combinations of two alkylating agents,[3)] which are considered to have essentially the same mechanism of action. The drugs classified as alkylating agents showed individually differences in other properties in experimental systems.[4)] There are possibilities to enhance drug effects by combinations of alkylating agents selected on the basis of these properties in certain tumor systems.

This paper deals with the combination effect of two alkylating agents, improsulfan and cyclophosphamide, on Yoshida sarcoma.

Experimental

Drugs——Iminodipropyldimethanesulfonate 4-toluenesulfonate (improsulfan), N-methyliminodipropyldimethanesulfonate 4,4′-biphenyldisulfonate (838-D), nitrogen mustard, nitrogen mustard N-oxide and busulfan synthesized in this laboratory, and the commercial product of cyclophosphamide (Shionogi Pharmaceutical Co.) were used. These drugs except busulfan were dissolved in saline solution immediately before use. Busulfan was suspended in saline solution containing 1% Tween 80. The solution was administered once daily at a dose of 10 ml/kg body weight of each rat.

Animal and Tumor——Male Donryu rats (6 and 7 weeks old) supplied from Nippon Rat Co., were used. The experimental procedures were the same as those described in the previous paper.[5)] One ml of cell suspension containing 10^6 cells of Yoshida sarcoma was inoculated intraperitoneally on day 0. Survival times of tumor-bearing rats were observed for 30 or 60 days after inoculation. In the experiment of the solid tumor, 5×10^6 cells were inoculated subcutaneously in the back of rats. The test solution was administered from day 3 to 6. On day 7, the tumors were removed and weighed. The percentage inhibition of tumor growth was calculated. The dose corresponding to 50% inhibition was determined graphically from the dose-response relation and expressed as ED_{50}.

1) This paper constitutes Part VIII of a series entitled "Studies on Carcinostatic Substances." Part VII: T. Okumoto and H. Imamura, *Yakugaku Zasshi*, **96**, 827 (1976). The outline of this study was presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
2) Location: *Nishigahara 1-26-1, Kita-ku, Tokyo, 114, Japan.*
3) *a*) W.R. Bruce, F.A. Valeriote, and B.E. Meeker, *J. Nat. Cancer Inst.*, **39**, 257 (1967); M. Inaba and Y. Sakurai, *Gann*, **65**, 465 (1974); *b*) H. Imamura, K. Ikegami, and T. Okumoto, *Gann*, **64**, 427 (1973); F. Kanzawa, A. Hoshi, and K. Kuretani, *Gann*, **65**, 55 (1974).
4) H. Imamura, *Chem. Pharm. Bull.* (Tokyo), **8**, 449 (1960); M.M. El-Merzabani and Y. Sakurai, *Gann*, **56**, 589 (1965); N. Brock and J. Kuhlman, *Arzeim. -Forsch.*, **24**, 1139 (1974); H. Imamura, K. Ikegami, and M. Takeuchi, *Yakugaku Zasshi*, **95**, 453 (1975).
5) H. Imamura, K. Ikegami, T. Okumoto, H. Hoshino, and Y. Sakurai, *Yakugaku Zasshi*, **93**, 47 (1973).

The minimum effective dose (MED) was determined cytomorphologically on day 5 according to the method described in the previous paper.[6] Single intraperitoneal administration of a drug was made on day 3 after intraperitoneal inoculation.

Results

Effect on the Ascites and the Solid Tumor by Intraperitoneal Administration (Table I)

The optimal dose of improsulfan was nearly equal to its ED_{50} and was larger than its MED. The MED was a similar value to the ED_{50} in the case of nitrogen mustard N-oxide and cyclophosphamide. The ED_{50} of 838-D or nitrogen mustard was a larger value than their MEDs and was less than their optimal doses, respectively. Busulfan showed no significant difference in these doses and did not prolonged significantly survival times of rats bearing the ascites tumor, and emaciated the animals at a dose of 10 mg/kg/day.

Table I. Antitumor Activity of Alkylating Agents on the Ascites and the Solid Tumor of Yoshida Sarcoma by Intraperitoneal Administration

Drug	Ascites tumor		Solid tumor
	MED mg/kg	Optimal dose mg/kg/day	ED_{50} mg/kg/day
838-D	1	5—10	2.5
Improsulfan	1	10—25	20
Busulfan	5—10	10	7.5
Cyclophosphamide	1	5—10	0.76
Nitrogen mustard	0.05	≦0.5	0.19
Nitrogen mustard N-oxide	1	5—10	0.98

Optimal doses were obtained from the treatments from day 3 for 2 weeks.

Effect on the Solid Tumor by Intraperitoneal or Subcutaneous Administration (Table II)

The dose corresponding to the ED_{50} of each drug, as shown in Table I, was administered for 4 days. The subcutaneous treatment of improsulfan resulted in a marked effect compared with that of the intraperitoneal treatment. The effect of busulfan or cyclophosphamide was similar, irrespective of the route of drug administration.

Table II. Antitumor Activity of Alkylating Agents on the Solid Tumor of Yoshida Sarcoma by Intraperitoneal or Subcutaneous Administration

Drug	Dose mg/kg/day	Growth rate (T/C, %)	
		i.p.	*s.c.*
Improsulfan	20	55.3	18.8
Busulfan	7.5	73.9	62.2
Cyclophosphamide	0.8	39.6	32.8

Administration was made from day 3 to 6. In the case of subcutaneous administration, the drugs were given in the near part of the solid tumor. Each value shows the average of 6 rats.

Effect on the Early or the Advanced Stage of the Ascites Tumor (Fig. 1)

Single administration of improsulfan or cyclophosphamide was made intraperitoneally at a dose of 5 mg/kg. The mean survival time of the group received improsulfan on day 0 was 23 days. Administration of improsulfan on day 5 showed no effect. The single treatment of cyclophosphamide on day 0, 3, or 5 was effective. Against the advanced tumor, improsulfan was less effective than cyclophosphamide.

6) T. Yoshida, M. Ishidate, Y. Sakurai, H. Satoh, and H. Imamura, *Gann*, **45**, 484 (1954).

Combination Effect of Improsulfan with Cyclophosphamide on the Ascites Tumor (Table III and IV)

The optimal dose of improsulfan (10 mg/kg/day) or cyclophosphamide (5 mg/kg/day) was administered intraperitoneally. In the treatment from day 3, the mean survival time of the group treated simultaneously with the both drugs was similar to that produced by each drug alone. The group treated with improsulfan for the first week and then with cyclophosphamide for the second week, lived the longest among the groups tested. The toxicity, checked by change in body weight of the rats during the period of administration, was not enhanced by this sequential treatment.

As shown in Table IV, the treatments from day 5 had a tendency to be less effective than those from day 3. The mean survival times of the groups received the combined treatments with improsulfan and cyclophosphamide were not longer than that treated with each drug alone.

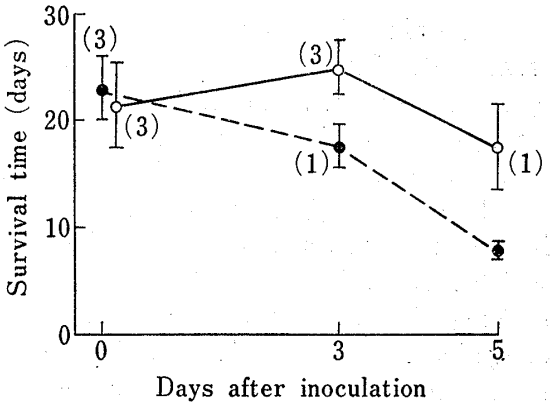

Fig. 1. Effect of Single Intraperitoneal Administration of Improsulfan and Cyclophosphamide at a Dose of 5 mg/kg on the Early and the Advanced Stage of Yoshida Ascites Tumor

Administration of each drug on day 0 was made immediately after inoculation. Each value shows the mean survival time with its standard error of 6 rats. Rats surviving over 30 days were calculated as 30 day survivors and given in parentheses. The survival time of the untreated group was 7.3±1.0 days.
—●—: improsulfan —○—: cyclophosphamide

TABLE III. Survival Times of Rats Bearing Yoshida Ascites Sarcoma by Treatments with Improsulfan and Cyclophosphamide from Day 3

Drug and dose (mg/kg/day, *i.p.*)		Survival time days[a]
First week (day 3—8)	Second week (day 10—15)	
Improsulfan (10)	——[b]	30.8±4.2 (3)
Improsulfan (10)	improsulfan (10)	32.2±5.2 (3)
Cyclophosphamide (5)	——	26.4±3.5 (2)
Cyclophosphamide (5)	cyclophosphamide (5)	31.7±5.1 (3)
Improsulfan (5)+ Cyclophosphamide (2.5)	improsulfan (5)+ cyclophosphamide (2.5)	35.0±5.4 (4)
Cyclophosphamide (5)	improsulfan (10)	31.8±6.1 (4)
Improsulfan (5)	cyclophosphamide (5)	46.3±5.4 (7)
Untreated control		7.5±0.6 (0)

a) mean ± standard error (n=12) Rats surviving over 60 days were calculated as 60 day survivors and given in parentheses.
b) no treatment

TABLE IV. Survival Times of Rats Bearing Yoshida Ascites Sarcoma by Treatments with Improsulfan and Cyclophosphamide from Day 5

Drug and dose (mg/kg/day, *i.p.*)		Survival time days[a]
First week (day 5—10)	Second week (day 12—17)	
Improsulfan (10)	——[b]	17.3±5.0 (1)
Improsulfan (10)	improsulfan (10)	26.8±6.4 (2)
Cyclophosphamide (5)	——	21.0±6.9 (3)
Cyclophosphamide (5)	cyclophosphamide (5)	29.1±6.1 (3)
Improsulfan (5) + Cyclophosphamide (2.5)	improsulfan (5) + cyclophosphamide (2.5)	24.3±6.6 (3)
Cyclophosphamide (5)	improsulfan (10)	28.9±5.8 (2)
Improsulfan (10)	cyclophosphamide (5)	26.1±6.3 (2)
Untreated control		7.2±1.4 (0)

a), *b*) See Legend for Table III.

Discussion

The aim of combination chemotherapy against tumor is to find effective modalities of treatment. The synergistic action of the simultaneous combination of two alkylating agents was reported with respect to inhibition of growth of experimental tumors.[3b] Therapeutic synergism was found in combinations of two alkylating agents, 1,3-bis(2-chloroethyl)-1-nitrosourea with other alkylating agents.[3a]

In this study, the combination of improsulfan and cyclophosphamide was examined for increase in the lifespan of rats bearing Yoshida ascites sarcoma. An optimal dose of each drug which was obtained from the treatment from day 3 for 2 weeks, 10 mg/kg/day for improsulfan or 5 mg/kg/day for cyclophosphamide was given intraperitoneally to the rats as a dose of each drug alone. Therapeutic synergism was obtained by the sequential treatment from day 3 with improsulfan for the first week and with cyclophosphamide for the second week, but not obtained by the treatment of cyclophosphamide followed by improsulfan. It was found that there were differences in biological activities of the two drugs against Yoshida sarcoma, as shown in Table I and II, and Fig. 1. Improsulfan had the topical antitumor activity against Yoshida sarcoma cells, in comparison with cyclophosphamide. It is likely that improsulfan is consumed rapidly by reaction with body components after parenteral administration,[7] and cyclophosphamide distributes in an active form to various tissues. A possible mode of this therapeutic synergism is the cell-killing process like "priming-dose therapy.[8]" The treatment of improsulfan may lower initially large populations of the tumor cells in the peritoneal cavity, and the subsequent doses of cyclophosphamide may inhibit the tumor cells disseminated into various tissues.

The tumor cells inoculated intraperitoneally grow in the peritoneal cavity and disseminate into various tissues.[9] These properties of the tumor cells seem to be favorable to the sequential treatment of the two drugs.

Therapeutic synergism was not observed by the combined treatment of the two drugs from day 5, which was the advanced stage of the tumor.

The antitumor activity of improsulfan is considered to be the most topical among the alkylating agents tested in this study. Improsulfan is effective in the clinical therapy of chronic myeloid leukemia and polycythemia vera by oral administration,[10] and will be useful as a drug for regional chemotherapy. Cancer chemotherapy may be improved by combinations of certain alkylating agents, which have different properties.

7) T. Okumoto and H. Imamura, Unpublished data.
8) F.M. Schabel, *Cancer Res.*, **29**, 2384 (1969); A. Goldin, *Cancer Chemother. Rep.*, **4**, 189 (1973).
9) H. Sato, *Nippon Byori Gakkai Kaishi*, **56**, 9 (1967).
10) M. Hirano, M. Miura, H. Kakizawa, A. Morita, T. Uetani, R. Ohno, K. Kawashima, H. Nishiwaki, and K. Yamada, *Cancer Chemother. Rep.*, **56**, 335 (1972); Y. Wakabayashi and F. Takaku, *Rinsho Ketsueki*, **16**, 39 (1975).