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Antitumor Activity of Improsulfan by the Fractionated Dose Schedule on Rat Ascites Tumors[1)]

Takeki Okumoto and Hiroshi Imamura

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd.[2)]

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The antitumor activity of improsulfan was examined with respect to an increase in the lifespan of rats bearing ascites tumors when a relatively low dose (5 and 20 mg/kg) was given intraperitoneally in two equal fractions and in a single dose. The fractionated dose schedule, even when given in a time interval of over 24 hr, was more effective against tumors sensitive to alkylating agents, Yoshida sarcoma and AH-272. Against other tumors, the fractionated dose schedule was more effective only when given in an interval of within 3 hr or was equally effective at all of the intervals tested as the single dose schedule. The toxicity of improsulfan to normal healthy rats was severer in a single dose than in two equal fractions of the same total dose, 150 mg/kg.

Therapeutic synergism was observed against Yoshida sarcoma when intraperitoneal administration of improsulfan and oral administration of cyclophosphamide were made simultaneously. The pharmacokinetic process in tissue concentrations of alkylating agents like that presumed in the fractionated dose schedule of improsulfan may occur by this combination.

Improsulfan, a derivative of methanesulfonic acid ester of aminoglycols, was reported to have a wide antitumor spectrum in experimental tumors,[3,4)] and to exhibit no cross-resistance with other alkylating agents in some clinical cases.[5,6)] The survival of cultured HeLa cells treated with improsulfan decreased exponentially with increase of its concentration. No difference was observed in the cell survival when the same total dose of improsulfan was given in two equal doses at a short interval and in a single dose.[7)] This result suggested that tumor growth was more affected by the fractionated dose schedule than by the single dose schedule of the same total amount in certain therapeutic models, if the fractionated dose schedule lowered host toxicity. The present paper describes the antitumor activity of improsulfan by the fractionated dose schedule against several rat ascites tumors and the combination effect of improsulfan and cyclophosphamide against Yoshida sarcoma.

Materials and Methods

Animals and Tumors——Yoshida sarcoma, its sublines with resistance to nitrogen mustard, and rat ascites hepatomas (AH-66, AH-272, and AH-109A) were used. The experimental procedure was the same as described in the previous paper.[4)] One ml of cell suspension containing 10^6 cells was inoculated intraperitoneally into male Donryu rats (6 and 7 weeks old) supplied from Nihon Rat Co., Urawa. Survival period of tumor-bearing rats was examined for 30 or 60 days. The result was expressed as the mean survival period and standard error. Rats surviving over 30 or 60 days were calculated as 30- or 60-day survivors.

1) This paper constitutes Part IX of a series entitled "Studies on Carcinostatic Substances." Part VIII: T. Okumoto and H. Imamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 1913 (1976). The outline of this study was presented at the 34th Annual Meeting of the Japanese Cancer Association, Osaka, October, 1975.
2) Location: *Nishigahara 1-26-1, Kita-ku, Tokyo*, 114, *Japan*.
3) M.M. El-Merzabani and Y. Sakurai, *Gann*, **56**, 589 (1965).
4) H. Imamura, K. Ikegami, T. Okumoto, H. Hoshino, and Y. Sakurai, *Yakugaku Zasshi*, **93**, 47 (1973).
5) N. Gad-El-Mawla, M. Abul-Enien, M.R. Hamza, M.M. El-Merzabani, and A.L. Abul-Nasr, *Japan, J. Clin. Oncol.*, 3, 95 (1973).
6) S.J. Altman, W. Fletcher, N. Andrews, W.L. Wilson, and T. Pischer, *Proc. Am. Assoc. Cancer Res.*, **15**, 161 (1974).
7) T. Okumoto and H. Imamura, *Yakugaku Zasshi*, **96**, 827 (1976).

The sublines of Yoshida sarcoma, RAc_{-13} and YSc_{-20}, show about 100-fold resistance to nitrogen mustard compared to the original Yoshida sarcoma at present when the resistance is determined by the *in vitro* procedure reported by Sakurai.[8]

Drugs——Improsulfan (iminodipropyldimethanesulfonate 4-toluenesulfonate) synthesized in this laboratory, and cyclophosphamide obtained commercially were each dissolved in saline solution just before use. The solution was given at a dose of 10 ml/kg body weight of each rat. The dose of improsulfan, which killed 50% of normal healthy rats[4] or produced about 1.5- to 2-fold increase in the mean survival time of rats bearing each tumor, was divided into two equal fractions. The fractionated dose schedule at the time interval of 0 corresponds to the single dose schedule. Improsulfan or cyclophosphamide was given intraperitoneally or orally, respectively. Administration of the drug was begun 3 hr after tumor inoculation.

Results

Antitumor Activity of Improsulfan by the Fractionated Dose Schedule

Figs. 1 and 2 show the effect of improsulfan against Yoshida sarcoma and AH-272 when a dose of 5 mg/kg was given in two equal fractions and in a single dose. The fractionated dose schedule at the intervals of 1 to 48 hr was significantly more effective than the single dose schedule against Yoshida sarcoma. The rats surviving over 60 days were observed in all the groups treated with the fractionated doses of improsulfan, while the single dose schedule gave no survivor. Three or 4 rats in each group of 6 animals survived over 60 days in some cases of the fractionated dose schedule.

Against AH-272, the fractionated dose schedule at the intervals of 6 and 24 hr was significantly more effective than the single dose schedule, and gave survivors over 60 days.

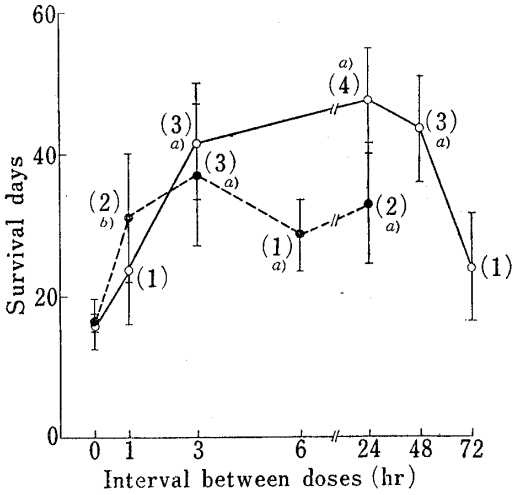

Fig. 1. Autitumor Activity of Improsulfan on Yoshida Sarcoma

A total dose of 5 mg/kg was given intraperitoneally in two equal fractions and in a single dose. The results of 2 experiments are expressed as the mean±S.E. of 6 rats. The number of rats surviving over 60 days is given in parentheses.
a) $p<0.05$
b) $p<0.1$ (statistical significance of difference from the group receiving the same total dose of improsulfan given in a single dose)
untreated; ○: 8.0±0.8 days, ●: 7.6±0.8 days

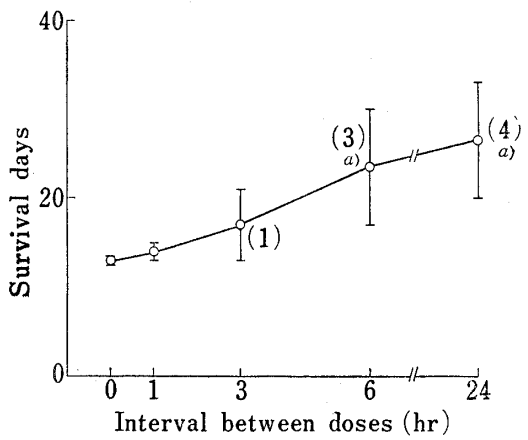

Fig. 2. Antitumor Activity of Improsulfan on AH-272

A total dose of 5 mg/kg was given intraperitoneally in two equal fractions and in a single dose. For each group, 12 rats were used. The results are expressed as described in Fig. 1.
untreated: 7.7±0.5 days
a) $p<0.05$

Fig. 3 and 4 show the effect of improsulfan against AH-66 and AH-109A, and the sublines of Yoshida sarcoma with resistance to nitrogen mustard. A dose of 20 mg/kg was given in fractions and in a single dose. The fractionated dose schedule at the interval of 1 or 3 hr was more effective than the single dose schedule against AH-66 and RAc_{-13}. There was no

8) Y. Sakurai, *Natl. Cancer Inst. Monogr.* **16**, 207 (1964).

significant difference in the antitumor activity of improsulfan between the two dose schedules against AH-109A and YSc-$_{20}$.

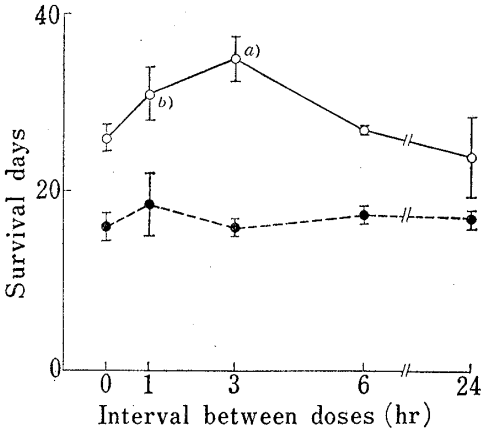

Fig. 3. Antitumor Activity of Improsulfan on AH-66 and AH-109A

A total dose of 20 mg/kg was given intraperitoneally in two equal fractions and in a single dose. For each group, 6 rats were used. The results are expressed as described in Fig. 1.
○: AH-66 (untreated: 18.5±2.7 days)
●: AH-109A (untreated: 11.5±1.1 days)
a) $p<0.05$ *b*) $p<0.1$

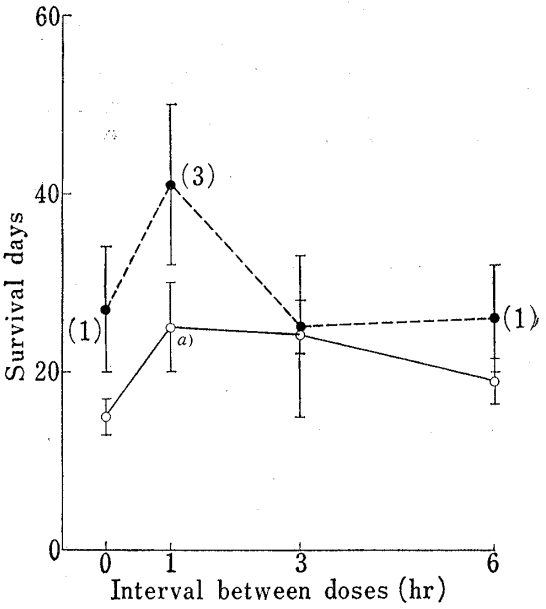

Fig. 4. Antitumor Activity of Improsulfan on the Sublines of Yoshida Sarcoma with Resistance to Nitrogen Mustard

A total dose of 20 mg/kg was given intraperitoneally in two equal fractions and in a single dose. For each group, 6 rats were used. The results are expressed as described in Fig. 1.
○: RAc-$_{13}$ (untreated: 8.8±0.4 days)
●: YSc-$_{20}$ (untreated: 12.8±0.3 days)
a) $p<0.05$

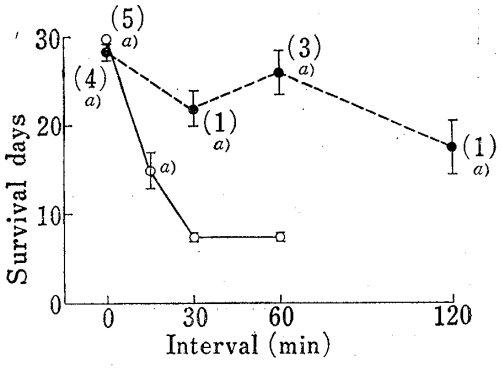

Fig. 5. Lifespan of Rats Inoculated with Tumor Cells after Drug Administration

At various time intervals after administration of 10 mg/kg of improsulfan or cyclophosphamide, 10^6 cells of Yoshida sarcoma were inoculated intraperitoneally. For each group, 5 rats were used. The results are expressed as described in Fig. 1.
a) $p<0.05$ (statistical significance of difference from the untreated tumor-bearing rats, 7.2± 0.8 days)
○: Intraperitoneal administration of improsulfan
●: Oral administration of cyclophosphamide

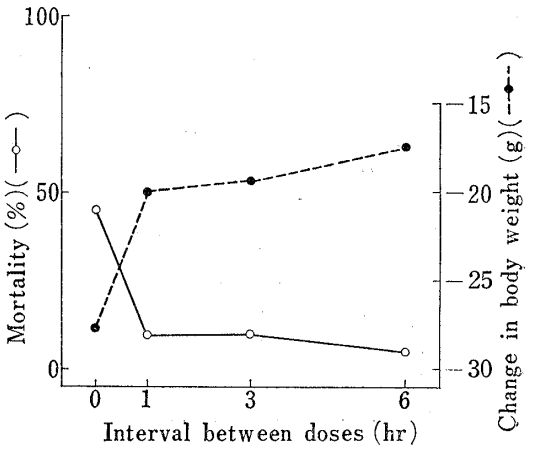

Fig. 6. Toxicity of Improsulfan to Normal Healthy Rats

A total dose of 150 mg/kg was given intraperitoneally in two equal fractions and in a single dose. For each group, 20 male Donryu rats at 6 weeks of age were used.
○: mortality (for 2 weeks after drug administration)
●: change in body weight (for 3 days after drug administration)

Lifespan of Rats Inoculated with Tumor Cells after Drug Administration and Host Toxicity of Improsulfan

Fig. 5 shows the survival period of rats inoculated intraperitoneally with 10^6 cells of Yoshida sarcoma at various time intervals after drug administration. All the rats that received tumor inoculation immediately after intraperitoneal administration of 10 mg/kg of improsulfan survived over 30 days. Improsulfan showed an antitumor activity only at the time interval of within 15 min. Oral administration of 10 mg/kg of cyclophosphamide significantly increased the mean survival period of the rats when the tumor was inoculated with an interval of over 120 min.

The toxicity of improsulfan to normal healthy rats, checked by changes in body weight and mortality, is shown in Fig. 6. These toxic signs were severer by the single dose schedule than by the fractionated dose schedule of the same total dose, 150 mg/kg. The increasing time intervals of the fractionated dose schedule gradually reduced these toxic signs.

Combination Effect of Improsulfan and Cyclophosphamide

Improsulfan or cyclophosphamide was given intraperitoneally or orally to rats bearing Yoshida sarcoma. The rats treated simultaneously with both drugs lived much longer than those with each drug alone. Combination of these two drugs showed the survival over 60 days in 67% of the animals, while 60-day survival was not more than in 33% by the treatment with each drug alone. These results are shown in Table I.

Table I. Combination Effect of Improsulfan and Cyclophosphamide on Yoshida Sarcoma

Drug (mg/kg)	Days mean±S.E. (n=12)
Improsulfan (5)	18.5±3.9 (1)
Cyclophosphamide (10)	31.8±6.2 (4)
Improsulfan (2.5)+cyclophosphamide (5)	49.5±6.2[a] (8)
Untreated	7.3±0.3 (0)

Improsulfan or cyclophosphamide was given intraperitoneally or orally, respectively, 3 hr after intraperitoneal inoculation of Yoshida sarcoma. The number of rats surviving over 60 days is given in parentheses.
a) $p<0.05$ (statistical significance of difference from the group treated with each drug alone).

Discussion

The optimal dose schedules of antitumor drugs have been examined. For alkylating agents, a single maximum torelated dose appeared optimal in the L-1210 leukemic model.[9]

In the present study, the antitumor activity of improsulfan was examined with respect to the increase in the lifespan of rats bearing ascites tumors at a relatively low dose (a total dose of 5 or 20 mg/kg) given intraperitoneally in two equal fractions and in a single dose. Administration of improsulfan was begun 3 hr after tumor inoculation. The lifespan of rats bearing different kinds of tumors was more prolonged by the fractionated dose schedule than by the single dose schedule of the same total amount. Namely, in the experimental model such as intraperitoneal administration to rats having tumors localized in the peritoneal cavity, tumor growth was more affected by the fractionated dose schedule than by the single dose schedule of improsulfan. A possible interpretation for the antitumor effectiveness of the fractionated dose schedule is that the host toxicity of improsulfan varies with the dose sched-

9) H.E. Skipper, F.M. Schabel, B. Mellett, J.A. Montogomery, L.J. Wilkoff, H.H. Lloyd, and R.W. Brockman, *Cancer Chemother. Rep.*, **54**, 431 (1970).

ules, since no difference in the survival of cultured mammalian cells was observed when the same total dose of improsulfan was given in two equal fractions at a short time interval and in a single dose.[7] Evidently the toxic signs of improsulfan to normal healthy rats, checked by changes in body weight and mortality, were severer by the single dose schedule than by the fractionated dose schedule of the same total dose, 150 mg/kg. These toxic signs were markedly decreased and the antitumor activity against certain tumors was significantly increased by the fractionated doses of improsulfan given in an interval of 1 hr.

The fractionated dose schedule even with an interval of over 24 hr was more effective against tumors sensitive to alkylating agents, Yoshida sarcoma and AH-272. Against ascites hepatomas naturally resistant to most of alkylating agents, AH-66 and AH-109A, or the sublines of Yoshida sarcoma with resistance to nitrogen mustard, RAc_{-13} and YSc_{-20}, the fractionated dose schedule was more effective only at the interval of 1 or 3 hr, or was equally effective at all of the intervals tested as the single dose schedule. Variation in the antitumor activity of the fractionated dose schedule among the tumors tested may be due to the differences in the extent and recovery of cell damage induced by improsulfan, and in the other properties of the tumors.

The dose of 10 mg/kg improsulfan showed no antitumor activity by intraperitoneal administration 30 min before tumor inoculation as shown in Fig. 5, because its tissue concentration decreases rapidly.[10] The tissue concentration of improsulfan in an active form may show two equal peaks when 5 or 20 mg/kg was given in two equal fractions even at the interval of 1 hr. It can be seen from the result shown in Fig. 5 that the tissue concentration of cyclophosphamide is sufficient for the increase in the lifespan of rats bearing Yoshida sarcoma over 2 hr after its oral administration. Therapeutic synergism against Yoshida sarcoma was obtained when intraperitoneal administration of improsulfan and oral administration of cyclophosphamide were made simultaneously. The pharmacokinetic process in the tissue concentrations of alkylating agents like that presumed in the fractionated dose experiment of improsulfan may occur by this combination, because there are possibilities that improsulfan in an active form disappears before the tissue concentration of cyclophosphamide increases sufficiently to exert an antitumor activity.

The therapeutic effect is possibly improved in certain tumor systems by the fractionated dose schedule of an alkylating agent and the combination of two alkylating agents selected on the basis of difference in pharmacokinetics.

Acknowledgement The authors are grateful to Drs. S. Tsukagoshi and T. Tashiro, Department of Cancer Chemotherapy, Cancer Institute, Tokyo, for their kind supplies of Yoshida sarcoma and its sublines.

10) T. Kuriyama and K. Kishiro, Personal Communication.