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STUDIES ON ANTI-TUMOR ACTIVITY OF SOEDOMYCIN, A NEW ANTI-TUMOR AGENT

II. Anti-tumor effect on Ehrlich ascites tumor and Yoshida sarcoma, and an outline of clinical application to human patients with carcinoma

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新抗腫瘍物質 Soedomycin の抗腫瘍性に

関する研究 (第2報)

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前報に於て本物質の Ehrlich 腹水癌並に Sarcoma 180に対する抗腫瘍性に就いて報告し, EAC マウス腹水の遠心上清中に著者の発見した carcinogenic agent (CA) が存在し, 之がマウスに腹水癌を誘発し得ること. *in vitro* に於てCAを本物質で処理することによりCAは活性を失うこと. 活性を失ったCAは腹水癌を誘発し得ないこと等を実験的に証明し, Soedomycin の抗腫瘍性は抗-CA 性と考えるのが合理的であると云う意

見を述べた.

本論文に於ては前報に続いてEAC並に吉田肉腫に対する抗腫瘍性を検討し, 併せて本物質による治療の結果発病を免れた動物の脾細胞の腫瘍発育阻止力を検討し腫瘍に対する免疫機構解明の一助とした. また Soedomycin を13例の癌症例に治療剤として長期に亘つて用いた所見の outline を記載し中毒作用が殆んど皆無の原因が本剤の抗-CA 性の為であろうとする著者の見解を述べた.

Synopsis

In the previous paper, anti-tumor activity of Soedomycin against Ehrlich ascites tumor and Sarcoma 180 of mice was reported and the mechanism by which Soedomycin exerts its inhibitory activity against tumor was discussed. This time, therapeutic effect of Soedomycin on s.c. implanted Ehrlich ascites tumor and i.p. implanted Yoshida sarcoma cells was tested. Furthermore clinical application of this agent to human carcinoma has been tried in several hospitals since around 2 years before. On the details of its therapeutic effectiveness on human carcinoma will be reported later by clinical specialists of those hospitals, but an outline of its clinical usefulness tested during recent 2 years will be described here and an outstanding characteristic of Soedomycin for anti-cancer therapy of human patients is briefly discussed.

Introduction

In the previous paper, I have reported on anti-tumor activity of Soedomycin against Ehrlich ascites tumor (EAC) and Sarcoma 180 of mice and presumed that this agent may be regarded as a member of the

anti-CA group of anti-tumor agents.

Similar experiments have been carried out with EAC of mice and Yoshida sarcoma (YS) of rats and, in addition clinical application of this agent has been done in several hospitals to examine its activity against human tumors.

In 1961, cell free supernatant of ascites fluid of EAC mice was chemically treated and a modified preparation of CA of Ehrlich ascites tumor was obtained in our laboratory. It was named EAD (Soeda), which was shown capable of inducing an acquired type of immunity against the original tumor, if i.p. given to mice as a vaccine. Similar experiments were done with Sarcoma 180 of mice and Yoshida sarcoma of rats and it was shown that this was also true even in the cases of these animal tumors.

Getting a hint from these findings, the same process was applied to peritoneal exudate of patients with peritonitis carcinomatosa, in the earlier studies, to obtain a similar preparation to EAD for mice. But this attempt was soon given up because of difficulties in obtaining materials for the purpose. Later the urine of patients with cancer were adopted as a source for this purpose and a modified preparation of human urine was obtained and named HUD (Soeda, 1961), which means "human urine agent derived from urine". The effectiveness of HUD as a vaccine will be reported on some other day, however, patients with cancer were treated with i.m. injection of HUD in the early postoperative days and later they were treated with Soedomycin alone or in combination with HUD.

I will report here on an outline of a therapeutic effect of Soedomycin on human carcinoma when i.m. given after the primary tumors were surgically removed.

Materials and Methods

Animals: Male mice of DDS strain weighing about 20 g. and male rats with body weight of about 100 g. were used for studies.

Tumor cells: EAC cells and YS cells were adopted as target cells for animal experiments. The tumor cells have been maintained by serial transplantations from one group of mice or rats to another at appropriate intervals for more than 5 years.

EAD: Ascites fluid was collected from EAC mice and centrifuged at 4,000 r.p.m. for 30 minutes. Fifty per cent solution of zinc chloride was added to the supernatant in the proportion of 1:50 to 1:100 and developed precipitate was removed by centrifugation at 3,000 r.p.m. for 15 minutes. This precipitate was extracted with 10 per cent solution of disodium phosphate which was then dialyzed against distilled water to completely eliminate zinc phosphate and other inorganic salts. Dialyzed solution was passed through a sterile Seitz filter and distributed to ampoules and lipoilyzed. EAD can be obtained in an amorphous, white and powdery form.

HUD: HUD can be prepared according to almost the same method as that for preparation of EAD. The urine of patients with carcinoma was used as a source of HUD instead of ascites fluid. It is also obtained in an amorphous, white and powdery form.

Soedomycin: This agent is also called by the name of M 3 substance, because it is the 3rd anti-tumor agent found in our laboratory. An outline of preparation procedures of this agent was described in the previous report.

Marimycin: In animal experiments, this agent was used in combination with Soedomycin. This substance is also called by the name of M-2 substance, which was isolated from agitation cultures of

Streptomyces mariensis n. sp. (Soeda, 1957). An outstanding characteristic of M-2 is its activity to increase the white cell count when given s.c. or i.v. to man and animals. This agent proved capable of maintaining leukocyte count of patients with malignant tumor within the normal range, even if they were treated with X-ray or Co-60 irradiation, and thus permitting such therapy to continue for a longer period than usual.

In animal experiments, tumor cells were i.p. or s.c. implanted into mice or rats and after 1 or 2 days they were treated with various doses of Soedomycin, once or twice a day, for 2 to 4 days. Soedomycin and Marimycin were given only by i.p. or s.c. route. In some experiments, spleen cells of immunized animals were given i.p. to mice or rats for examination of their inhibitory effect upon tumor growth in the recipient.

In clinical application to 13 human patients with gastric or ovarian cancer, Soedomycin was daily given s.c. or i.m. for 20 to 333 days, and in some cases by spacing injections more widely, e.g. 2 to 3 times weekly, treatment was continued for as long as possible. In some other cases, patients were at first treated with i.m. injection of HUD for 2 to 3 months and then Soedomycin was used alone or in combination with HUD.

A daily therapeutic dose of Soedomycin was in the range of 20 to 30 mg, while that of HUD was about 80 mg.

Experimental Results

Experiment 1. Treatment of mice with i.p. administration of M 3

Groups of mice were i.p. inoculated with 10^4 EAC cells and 2 days later they were treated with i.p. injection of either 65 mcg M 3 or 65 mcg M 3 plus 145 mcg M-2, twice a day, for 4 consecutive days. Two of 4 mice of the former group and 3 of 3 mice of the latter group did not develop ascites tumor and survived for more than 3 months. Since a dose of 10^8 EAC cells can be regarded as absolutely fatal for usual mice of DDS strain, daily administration of 130 mcg M 3 for 4 days seems to be considerably active against EAC, if it was given immediately after tumor cell transplantation.

Simultaneous use of a daily dose of 300 mcg M-2 seems to enhance the inhibitory effect of

Table 1 M 3 therapy of mice inoculated with EAC cells

Group of tests	Inoculation of EAC cells		Administration of M-3 or M-2 (mcg) \times (times a day) \times (days)	No. of test mice	dead due to tumor	Surv. mice
	i.p.	s.c.				
Exp. 1	10^4		(65M 3) \times 2 \times 4 i.p.	4	2	2
	10^4		(65M 3 plus 145M-2) \times 2 \times 4 i.p.	3	0	3
Exp. 2		10^5	(2000M 3) \times 1 \times 2 s.c.	3	2	1
		10^5	(2000M 3 plus 1000M-2) \times 1 \times 2 s.c.	3	2	1
		10^5	(150M 3) \times 1 plus (1500M-2) \times 1 s.c.	4	3	1
		10^5	(150M 3 plus 2000M-2) \times 1 plus (1500M 3 plus 2000M-2) \times 1 s.c.	4	1	3
		10^5	(100M 3) \times 1 \times 4 s.c.	3	0	3
		10^5	(100M 3 plus 1000M-2) \times 1 \times 4 s.c.	3	0	3
		10^5	/	3	3	0
Control	10^4		/	3	3	0
		10^4	/	10	10	0

Soedomycin (Table 1).

Experiment 2 Treatment of mice with s.c. administration of M 3

Groups of mice were inoculated s.c. with 10^6 EAC cells and 2 days later they were treated with s.c. injection of a daily dose of either 2,000 mcg M 3 alone or 2,000 mcg M 3 plus 1,000 mcg M-2 for 2 days. Each 2 mice of both groups developed solid tumors and died within 39 days, but each one mouse could escape from death due to s.c. solid tumors (Table 1).

Similar experiments were carried out according to various schedules of M 3 administration. A group of mice were s.c. inoculated with 10^6 EAC cells and 2 and 4 days later they were s.c. given 150 mcg and 1,500 mcg M 3 respectively. Mice of the 2nd group were s.c. inoculated with 10^6 EAC cells and 2 days later they were treated with s.c. injection of 150 mcg M 3 plus 2,000 mcg M-2 and again they received s.c. injection of 1,500 mcg M 3 plus 2,000 mcg M-2 2 days thereafter. Mice of the 3rd group were inoculated s.c. with 10^6 EAC cells and 2 days later they were treated with s.c. injection of a daily dose of either 100 mcg M 3 or 100 mcg M 3 plus 1,000 mcg M-2 for 4 consecutive days. In the 3rd group, s.c. injection were done at the sites of tumor cell implantation.

One of 4 mice of the 1st group, 4 of 8 mice of the 2nd group and 3 of 3 mice of the 3rd group were completely protected against s.c. growth of solid tumors and could survive in good health. When ascites tumor cells were again implanted into those survived mice at the same site of previous cell implantation, it was of much interest that local development of solid tumors was, in general, completely suppressed from the beginning and small tumors rarely developed at the site of the 2nd cell implantation usually regressed sooner or later and disappeared within about 3 months.

The mice in which s.c. tumor growth after the 2nd cell implantation was completely suppressed were sacrificed 60 days thereafter and the spleens were removed to prepare spleen cell suspensions. Groups of

Table 2 Inhibition of EAC growth due to spleen cells of mice protected against solid tumor deaths by M 3 therapy

Test mice No.	Dose of spleen cells	Challenge dose of EAC cells	Dead or survived (day)	2nd chal on 59th day	Dead or survived (day)	3rd chal. on 86th day	Dead or survived (day)
1	5×10^8	10^4	Nil	10^5	Nil	10^6	dead (38)
2	5×10^8	10^4	dead (32)	/		/	
3	5×10^8	10^4	Nil	10^5	Nil	10^6	Nil
4	5×10^8	10^4	Nil	10^5	Nil	10^6	Nil
5	5×10^8	10^4	dead (28)	/		/	
Control	6	/	dead (23)				
	7	/	dead (27)				
	8	/	dead (27)				
	9	/	dead (29)				
	10	/	dead (36)				

mice were i.p. inoculated with 10^4 EAC cells and after 2 days they received a single i.p. injection of 5×10^8 spleen cells. Three of 5 mice were protected against this test and survived without any signs of ascites tumor. After 58 days they were again challenged with 10^5 EAC cells, but 2 mice remained alive without any signs of ascites tumor. A 3rd challenge with 10^6 tumor cells failed to kill them due to ascites tumor (Table 2).

It seems likely that s.c. implantation of EAC cells into susceptible animals can also induce a certain

degree of specific immunity against tumor growth, which may probably be due to an anti-tumor antibody produced and carried by lymphoid cells of host animals.

Experiment 3. Therapeutic effect of M-3 on rats inoculated with YS cells

Groups of rats were i.p. inoculated with 10^6 YS cells and after an hour they received i.p. injection of M 3 alone, or in combination with M-2, according to various schedules of administration. Rats in the 1st group were treated with a daily dose of 250 mcg M 3 plus 1,700 mcg M-2 for 3 days, while those of the 2nd group were treated with a daily dose of 2,000 mcg M 3 alone for 3 days. Rats of the 3rd group were treated with a daily dose of 50 mcg M 3 plus 2,000 mcg M-2, twice a day, for 3 days. In spite of such variable methods of administration of both agents, all rats were completely protected against YS and survived for more than 3 months. On the contrary, control rats which had received tumor cell implantation and nothing else developed the typical disease and died within 14 days (Table 3).

Then the survived rats were killed and spleen cell suspensions were made. Normal 8 rats were i.p. given 5×10^8 spleen cells and 2 days later they were challenged i.p. with 10^6 YS cells. Two rats developed the typical disease and died after 11 and 14 days respectively, but the other 6 rats well tolerated not only this

Table 3 M 3 therapy of rats inoculated with YS cells

Group of rats	Inoculation of YS cells i.p.	Administration of M 3 or M-2 (mcg) \times (times a day) \times (days)	No. of test rats	Dead due to tumor	Survived rats
Exp. 3	10^6	(250M 3 1700M-2) \times 1 \times 3	3	0	3
	10^6	(2000M 3) \times 1 \times 3	3	0	3
	10^6	(50M 3 2000M-2) \times 2 \times 3	3	0	3
Contr.	10^6	/	3	3	0

Table 4 Inhibition of YS growth due to spleen cells of rats protected against YS by M 3 therapy

Test rats No.	Dose of spleen cells	Challenge dose of YS cells	Dead or survived day	2nd chal on 32th day	Dead or survived (day)	3rd chal. on 50th day	Dead or survived (day)
1	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
2	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
3	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
4	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
5	5×10^8	10^6	dead (11)	/			
6	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
7	5×10^8	10^6	dead (14)	/			
8	5×10^8	10^6	Nil	10^6	Nil	10^7	Nil
Control	9	10^6	dead (14)	/			
	10	10^6	dead (10)	/			
	11	10^6	dead (12)	/			
	12			10^6	dead (11)		
	13			10^6	dead (9)		
	14			10^6	dead (12)		

test, but also 2 further tests with 10^6 and 10^7 YS cells (Table 4).

It seemed reasonable to consider that rats had acquired a certain degree of active immunity during the first challenge test, which may have protected the animals against tumor growth after the 2nd and 3rd implantations of tumor cells.

In contrast to anti-tumor activity against EAC, M 3 seems far more effective on Yoshida sarcoma of rats. The grade of acquired immunity against both tumors induced in respective animals seems also to be considerably variable, which may be there due to variable immunological competence of host cells to respond to respective tumor antigens, or depend upon sensitivity of respective tumor cells to M 3 therapy.

Clinical application of Soedomycin to human patients with carcinoma

On the details of the therapeutic activity of Soedomycin to human carcinoma will be reported later by clinical specialists of several hospitals. Here I will report on an outline of the therapeutic effect of Soedomycin on patients with gastric or ovarian cancer. Almost all of them were treated with i.m. injection of HUD alone in the early stage of postoperative course and then they were daily given i.m. a dose of 20 to 30 mg M 3 for more than 1 month. In the following 4 cases, daily administration of M 3 was continued for at least 3 months.

No. 10 case: ovarian cancer, female 69 years of age

This patient had been 5 times operated because of ovarian cancer and postoperative metastasis to the abdominal wall and the right axillar lymph nodes. She was treated thereafter with daily i.m. injection of a dose of 100 mg HUD prepared from her own urine for 91 days, during which period metastatic tumors gradually regressed and almost completely disappeared, and since then she received daily i.m. injection of 20 to 30 mg M 3 for 240 days. The indurated abdominal skin due to tumor cell infiltration gradually reduced its intensity and almost completely restored its normal touch during the further 3 months and subsequent clinical course has been quite favorable. Twelve months have passed now since the Soedomycin therapy was terminated and now-a-days she is almost completely well and enjoying her retired life.

No. 11 case: gastric cancer, male, 64 years of age

The patient was treated with daily i.m. injection of a dose of 20 mg HUD prepared from his own urine since the primary lesion had been totally resected. After the therapy with HUD for 90 days, the patient was daily given a dose of 20 to 30 mg M 3 for 3 months. Subsequent clinical course has been quite favorable and the patient has almost completely recovered his health and returned to his former job. More than one year has passed now since the patient was treated with Soedomycin and now-a-days he is entirely asymptomatic.

No. 12 case: ovarian cancer, 60 years of age, female

This was an inoperable case of ovarian cancer. The patient was treated with daily i.m. injection of 20 to 30 M 3 alone from the beginning for 86 days, by which the tumor gradually decreased in size and no further metastases have occurred since the treatment was started. Her clinical course has been favorable and eventless and now-a-days she is almost asymptomatic.

Only 7 months have passed now since the therapy with Soedomycin was started, so we must carefully follow up her clinical course hereafter however, it seems likely that this case is now progressing favorably.

No. 13 case: gastric cancer, female, 34 years of age

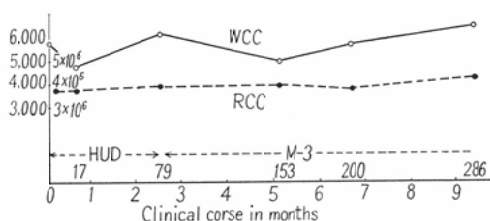
This patient was treated with daily i.m. injection of 20 to 30 mg M 3 alone for 33 days, after total resection of the primary lesion had been performed. Clinical course thereafter has been quite favorable

and eventless and during 6 months since Soedomycin therapy was started she has gradually recovered her health and discharged from hospital in a favorable condition. About 10 months have passed now since she returned to her former job. Now-a-days she is almost entirely asymptomatic.

The other 9 cases of stomach cancer were treated with daily i.m. injection of 20 to 30 mg M 3 for less than 3 months after total resection of the primary lesions had been performed. In 4 of 9 cases, only 2 to 5 months have passed now since Soedomycin therapy of relatively short duration, was terminated, so that we should carefully follow up their further clinical courses before a reasonable appreciation of therapeutic effect of Soedomycin can be obtained.

As for the remaining 5 cases, more than 6 months have passed now since they were treated with daily administration of 20 to 30 mg M 3 for 25 to 65 days. At present they are progressing favorably and almost asymptomatic.

Fig. 1 Variation in the white cell and red cell count



Undesirable side effects such as leukopenia, anemia and dysfunction of the liver have not been recognized even in the course of Soedomycin therapy for as long as 240 days (No. 10 case). Fig. 1 shows the variations in the white cell count and the red cell count of this case.

Thus even a long-term therapy with Soedomycin has been shown to lack any undesirable toxic effects upon hematopoietic organs as well as upon the liver which strikingly differs from other cytotoxic anti-tumor agents now available.

In view of this property, Soedomycin seems to be definitely superior for the purpose of anti-tumor therapy to many other anti-tumor agents to date, because the clinical use of so many anti-tumor agents which have been discovered up to the present time was to a great extent restricted because of their severe toxic effect inherent in their therapeutic activities. The fact, that daily administration of this new anti-tumor agent for as long as 8 to 11 months have not caused any appreciable toxic reactions in human patients, may indicate that it may throw much light on the chemotherapy for human malignant tumors.

Discussion

As shown in the previous report, about 60 mcg M 3 proved capable of inactivating, in vitro, CAE corresponding to 10^6 EAC cells, while about 50 mcg M 3 was required for complete inactivation of 10^6 washed cells of Sarcoma 180, in vitro. In contrast, by far a larger dose of M 3 seemed to be necessary for rescue of mice which had been i.p. inoculated with 10^5 to 10^6 EAC cells.

Roughly speaking, i.p. administration of 400 mcg M 3 for at least 3 days may be necessary for rescue of the majority of mice which had been i.p. inoculated with 10^5 EAC cells.

This time, mice were s.c. inoculated with 10^6 EAC cells in an attempt to produce s.c. solid tumor in them. Therapeutic effect of M 3 on tumor growth at the site of cell implantation was tested by several

methods of medication and it was confirmed that s.c. daily administration of 100 mcg M 3 for 4 days at the site of cell implantation is most effective to prevent tumor growth in mice. In general, repeated injection of a relatively small dose of M 3 seemed to be apparently superior for anti-tumor therapy to a single injection of a large dose of M 3. To prevent s.c. solid tumor growth, local injection of M 3 near the site of tumor cell implantation seemed superior to s.c. injection done anywhere else.

It was of interest that reinoculated tumor cells at the site, where tumor growth had been suppressed by Soedomycin therapy, usually failed to grow from the beginning. Rarely, however, small tumors developed locally, but they later decreased in size gradually and disappeared within 3 months.

Similar phenomenon has been also observed when a relatively small dose of EAC cells at the level of 10^3 or 10^4 was s.c. reinoculated at the site apart from original implantation of tumor cells. In viewing these facts, it seems likely that failure of tumor growth at the site where tumor growth once suppressed by M 3 medication may be primarily due to specific immunity induced by the former implantation of tumor cells and secondarily due to a specific local arrangement for response to tumor cell antigen.

Soedomycin proved to be also effective against Yoshida sarcoma of rats. By i.p. injection of various doses of M 3, either alone or in combination with M-2, almost all rats were protected against tumor deaths due to i.p. implantation of 10^5 YS cells. For instance, immediate daily administration of 2.0 mg M 3 alone for 3 days was enough to help all such rats from tumor deaths. Spleen cells of such rats were tested on inhibitory effect on tumor growth in normal rats. A standard dose of 5×10^8 spleen cells was given i.p. to normal rats and 2 days later they were challenged with i.p. implantation of 10^6 YS cells. About 75% of rats were completely protected against YS tumor deaths, and furthermore they all tolerated the other 2 successive challenges with 10^6 and 10^7 YS cells. It seems likely that the grade of anti-YS immunity due to implantation of YS cells in rats may be considerably higher than that of anti-EAC immunity induced in mice by similar procedure.

Soedomycin was applied to human carcinoma cases consisting of 11 patients with stomach cancer and 2 patients with ovarian cancer. It was daily injected i.m. for 20 to 333 days after the primary lesions had been totally resected in several hospitals. Only exception was an inoperable case of ovarian cancer. There were 4 cases, in which daily administration of M 3 was done for more than 3 months. In No. 10 M 3 was daily injected for 240 days, while in No. 13 case it was daily given for as long as 333 days. Even in such cases we could not recognize any undesirable toxic effects including leukopenia, anemia and dysfunction of the liver.

Thus Soedomycin was shown to lack any undesirable side effects even if medicated in the therapeutic dose for as long as one year.

This outstanding characteristic differs strikingly from those of the ordinary anti-tumor agents discovered up to the present time, because clinical application of them has been restricted to a great extent because of their severe toxic side-reactions inherent in their anti-tumor activities. In viewing these facts Soedomycin seems to be by far advantageous for anti-tumor therapy of the human patients.

As has been discussed in the previous paper, anti-tumor activity of Soedomycin does not seem to depend upon its interaction with vital cellular constituents which will disturb cell divisions or cellular functions of tumor, but it may exert its anti-tumor activity by interfering with vital capacity of carcinogenic agent responsible for malignant transformation of host cells.

On the contrary, powerful agents such as 6-MP, aminopterin and azaserine are believed to exert their anti-tumor activity by interfering with synthesis of vital cellular constituents which will disturb cell reproduction, while it is presumed that the alkylating agents may exert their anti-tumor action by interaction with vital cellular constituents which, if extensive enough, will disturb cellular function and thus produce toxic manifestations.

It seems reasonable to consider that we should classify anti-tumor agents into at least 2 groups. The agents, which can exert their anti-tumor action by interaction with vital cellular constituents responsible for essential cellular function or cellular reproductions, should be classified as members of anticellular group, while the agents like Soedomycin which may exert their anti-tumor action by interaction with vital capacity of carcinogenic agent should be classified as members of another group. We called the latter group by the name of anti-CA group in contrast to the anti-cellular group.

Summary and Conclusion

An anti-tumor agent, named Soedomycin, was prepared from culture broth of *Streptomyces hachijoensis* by Soeda (1965). This was called also by the name of M 3, because it was the third anti-tumor agent found in our laboratory. It has neither antibacterial nor antifungal activity, but it was shown to be active against malignant tumors such as Ehrlich ascites tumor or Sarcoma 180 of mice and Yoshida sarcoma of rats.

1) Groups of mice were i.p. inoculated with 10^4 EAC cells and therapeutic effect of M 3 was examined. Daily administration of 130 mcg M 3 for 4 days starting 2 days after tumor cell implantation protected the majority of mice against ascites tumor deaths. Simultaneous use of a daily dose of 300 mcg M-2 seemed to exert a synergetic inhibitory effect upon tumor growth.

2) Groups of mice were s.c. implanted 10^6 EAC cells and therapeutic effect of M 3 on s.c. growth of solid tumor was examined. Daily dose of M 3 in the range from 100 to 1,500 mcg was given to mice, either alone or in combination with 1,000 to 2,000 mcg M-2 according to various medication schedules of M 3. The best result was achieved in the group where a dose of 100 mcg M 3 alone was daily injected for 4 successive days, starting 2 days after s.c. implantation of tumor cells. Repeated injections of a relatively small dose for a longer period are surely superior to a few injections of a larger dose for a short period. Spleen cell suspensions were prepared from mice rescued from tumor deaths by medication of M 3 for the following test.

3) Inhibitory effect of such spleen cells on ascites tumor growth in the recipient was examined according to the Stuart's method. A single injection of 5×10^8 cells protected the majority of mice against i.p. inoculation of 10^4 EAC cells, and such mice proved later to be immune against 10^5 to 10^6 tumor cell implantation.

4) Therapeutic effect of M 3 on growth of YS in rats was also examined. Groups of rats were i.p. inoculated with 10^6 YS cells and immediately thereafter they were treated with i.p. injection of M 3, either alone or in combination with M-2, for 3 days. A daily dose of M 3 was in a range from 250 to 2,000 mcg. In spite of variable medication schedules of M 3 and M-2, rats were protected against tumor growth of YS without exception. Immediate use of a daily dose of 2.0 mg M 3 for 3 days seemed to be enough to protect almost all rats against onset of YS following i.p. implantation of 10^6 tumor cells.

5) Spleen cell suspensions were prepared from above rescued rats and inhibitory action of such cells against YS growth in rats was tested. They were also highly effective and a single injection of a standard

dose of spleen cells protected the majority of rats against i.p. implantation of YS cells at the level of 10^6 and 10^7 .

6) Clinical application of M 3 to human carcinoma has been tried since about 2 years before. Total 13 patients with stomach or ovarian cancer have been treated with M 3, either alone or in conjunction with HUD, after the primary lesions had been surgically resected except for an inoperable case of ovarian cancer.

It is not yet the time to evaluate the over-all therapeutic effect of this agent, but it has proved to be considerably effective against human cancer, because clinical courses of almost all patients after start of Soedo mycin therapy have been favorable and 4 patients became asymptomatic, 2 of which have already returned to their former jobs in quite favorable conditions. The most beneficial property of Soedomycin is that it entirely lacks undesirable toxic reactions, even if it is daily given for as long as 11 months (No. 13 cases). In contrast to the other anti-tumor agents, whose clinical use has been greatly restricted because of their severe toxic reactions, Soedomycin has almost no such toxic reactions, because the anti-tumor action of this agent does not seem to be due to interaction with vital cellular constituents essential for cellular functions or cell divisions.

Anyway, this beneficial property of Soedomycin may be definitely advantageous for a long-term therapy of malignant tumors in human patients.

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