Studies on a Leukocytosis-Inducing Antibiotic Agent, Marimycin

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抗白血球減少剤，Marimycin に関する研究

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化学的放射能防護剤に関する研究はCysteine に関する Patt 等の研究（1949）以来多数の研究者により行なわれ Cysteamine, Cystamine, AET 等を始めとし，多くの優れた化学剤が合成されたが，臨床試験の結果放射線療法により誘発される白血球減少症を予防又は治療し得る効果，或は radiation sickness を防止し得る効果を認められたものは今日迄皆無である．殆んどの化学剤の有効量（動物実験）の使用は人体に対して副作用が強く，遙かに安全性の域を越えるため，危険で臨床上の使用に堪えないのが例である．

私は1961年 Streptomyces の新種 Str. mariensis の培養濾液から正常 rabbit に白血球増多症を確実に誘発する物質を抽出し Marimycin と命名した．此の報告は本剤を用いた動物実験並に臨床試験の成績の概要に関するものであり，殊に臨床的に用いて，副作用が殆んど皆無のため反復使用が可能である点，抗白血球減少効果が確認された点に於て注目に値する物質であると思う．

Introduction

Since Patt et al. reported, in 1949, on the protective activity of cysteine against radiation damage of experimental animals[1], numerous attempts have been made by many investigaters with related compounds to cysteine and other chemical substances to enhance survival of animals exposed to sublethal or lethal doses of radiation. Cysteine is capable of reducing the effects of radiation in a dose of 200 to 1200 mg/kg[5], and its dose-reduction rate for mice and rats is around 1.7 and 1.5 respectively[8]. Intravenous injection of a dose of 600 mg/kg protects rabbits against whole body irradiation with 1000 r, while a dose of 500 mg/kg protects dogs against 500 r[6]. Cysteine is itself almost lethal at doses around 1500 mg/kg, and it is almost useless when given orally[3)5].

In 1951, Bacq et al. reported on the effectiveness of cysteamine and cystamine[12]. Both compounds are somewhat more effective than cysteine when given i.p., but not more effective than cysteine when given intravenously. On a weight basis, however, cysteamine is considerably more effective than cysteine, since 150 mg/kg of cysteamine hydrochloride produced nearly the same degree of protection as 1200 mg/kg of cysteine hydrochloride[15].

Cystamine is said to be as effective as cysteamine on a molar basis when given parenterally and somewhat superior to cysteamine when given orally.

AET is the next compound introduced by Doherty and his co-workers in 1955, who pointed out its

superiority to above 3 compounds, particularly in respect to its effectiveness after oral administration[27]. It appears to be slightly superior to both cysteine and cysteamine in the extent to which it can enhance the LD_{50} in mice when given intravenously. In some strains of mice, a maximum dose-reduction rate of more than 2.0 was obtained with about 400 mg/kg, but in another strain of mice it was 1.45[34]. An additive toxic effect of radiation and AET was noticed by Preston et al., since a dose of 300 mg/kg was very effective in increasing survival of rats exposed to whole body irradiation with 900 r, but 400 mg/kg killed almost all rats within the first three days[32]. The most important advantage of AET or its derivatives over cysteine and cysteamine may be its effectiveness after oral administration. A single oral dose of AET is reported to protect mice against X-radiation for at least 6 hours, but not for 16 hours[35].

Cysteamine was clinically tested for its protective activity against therapeutic X-irradiation by Baldini et al. with i.v. administration of a dose of 200 mg, which failed to modify post-irradiation leukopenia[19]. Similar results were obtained with orally administered cysteamine and it proved also of no value in prevention of radiation sickness in human patients[17][22].

AET was also clinically tested by Andrews et al. and shown that its effect as a protective agent was highly limited by its remarkable toxicity. Oral or i.p. administration of a dose of only 10 to 20 mg/kg could not be tolerated and in some cases circulatory disturbances were noticed[30].

Hundreds of related derivatives of above three compounds[7)-11)23)-26)37)42)-46)] have been synthesized and tested to find a superior compound and to establish the essential chemical structures with respect to protective effectiveness, however, at present none of the agents hitherto known can be considered to be of value except as adjuvants to bone marrow therapy.

The ideal protectant should not produce untoward side effect. It should not exert cumulative effects on repeated application, or it should have a large margin of safety. Unfortunately, there have been no such radiological protecters which being known to satisfy these criteria.

In 1961, an antibiotic substance, named Marimycin, was isolated from culture broth of a new strain of Streptomyces in our laboratory. According to the chemical characterization to date it appears to be a polysaccharide substance and it is readily soluble in water, but not soluble in organic solvents. It is quite stable in a lyophilized form and its i.p. LD_{50} for mice is around 200 mg/kg. It is capable of inducing leukocytosis in normal rabbits when it is given parenterally at doses of 1 to 5 mg/kg. It is also effective in increasing the white cell counts of sublethally Co^{60}-irradiated rabbits. It has both preventive activity against leukopenia caused by irradiation or the use of anticancer agents and recoverying activity from leukopenia already induced by the same causes.

In mice, a single i.p. administration of 1.25 mg/kg after irradiation was shown to increase survival from 0 to 40 per cent of animals exposed to 800 r, while a single s.c. injection of 2.5 mg/kg increased survival of mice from 0 to 50 per cent after exposure to 800 r.

Studies on human patients given therapeutic Co^{60}- irradiation have indicated that repeated administration of a dose of 20 to 60 mg of this agent is considerably effective in modifying post-irradiation leukopenia without showing any untoward influences upon the functions of the liver, heart, kidney and gastrointestinal tract, and as well as in inducing recovery from leukopenia already developed after irradiation or the use of anti-cancer drugs. This agent has been tested for its anti-leukemic effect in over 20 patients with malignant tumors receiving radiation therapy. The purpose of this paper is to present both experimental and clinical results obtained from application of Marimycin to experimental animals and human patients.

Materials and Methods

1) Experimental animals

Mice of ddS strain, weighing about 20 g, were used to test for the protecting activity of Marimycin against Co^{60} irradiation. Rabbits, about 3.5 to 4.0 kg of body weight, were irradiated with graded doses of Co^{60} radiation and the anti-leukopenic effect of Marimycin was tested by periodic examination of the leukocyte counts in the peripheral blood.

Anti-cancer drugs such as Mitomycin C, Toyomycin and Nitromin were paranterally given to rabbits, and Marimycin was i.v. injected several times immediately thereafter or after a significant leukopenia had developed due to such drugs.

2) Clinical test

Total 21 cases of malignant tumors, admitted to Kanto Teishin Hospital, were subjected to clinical application of Marimycin during the course of radiation therapy. In operable cases, preoperative Co^{60} irradiation was usually done in parallel with s.c. administration of this agent, and after operation the same procudure was repeated to maintain the leukocyte count within the normal range throughout the course of radiation therapy.

A daily dose of 20 to 60 mg was s.c. given for variable days (7 to 48 days), consecutively or at variable intervals. The blood picture was repeatedly examined during and after the course of radiation therapy and its shift was followed up carefully.

3) Marimycin

It is chemically extracted from culture broth of Streptomyces mariensis (Soeda, 1957) according to the following procedure.

Culture broth incubated at 27 to 29°C for 4 days is centrifuged at 3000 rpm for 30 minutes. The reaction of the supernatant is usually within the range between 8.2 and 8.6. One twentieth volume of 50% zinc chloride solution is gently added to the supernatant and stirred carefully, and the developed precipitate is removed by centrifugation. It is extracted with 10% disodium phosphate solution, and after removal of the non-extractable fraction, 4 volumes of cold methanol is added to the extract. The resultant precipitate is then dissolved into water and the solution is passed through a Seitz filter and dialyzed for less than one hour against cold destilled water. This filtrate is again passed through a sterile Seitz filter and freeze-dried.

By this procedure, about 2 g of crude active material can be obtained from 1000 ml of culture broth.

To further purify this crude substance, lyophilized material is dissolved into destilled water and again precipitated by addition of cold methanol, which is dissolved into destilled water. This solution is desalted by treatment with a mixture of Amberlite IR120 and IRA 400 in free type and then passed through a column of Sephadex G-25, and the effluent is lyophilized.

Antileukopenic effect of Marimycin

The effect on the peripheral WBC of normal rabbits

The leukocyte count of the peripheral blood was daily examined in normal rabbits during a course of one month. Usually it was in the range between 6,000and10,000 throughout the course of examination.

It was shown that when a dose of 1.0 to 200 mg of Marimycin is i.v. given, a significant leukocytosis occurs in almost every rabbits, reaching a maximum level within 24 hours after injection, and the WBC

gradually returns to the normal level after 3 to 7 days. It was also shown that if the same procedure is repeatedly done on the same animal, there occurs almost invariably a significant rise in the leukocyte count. Increment of the dose of Marimycin from 1 to 200 mg does not seem to result in a significant rise in the effect of this agent as shown in the following table (Table 1), although a dose less than 0.5 mg seems ineffective in inducing a significant leukocytosis in rabbits.

Table 1. The white blood cell count 24 hours after i.v. injection of MM

Group	Dose of MM(mg)	The leukocyte count* 1st	2nd	3rd
1	0.5	7,900	8,100	7,500
2	1.0	14,200	13,400	18,100
3	2.0	14,000	15,600	21,900
4	5.0	15,500	16,300	15,700
5	10.0	12,300	14,200	14,400
6	50.0	14,400	13,800	14,200
7	100.0	14,500	18,000	14,500
8	200.0	12,900	14,300	18,300

*The above count is the average of 3 rabbits in each group.

In view of the above fact, it seems likely that Marimycin itself may not directly stimulate a rise in the peripheral WBC, but it may primarily bring some unknown mechanism into play which secondarily increases the WBC in the peripheral blood.

A large dose of Marimycin seems unnecessary, and only a dose as small as 1 to 2 mg seems sufficient to pull the trigger for bringing such mechanism into play in animals.

The effect on the WBC-count of X- or Co^{60}-irradiated rabbits with 300 or 400 r

A significant degree of leukopenia usually develops after a single whole-body exposure of rabbits to 300 or 400 r. Ordinary patterns are shown in Fig. 1. Development of leukopenia may be almost com-

Fig. 1. Shift pattern of WBC-count in rabbits exposed to 300 r or 400 r.

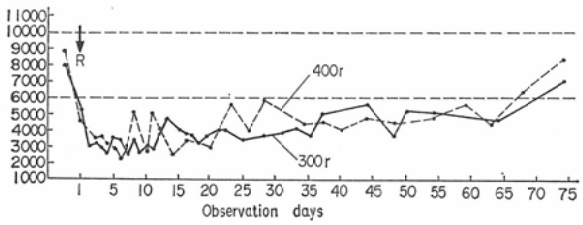
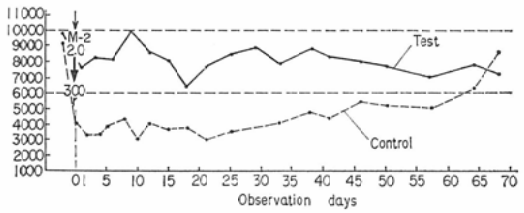


Fig. 2. Shift pattern of WBC-count of rabbits given MM 2 hours before exposure to 300 r.



pletely prevented by a single i.p. injection of 2 to 5 mg of Marimycin 2 to 4 hours before irradiation (Fig. 2). A single s.c. injection of the same dose does not seem so effective as i.v. injection, however, leukopenia induced by exposure to 300 r is relatively mild and does not last so long as in control animals. It usually takes about two months until the WBC-count of control rabbits returns to normal, while recovery from leukopenia in rabbits given a single s.c. injection of Marimycin 2 to 4 hours before exposure occurs within one month.

On the other hand, leukopenia cannot be inhibited by i.v. administration of this agent if it is done 2 days before irradiation (Fig. 3).

Repeated pretreatment with a dose of 2 mg of this agent seems effective both in preventing the oc-

Fig. 3. WBC-count of rabbits given MM 2 days before irradiation and treated 3 times with 2 mg of MM after irradiation.

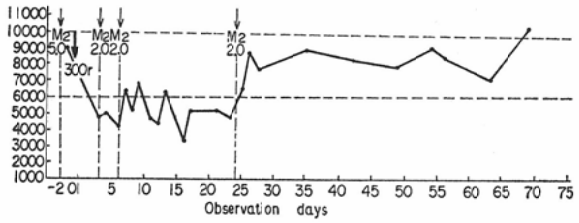
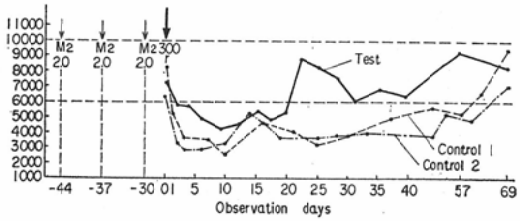


Fig. 4. Shift pattern of WBC-count of rabbits treated with MM 3 times before exposure to 300 r.



currence of a significant degree of leukopenia after exposure to 300 r and in accelerating the recovery from post-irradiation leukopenia A rabbit was i.v. given a dose of 2mg 3time at an interval of 7 days and exposed to 300 r 30 days after the third injection of Marimycin. As shown post-irradiation leukopenia. Arabbit was i.v. given a dose of 2 mg 3 times in Fig. 4, a milder leukopenia immediately appeared and lasted for about 3 weeks, but the recovery of the WBC-count was much accelerated in contrast with control animals.

Post-irradiation therapy with this agent seems also effective in accelerating the recovery from leukopenia. Rabbits were subjected to whole-body irradiation with 300 r and ↩reated with repeated i.v. or s.c. injection of this agent starting 3 to 6 days after exposure. A significant leukopenia developed in every animals, but the course of recovery was considerably shortened by such post-irradiation therapy. The shift patterns of the WBC-count in test animals are shown in Fig. 5 and 6.

Fig. 5. Shift pattern of WBC-count of rabbits treated with MM starting 6 days after exposure to 300 r.

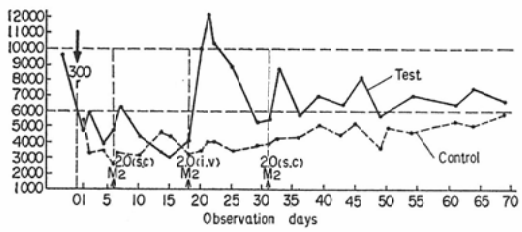
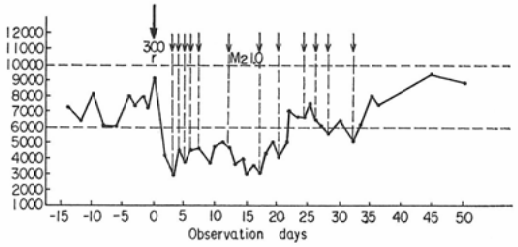


Fig. 6. Shift pattern of WBC-count of rabbits repeatedly treated with MM after exposure to 300 r.



If rabbits are i.v. given a single injection of 2 to 5 mg two hours before exposure to 300 or 400 r and treated therafter with repeated i.v. administration of 2 mg of Marimycin, the animals exposed to 300 r reveal almost no sign of leukopenia, while those exposed to 400 r show a mild degree of leukopenia which usually disappears within a relatively short period The shift patterns are shown in Fig. 7 and 8.

It is clear from the above facts, that the anti-leukopenic effect of Marimycin on irradiated animals is mainly due to its leukocytosis-inducing activity which being demonstrated in normal rabbits. The use of a dose larger than 5 mg of this agent does not result in a significant rise in its anti-leukopenic activity, and sometimes it seems less effective than the use of 2 to 5 mg, both in preventing and in treating post-irradiation leukopenia in rabbits.

I.v. administration of this agent immediately before irradiation proved apparently beneficial for suppression of occurrence of leukopenia. In animals exposed to 300 r, almost no leukopenia developed in

Fig. 7. WBC-count of rabbits given MM 2 hours before exposure to 300 r and treated 3 times with MM.

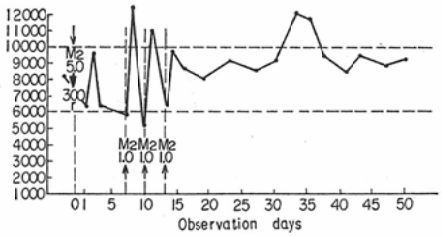
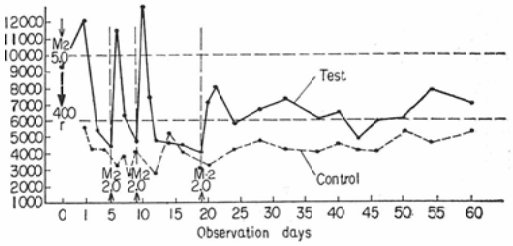


Fig. 8. Shift pattern of WBC-count of rabbits treated with MM 2 hours before exposure to 400 r and again treated with MM.



the post-irradiation course, while in those exposed to 400 r a mild degree of leukopenia usually developed after irradiation, but its recovery was considerably accelerated in contrast with control animals.

The injection by s.c. route seems less effective than by i.v. route, because it cannot prevent occurrence of leukopenia even in rabbits exposed to 300 r. However, the degree of leukopenia is usually mild and its course is, in general, considerably shortened by this procedure.

Repeated i.v. injection of Marimycin, starting 3 to 6 days after irradiation, proved also effective in accelerating recovery from leukopenia. Injection of a dose of 2 mg, 3 times at intervals of 3 to 5 days, proved almost sufficient to recover the normal level of the WBC-count of rabbits exposed to 300 r within about half the usual course of control animals.

Combined treatment with this agent, both immediately before and after irradiation, seems most effective in preventing or treating post-irradiation leukopenia in rabbits exposed to 400 r. If rabbits are i.v. given a dose of 5 mg of Marimycin 2 to 4 hours before exposure to 400 r and further given a dose of 2 mg 3 times after irradiation, the course of post-irradiation leukopenia may be considerably shortened and the animals may regain the normal range of the WBC-count within 3 weeks.

It is of much interest that the anti-leukopenic effect of this agent seems to be maintained at least for one month after its application, because it was demonstrated that if rabbits are i.v. given 3 successive injection of 2 mg of Marimycin at an interval of 7 days and one month later exposed to 300 r, the animals show only a mild degree of leukopenia with a shorter course than that of control animals. This maintenance of the anti-leukopenic effect of this agent does not seem to depend upon its leukocytosis-inducing activity, because the latter activity reaches the maximum level within 24 hours and then is completely subsided after 2 or 3 days as indicated previously. In pretreated animals, host response to a leukopenic state may have been reinforced by repeated injection of Marimycin and such a reinforced condition may have been maintained until they are exposed to Co^{60} -radiation.

In view of these facts, it seems likely that the anti-leukopenic effect of this agent may depend upon its specific activity to stimulate host leukocytosis-inducing mechanism and to alter its responsiveness to abnormal reduction in the WBC-count of the peripheral blood.

The effect on leukopenia induced by anti-cancer drugs

Many kinds of anti-cancer drugs have been used for treatment of various cancer diseases. The majority of such drugs have an untoward activity to induce a significant degree of leukopenia in the peripheral blood of man and animals. Marimycin was tested for its effect on leukopenia induced by anti-cancer agents, and proved to be also effective in both preventing and treating durgs-induced leukopenia.

If rabbits are i.v. given a dose of 2,500 units of Carzinophilin, two times at an interval of 1 to 2 weeks, the WBC-count in the peripheral blood gradually decreases and a moderate degree of leukopenia develops and lasts for about one month (Fig. 9).

Rabbits i.v. given a dose of 2 mg of Marimycin shortly before administration of Carzinophilin showed only a mild leukopenia and its recovery was considerably accelerated in contrast with control animals (Fig. 10).

Fig. 9. Shift pattern of the WBC-count of rabbits i.v. given Carzinophilin.

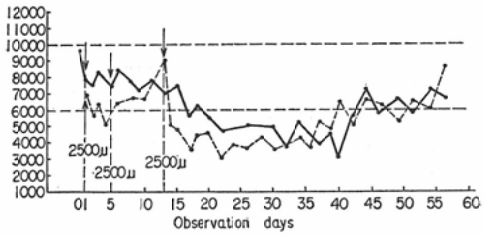
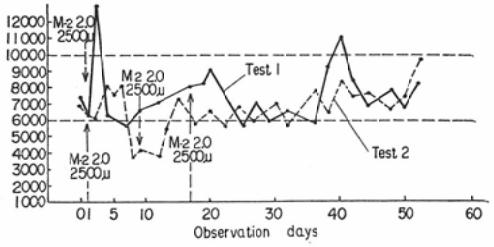


Fig. 10. Shift pattern of the WBC-count of rabbits given MM 2 hours before administration of CP.



Three successive i.v. injection of a dose of 2 mg of Mitomycin C at intervals of 3 to 5 days induces a mild degree of leukopenia in test rabbits, but if a dose of 2 mg of Marimycin is i.v. given simultaneously or shortly before administration of Mitomycin C, almost no leukopenia develops in such animals.

Similar tests were carried out with other anti-cancer agents such as Sarcomycin, Toyomycin, Nitromin and Tespamin. A daily dose of 200 mg of Sarcomycin was consecutively given to rabbits for 6 days by i.v. route, but any significant reduction in the WBC-count was not demonstrated in such animals. A daily dose of 0.25 mg of Toyomycin was i.v. given for 2 days, and 2 days later another dose of 0.5 mg was again given to rabbits, but the animals did not revela any significant degree of leukopenia.

On the other hand, a mild degree of leukopenia with a relatively short duration was demonstrated in rabbits which had been i.v. given a dose of 10 mg of Nitromin 3 times at intervals of 2 to 3 days. Similar results may be obtained by a single i.v. injection of 5 mg of Tespamin. If a dose of 2 mg of Marimycin is i.v. given to rabbits, simultaneously of shortly before administration of Nitromin or Tespamin, the animals do not show any reduction of the WBC-count in the peripheral blood.

Clinical application of Marimycin

As noted above, it was shown that Marimycin has a specific activity to induce a significant degree of leukocytosis in rabbits, and that such activity may be due to its characteristic effect to pull the trigger for an unknown mechanism which may directly increase the WBC-count in the peripheral blood. The duration course of post-irradiation leukopenia developed in rabbits exposed to 300 or 400 r may be considerably shortened by repeated injection of Marimycin, while a single i.v. injection of this agent shortly before exposure to 300 r may suppress the occurrence of post-irradiation leukopenia.

In an attempt to test Marimycin for its clinical usefulness for patients with malignant tumors therapeutically irradiated with Co^{60} radiation, a daily dose of 20 to 40 mg was s.c. given to total 21 patients for variable days, starting before or during the course of irradiation therapy, and the blood picture was periodically examined to follow its shift patterns in individual cases. Other 10 patients with similar diseases were included as control. All these cases were admitted to Kanto Teishin Hospital to receive operative or

radiologic therapy for malignant neoplasms (Table 2 and 3).

Several case reports will be presented here to show an apparently beneficial effect of Marimycin on inhibition of irradiation-induced leukopenia.

Case 5. Relapse of laryngeal cancer with lymph node metastasis

A male, 67 years of age, received radical operation of the laryngeal cancer lesion 5 years ago (1962). In July, 1966, the disease recurred and metastasis developed to lymph nodes of the neck, so that Tele Co^{60} irradiation therapy with total 4,800 r was initiated on 26th January, 1967. Subcutaneous administration of a daily dose 40 mg of Marimycin was initiated at the same time and continued for 52 days.

Table 2. Patients in the test group

Case No.	Age	Sex	Diagnosis	Radiation dose(r)	Marimycin mg(days)	WBC-count before	WBC-count after
1	68	M	Laryngeal cancer	5,800	40(39)	3700	4000
2	74	M	Laryngeal cancer	5,000	40(18)	6500	7500
3	68	M	Laryngeal cancer	6,000	40(34)	6000	6000
4	57	M	Laryngeal cancer	5,800	40(31)	7000	4000
5	67	M	Relapse of laryngeal cancer with lymph node metastasis	4,800	40(52)	6200	6000
6	54	M	Maxillary cancer(r)	6,000	40(28)	6500	7500
7	55	F	Maxillary cancer(r) with metastasis to cervical lymph nodes(r)	14,000	40(34)	5300	8600
8	42	F	Cancer of the tongue	5,000	40(25)	5400	5300
9	61	F	Reticulosarcoma of the left tonsil	5,000	40(32)	6300	8200
10	42	F	Malignant naso-pharyngeal melanoma	2,000	20(24)	5300	6400
11	61	M	Thymoma with metastasis to cervical lymph nodes(r)	12,000	40(20)	6300	6500
12	48	F	Uterine cancer (C. colli)	6,900	20(7)	4500	3400
13	59	F	Uterine cancer (C. colli)	9,000	20(12)	4000	3500
14	77	F	Uterine cancer (C. colli)	9,000	20(30)	7200	4900
15	19	F	Ovarian cancer	9,000	20(15)	5200	5000
16	47	F	Uterine cancer	9,000	20(13)	7300	6700
17	38	F	Uterine cancer	3,900	20(9)	2800	3400
				6,900	20(6)	3400	3500
18	58	M	Ascites of unknown origin	—	40(14)	2400	4000
19	64	M	Relapse of gastric cancer	9,500	20(17)	3500	6500
20	51	F	Cancer of the breast(r)	6,000	20(7)	5200	4100
21	61	M	Adenocarcinoma (1 neck)	5,100	20(20)	5100	2700

As shown in Fig. 11, the WBC-count was maintained almost in the normal range during the course of irradiation therapy and then somewhat decreased, but it was always maintained above 4000/cu.mm throughout the course of his stay in hospital. In this case, almost no untoward side-effect was recognized in spite of its repeated administration for as long as 52 days.

Case 7. Maxillary cancer(r) with cervical lymph node metastasis

A female, 55 years old, visited the hospital on February 6th, 1967, complaining of nasal obstruction,

Table 3. Patients in the control group

Case No.	Age	Sex	Diagnosis	Radiation dose(r)	Greenpole mg(days)	WBC-count before	WBC-count after
1	49	M	Laryngeal cancer	5,600	10(43)	8200	3300
2	59	M	Laryngeal cancer	9,000	10(72)	6500	4000
3	56	M	Laryngeal cancer	9,600	10(34)	7600	4600
4	68	M	Laryngeal cancer	7,400	—	10200	4700
5	75	M	Laryngeal cancer	167	—	4200	3400
6	59	M	Cancer of the tongue with metastasis to cervical lymph nodes	5,220	10(58)	3700	3400
7	64	M	Repapse of gastric cancer	1,500	—	3500	1900
8	51	F	Cancer of the breast(r)	2,500	—	5200	2600
9	44	F	Reticulosarcoma of the left chest	2,500	—	4600	2550
10	38	F	Uterine cancer	3,900	—	5800	2800

Fig. 11. No. 5, Relapse of laryngeal cancer with lymph node metastasis.

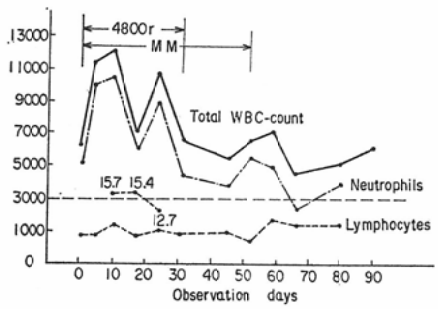
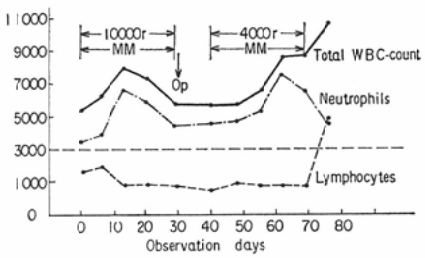


Fig. 12. No. 7, Maxillary cancer with metastasis.



nosebleed and swelling of the right maxillary region. Operation was performed on 10th March under the diagnosis of "maxillary cancer". Preoperative Co^{60} irradiation of both the primary lesion and the cervical lymph nodes with each 5,000 r was done during the course between February 7th and March 8th. After the operation both lesions were irradiated with each 2,000 r until April 17th. A daily dose of 40 mg of Marimycin was s.c. given in parallel with both irradiation courses.

As shown in Fig. 12, the WBC-count was maintained almost in the normal range during both courses of radiation therapy and no leukopenia was observed throughout her stay in hospital.

Case 9. Reticulosarcoma of the left tonsil

A female, 61 years of age, complained of a painful swelling of the pharyx and visited the hospital. The left tonsil was remarkably swollen and indurated with a highly uneven surface which was partially ulcerated. Histologically it was diagnosed as reticulosarcoma and the patient was immediately treated with Tele Co^{60} irradiation with toal 5,000 r during the course between December 20th and February 22nd, 1967.

A daily dose of 40 mg of Marimycin was s.c. given for 32 days in parallel with radiation therapy, which did not reveal any undesirable side-effect. The WBC-count was maintained in the normal range throughout the course of radiation therapy and the tumor was completely disappeared until the beginning of February and the patient was discharged under a considerably favorable condition. (Fig. 13)

Fig. 13. No. 9. Reticulosarcoma of the left tonsil.

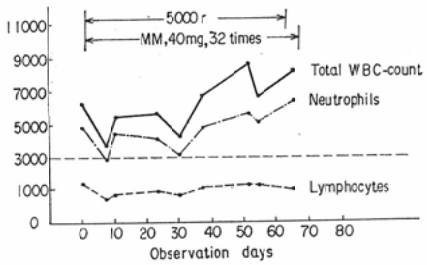
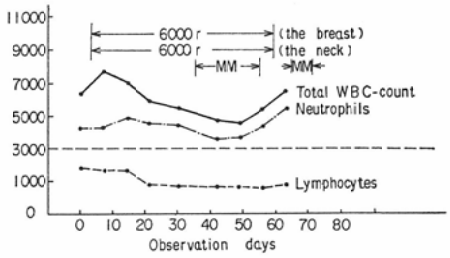


Fig. 14. No. 11. Thymoma.



Case 11. Thymoma with metastasis to cervical lymph nodes

A male, 61 years of age, complained of uncomfortable feeling of the stomach and loss of appetite since March, 1966. At the end of October he became aware of gradual swelling of the cervical and supraclavicular lymph nodes. On December 9th, test samples were taken from the cervical lymph nodes and it was established that thymoma had developed metastasis to lymph nodes of cervical and supraclavicular regions.

The patient was immediately treated with Tele Co^{60} irradiation until 2nd February, 1967. Subcutaneous injection of a daily dose of 40 mg of Marimycin was initiated on January 9th and continued up to 29th of that month and 10 days later a daily dose of 20 mg was again given for 8 days.

The WBC-count gradually decreased to the level less than 5,000/cu.mm, but it soon recovered the normal level within a short period (Fig. 14). The count of the neutrophils remarkably increased at the end of Marimycin therapy, but that of the lymphocytes remained very low throughout the course of his stay in hospital.

Case 8. Cancer of the tongue with metastasis to cervical lymph nodes

A female, 42 years old, was admitted to the hospital and treated under the diagnosis of "Cancer of the tongue" with Tele Co^{60} irradiation and radium needle therapy in October, 1965. The patient was once discharged but again admitted because of recurrent growth of the primary lesion and metastasis to cervical lymph nodes in January, 1966. Operation was performed on January 25th and Tele Co^{60} irradiation therapy was started on 9th of the next month and continued up to March 10th. Subcutaneous injection of a dose of 40 mg of Marimycin was done 25 times during the course of radiation therapy. The WBC-count decreased to the level of 4,000/cu.mm, but it soon became almost normal within a short period (Fig. 15). The count of thrombocytes was well maintained in the normal range, while that of the lymphocytes remained very low as in the case of No. 11. As shown also in the next case, Marimycin appears to increase the count of thrombocytes in the peripheral blood.

Case 10. Malignant nasopharyngeal melanoma

A female, 42 years of age, complained of nasal obstruction and nosebleed and was admitted on October 11th, 1966, under the diagnosis of "Malignant naso-pharyngeal melanoma." She was radiologically treated with total 3000 r during the course between 13th October and 2nd November, and then a dose of 100 mg of Endoxan was i.v. given 25 times on alternate days until the end of December. Operation was performed on 25th January, and then post-operative Co^{60} irradiation with total 2,000 r was done until February 7th.

Fig. 15. No. 8. Cancer of the tongue.

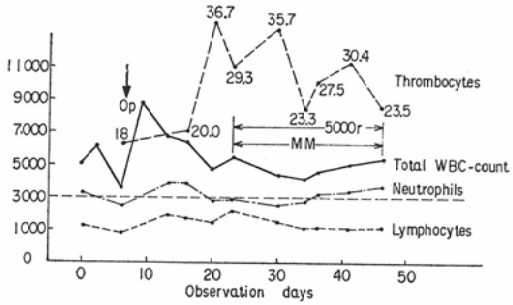
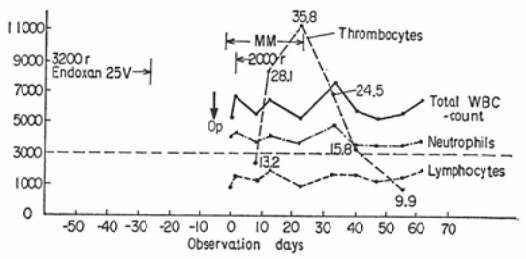


Fig. 16. No. 10. Malignant nasopharyngeal melanoma.

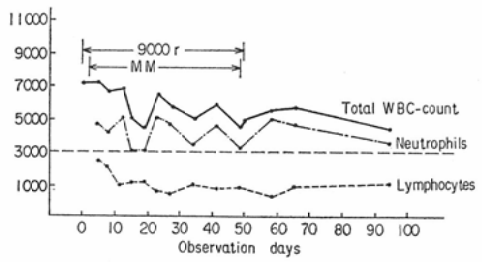


On the other hand, a daily dose of 20 mg of Marimycin was s.c. given for 24 days, starting 2 days before initiation of post-operative irradiation. The WBC-count was well maintained above the level of 5,000/cu.mm, and no significant leukopenia appeared throughout the course of her stay in hospital. Marimycin appears to increase the count of the thrombocytes in the peripheral blood, because as shown in Fig. 16 the thrombocyte-count rapidly increased after the start of Marimycin therapy and then decreased to the former level when its administration was discontinued.

Case 14. Uterine cancer

A female, 77 years of age, was diagnosed as suffering from uterine cancer and treated with Tele Co^{60} irradiation during the course between April 13th and May 31st, 1966 (total 9,000 r). As shown in Fig. 17, a dose of 20 mg of Marimycin was s.c. given 30 times in parallel with radiation therapy. In spite of very old age of the patient, the WBC-count was well maintained above the level of 4,5000/cu.mm, which made completion of the full course of radiation therapy possible.

Fig. 17. No. 14. Uterine cancer (Carcinoma colli).



Case 17. Uterine cancer

A female, 38 years old, was diagnosed as suffering from an advanced uterine cancer and radiologically-treated with Tele Co^{60} irradiation. Therapy was started on August 31st, 1966, but the WBC-count rapidly reduced from 5,800 to 2,800/cu mm within 2 weeks, so that the therapy was discontinued and s.c. administration of a daily dose of 40 mg of Marimycin was initiated at once. After 5 days the WBC-count increased to the level more than 3,000/cu.mm, so radiation therapy was again started and continued in parallel with daily injection of Marimycin until October 8th. Although the WBC-count was not so much improved by administration of Marimycin in this case, it always remained higher than 3,000/cu.mm throughout the course of radiation therapy. The count of the thrombocytes was not also improved by administration of Marimycin (Fig. 18).

Fig. 18. No. 17. A case of uterine cancer.

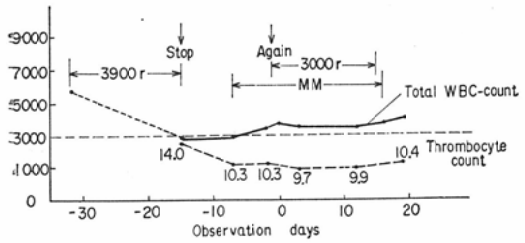
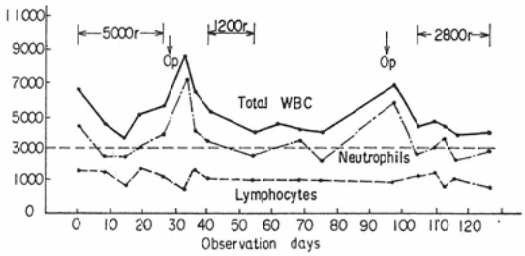


Fig. 19. No. C-2 Laryngeal carcinoma with metastasis to cervical lymph nodes.



Patients in the control group

Case C-2. Laryngeal carcinoma with metastasis to cervical lymph nodes

A male, 59 years of age, complained of violent cough and difficulty in swallow, and treated with Tele Co^{60} irradiation under the diagnosis of "Laryngeal cancer" during a course between February 4th and 20th, 1963 (total 3,000 r). Resection of the lerynx was performed on February 20th and radiation therapy was again applied up to March 20th, starting about 2 weeks after operation.

About 2 months later, metastatic lymph nodes in the right cervical region were thoroughly resected and then radiation therapy with total 2,800 r was applied immediately thereafter.

Although the WBC-count was maintained above the level of 3,500/cu.mm, it was readily lowered up to nearly the critical level by each application of radiation therapy as seen in Fig. 19.

Case C-6 Cancer of the tongue with metastasis to cervical lymph nodes

A male, 59 years old, was diagnosed as suffering from cancer of the tongue, and treated with radium needle therapy in September, 1959. Subsequent course for about 5 years was quite favorable, but the patient was again admitted in April, 1962, because of gradual swelling of cervical lymph nodes on the right side which were preoperatively treated with Tele Co^{60} irradiation during the course between May 18th and 31st. Resection of the metastatic lymph nodes was performed on May 30th and the local area was postoperatively irradiated with total radiation dose of 4,400 r.

The WBC-count rapidly decreased during the course of post-operative irradiation and approached the level of 3,000/cu.mm, and it was still less than 4,000/cu.mm on the day when the patient was discharged from the hospital.

Aside from these cases, there were 4 cases in which radiation therapy being inevitably discontinued halfway because of critical reduction in the leukocyte count during the course of radiological therapy. One patient with relapse of gastric cancer was radiologically treated with a radiation dose of 250 r for 6 consecutive days, but subsequent therapy was discontinued because of rapid reduction of the leukocyte count to 1,900 r/cu.mm.

The second patient with cancer of the breast was radically operated and postoperatively treated with a radiation dose of 200 r for 8 days, when the leukocyte count lowered to the level of less than 2,500/cu.mm, so that the radiation therapy was temporarily discontinued.

The third patient with reticulosarcoma of the thorax was radiologically treated with a daily dose of 250 r for 10 days, but subsequent continuation of radiation therapy became difficult because the leukocyte count dropped to the level of less than 2,500/cu.mm. The fourth patient with relapse of uterine cancer

was also treated with a daily radiation dose of 250 r for 13 days, but since the leukocyte count reduced up to 2,500/cu.mm, subsequent irradiation was temporarily discontinued.

Thus radiation therapy was inevitably discontinued temporarily or for fairly a long period in about half of patients in the control group because of a critical reduction of the leukocyte count in the blood. Even in the remaining cases where a nearly full course of irradiation therapy was feasible, the recovery from radiation-induced leukopenia was considerably delayed and the leukocyte count often remained very low for as long as one month after radiation therapy was over.

In the test groups, however, the majority of patients did not reveal any significant degree of leukopenia during a full course of radiation therapy and the caurse of the recovery from mild reduction of the leukocyte count was, in general, considerably shortened by repeated administration of Marimycin.

Summary and Discussion

Marimycin is a polysaccharide agent which was first extracted, in 1961, from culture broth of Streptomyces mariensis, a new strain isolated by the author in 1957. This agent has a specific activity to induce a significant degree of leukocytosis in normal rabbits, the peak of which being usually attained more than 20 hours after its i.v. administration. Since the grade of such activity does not seem to vary depending upon the dosage of Marimycin within the wide range from 1.0 to 200 mg, it seems reasonable to consider that leukocytosis due to Marimycin may not be induced by its direct activity, but may depend upon some yet unknown mechanism which may be brought into play by the direct activity of Marimycin. Its specific activity may probably serve to pull the trigger for bringing such mechanism into play which stimulates the increase of the white blood cells in the peripheral blood.

Leukocytosis induced by a single administration of Marimycin usually lasts at least for a few days and then the leukocyte count gradually returns to normal within several days. Post-irradiation leukopenia which usually develops in control rabbits exposed to whole-body irradiation with 300 r may be prevented if a single i.v. injection of a dose of 2.0 mg of Marimycin is done shortly before irradiation, whereas with 400 r such leukopenia connot be prevented by the same produdure, but its duration in the post-irradiation course may considerably be shortened by such pre-treatment with this agent.

On the other hand, if 2.0 mg of Marimycin is i.v. given to rabbits 3 times at an interval of 5 to 7 days, one month before exposure to 300 r, post-irradiation leukopenia cannot be avoided but the recovery from leukopenia may usually be accelerated in such animals. Repeated i.v. administration of Marimycin in the post-irradiation course appears to be also effective in accelerating the recovery from leukopenia developed in animals exposed to 300 to 400 r.

According to these experimental facts, Marimycin was clinically tested for its inhibitory effect on occurrence of leukopenia in patients receiving therapeutic irradiation. In the majority of total 21 cases of the test group, the leukocyte count was, in general, well maintained throughout the course of radiation therapy with the total doses ranging from 5,000 to 14,000 r. Of course, a mild degree of leukopenia was rarely recognized in some cases, but it was only temporary and soon followed by a rapid recovery from such state up to the normal range of cell count, except for a single case of uterine cancer. There were no cases where irradiation was inevitably discontinued halfway mainly because of critical reduction in the WBC-count. In contrast with this, irradiation was discontinued temporarily or for fairly a long period halfway in the course of therapy in about half of cases of the control group by reason of a rapid reduction in the leu-

kocyte count to the level less than 2,500 per cubic mm.

According to our clinical study to date, Marimycin appears to be mainly effective in enhancing the count of the neutrophil leukocytes, but of almost no beneficial effect on increasing the lymphocyte count. In certain cases, the count of the thrombocytes rapidly increased in parallel with administration of Maimycin and again returned to the former level as soon as its administration was over. In view of these above facts, this agent seems to be useful for clinical purpose to suppress the occurrence of a significant degree of leukopenia due to local irradiation with a daily therapeutic dose of 200 to 300 r and to prevent the danger of complications due to decrease of the leukocytes in the blood.

It was clinically demonstrated to be almost non-toxic and could be used in a daily dose of 20 to 40 mg consecutively for more than 50 days without any appreciable side-effects neccesitating immediate discontinuance of its administration.

As mentioned above, the ideal protectant should not produce untoward side effect. It should not exert cumulative effects on repeated application, or it should have a large margin of safety. Chemical agents such as cysteamine, cystamine, cysteine and AET have clinically been tested for their protective activities against therapeutic X-irradiation, but all have failed to modify post-irradiation leukopenia. AET was also tested by oral route, but its effect as a protective agent was highly limited by its remarkable toxicity. Handreds of related derivatives have been synthesized and tested, however, none of the agents hitherto known cannot be considered to be of value in safely modifying post-irradiation leukopenia.

In this meaning, Marimycin seems to be one of the promising agents which for the most part satisfy the essential criteria for ideal protectant, except for lack of evidence for the usefulness of its administration by oral route.

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