A CANCER CONTROL WITH SARKOMYCIN

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Cancer chemotherapy is nowadays considered as the use of a systematically administered agent which, while relatively nontoxic to the host, will interfer with, favorably modify, or destroy a neoplastic growth or alleviate its deleterious effects on the host. But clinically speaking, even the recent advancement in the chemotherapeutic control of malignant tumors are too behind to be comparable to that of bacterial infections. And one of the most important problems is, at present, to know if similar therapeutic effects as those confirmed by animal experiment can be expected in the treatment of human cancer.

As a most sensitive experimental neoplasma to sarkomycin we selected EHRLICH ascites carcinoma to study *in vitro* and *in vivo* activity of the drug. A saline suspension of EHRLICH carcinoma cells was mixed with varying amounts of sarkomycin in a prove tube and kept for 3 hours at 0°C. Then it was inoculated intrapretoneally into a d-d-N mouse. There were observed no carcinoma developments in mice, which received carcinoma cells exposed to more than 0.78 mg/cc of the drug, while other mice all died after manifesting their carcinomas.

The therapeutic effect for mice carcinomata was greatly dependent on the time at which the administration started. Early treatments were satisfactory, but the treatment after accumulation of remarkable asites allowed no good results. Exceptionally it was found effective when it was given intravenously twice a day.

The carcinoma cells treated with sarkomycin *in vitro* or *in vivo* were different from normal one in not only their biological properties but also in their morphological findings. Rapidly reduced mitosis and degenerative changes of their nucleous and cytoplasms were the forgoing pictures. In the fixed tumors we could find heavy bleeding and necrosis combined with in the neoplastic tissues.

The concentration of administered sarkomycin was assayed through a modified vertical diffusion method, a colorimetric method with Anthrone or a transplantation method using urine of the patients and dogs. An adult received 2,000 mg of intravenous sarkomycin will excrete approximately 18.4 of total administration into the urine. And the maximum excretion will be found 1~2 hours after injection, nearly a half of the amount being excreted by this time. Decreasing gradually thereafter, detectable excretion will continue as long as 5 to 13 hours after injection.

During a period from September 1953 to January 1955, 193 cases of 28 different kinds of malignant tumors, whose diagnosis had been confirmed histopathologically, received sarkomycin treatments. Summary of the clinical findings of 144 cases, excluding 49 for which sarkomycin was given for the purpose of prevention of postoperative recurrence, are reported. In 27 cases (19%) the size of tumor or its X-ray shadow became diminished or disappeared and 49 cases (34%) improvements rare noted as to pain, appetite, body weight, ascites, sputum, stricture, and serum protein rates etc., while in 68 cases (47%) the treatment was either fruitless or only temporary.

A NEW ANTITUMOR SUBSTANCE, CARCINOMYCIN, ISOLATED FROM STREPTOMYCES CARCINOMYCICUS

SEIGO HOSOYA

A number of antibiotic substances, such as actinomycin C, sarkomycin, puromycin, trichomycin, carcinophilin, azaserine and mitomycin, proved to be active against tumor cells.

HOSOYA and SOEDA isolated 60 strains of *Streptomyces* species active against *Aspergillus niger* from soil samples taken at Hachijo Jima, and 25 out of them were found to show marked *in vitro* activities (according to the so-called NIINOMI method) against EHRLICH's ascitic cancer cells.

A new antitumor substance, designated as carcinomycin (trade-name : gannmycin), has been isolated from H-5342 strain, one of the above-stated 25 strains. Mycological characteristics of H-5342 strain, both *in vitro* and *in vivo* activities of carcinomycin against EHRLICH's ascitic cancer cells, methods of production, isolation and purification, and its toxicity to mice are presented here. The strain has proved to be a new species of *Streptomyces* from the results of mycological investigation and has been named as *Streptomyces carcinomycicus* HOSOYA and SOEDA *n. sp.*

Pathological and histopathological researches on the action of carcinomycin were carried out by Dr. KIYOSHI SATO and the original papers will be published in J. Antibiotics, Ser. A.

ON THE ACTION MODE OF SARKOMYCIN
(A New Antitumor Antibiotic derived from Streptomyces erythrochromogenes)

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The authors have cleared up the following facts from the standpoints of laboratorial experiments and clinical investigations on the action mode of sarkomycin against cancer.

A) Direct action of sarkomycin against EHRLICH carcinoma cells :-

The effects of sarkomycin in various doses were

studied on transplanted EHRLICH ascites tumor cells of mice. A single dose of 1 mg sarkomycin administered intraperitoneally damages selectively carcinoma cells under mitosis, but its effects prolongs only two to four hours and when the dose is increased up to 3 mg both resting and mitotic carcinoma cells are damaged in various degrees, in spite of little changes of normal cells, having been reduced to normal conditions within 18 hours.

The same effects are also demonstrated by the substitution of the drug to the filtrate of *Escherichia coli.*

The authors assume that the direct action of sarkomycin against tumor cells are displayed rather by denaturalization than by inhibition of synthetic process of nucleoprotein.

B) Action mode of sarkomycin against stroma of tumor tissues, especially against its blood vessels.

The autopsy findings of a patient with stomach cancer, who had received 150 g sarkomycin, revealed such remarkable degenerative changes of blood vessels as infarct of spleen or thrombophlebitis of liver.

From this point of view, that we have here demonstrated, sarkomycin seems to contain Shwartzman factor, but it was quite unable to domonstrate this real fact experimentally in sarkomycin with rabbits.

(2)

Therefore the authors have pursued changes of the permeability of blood vessels after the administration of sarkomycin eitherby the chinese ink method or by the injection method of fluorescein albumin derived from bovine serum, resulting in the demonstration of their increased permeability in the tumor tissues.

On the other hand, experimentally irradiated mice demonstrated always the arrest of chinese ink in the blood vessels of intestinal wall. But if the animals were administered sarkomycin just before the irradiation, the arrest of chinese ink in the blood vessels was not detected.

Furthermore the authors investigated the effect of sarkomycin to modify Shwartzman phenomenon. Its normal reaction, that is, the hemorrhage did not occur, when sarkomycin was injected intravenously before the second intravenous injection of the filtrate of *Escherichia coli* just same as Gross-Ogata's inhibition reaction. However when the animals were injected with sarkomycin intravenously two hours after the positive development of Shwartzman phenomenon, they were apt to show remarkable wide spread hemorrhagic zone in its peripheral area. This fact shows that sarkomycin has affected the increased permeability of the blood vessels in tumor tissues so much as the hemorrhage has occurred.

In conclusion, the authors emphasize that the most important action mode of sarkomycin must be its modifying effect on the permeability of more or less damaged blood vessels in the stroma of tumor tissues.

CLINICAL STUDY OF CARZINOPHILIN

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The report is the clinical result of Carzinophilin, anti-tumor substance, which was used for 65 cases of cancer, sarcoma, and other disease. This series was composed of the inoperable 48 cases and 17 cases to which radical operation were performed.

The result of the latter group was not clear because the post operative observating period is not so long. The clinical finding which was improved by Carzinophilin in carcinoma are reduction of tumor or ulcer, diminution of ascites and improvement of appetite, but these signs were found in only few cases. In the cases of sarcoma, reduction of tumor, softening or necrosis which were curretaged or excised, were produced in all the cases except one case of fibrosarcoma.

In 3 cases, pain was reduced markedly or disappeared. Namely, improvements of subjective sign were found in 9 cases, objective signs were improved in 7 cases.

The histo-pathologic alteration were decrease of mytosis, appearance of acidophile cells, vacuole degeneration of protoplasma, tendency to fibrination, and in sarcoma, necrosis of tumor, disappearance of tumor cell were prominent. In the one case of bone sarcoma, obvious repairment of bone were recognized by X-ray.

The latest 6 cases in these cases which were affected effectively by Carzinophilin were reported.

At present, intravascular administration, intraabdominal instilment, local injection and compress, or per oral administration are used, because by aubcutaneous or intramuscular injection the necrosis or indulation are produced occasionally.

Appearance of urine urobilin, inpairment of liver function or decrease of leucocyte count were recognized in intravenous or intramuscular administration. Myeloblasts, promyelocytes, myelocytes, lymphocytes and plasma cells in myelocells are reduced by Carzinophilin but are curable as leucocyte by blood-transfusion or maedication.

Decrease of leucocyte cells are marked in the case in which big daily doses are given and now intravascular injection of 1,000-2,000 u. diluted to 100 u/cc are used.

The finding of autopsy which was impressed as efficient and histologic finding of liver, spleen and kidney were reported.

The histo-pathologic alterations of liver specially,

impaired circulation and difference of prothrombin index when big dosis or small dosis of Carzinophilin were injected into the portal vein of dog were discussed.

STUDIES ON PENICILLIN ALLERGY

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1. Intracutaneous test

The test was carried out with the intracutaneous injection of 0.1 cc of saline solution of crystalline penicillin G containing 10,000 u/cc for the persons of different groups : those who had never been given penicillin ; those who had been given penicillin before and those who were working in a penicillin producing factory.

From the above tests, the local inflammatory area, the diameter of which are over 20 mm at 15 minutes after the injection of penicillin seems to indicate the positive reaction.

2. Blood picture before and after the penicillin injection

Intracutaneous injection was carried out with 0.05 ~0.1 cc of saline solution of crystalline penicillin G containing 200~1,000 u/cc in nine penicillin hypersensitive persons : four persons with anaphylactic systemic reaction after the injection of penicillin (which may be called "penicillin-anaphylactic case") and five persons with urticaria and other allergic symptoms after the use of penicillin (which may be called "penicillin-allergic case").

As the control, 0.1 cc of saline solution of penicillin G containing 1,000 u/cc was intracutaneously injected in persons : five persons who had ever given penicillin repeatedly without hypersensitive signs and five persons who had never received penicillin treatment.

In every cases the intensity of local penicillin reaction was recorded and in case any systemic reaction appeared after penicillin test, symptoms were also recorded.

It was recognized from the above tests that there were some cases, in which the intensity of the intracutaneous reaction did not parallel the intensity of the systemic reaction and in such a case the intracutaneous test alone did not seem to have any diagnostic value.

The change of the blood picture before and after the penicillin injection also has no significant diagnostic meanings.

3. Collodion particles agglutination test

Aqueous solution with different concentrations of crystalline penicillin G and the serum from penicillin-anaphylactic and/or penicillin-allergic persons were mixed in the suspensions of collodion particles according to SCHEIFFARTH's method.

With only one of three penicillin-anaphylactic cases the serum agglutinated collodion particles but with other two cases and with six penicillin-allergic cases no agglutination was observed.

4. Antigenic property of penicillin G

Guinea pigs were given penicillin in various forms with different kinds of application. Three to five weeks after the last application the lower iluem were extirpated and according to SCHLTZ-DALE's technique the antigenic property of crystalline penicillin G was examined.

Five of eight animals, which had previously deceived five times alternative treatment with subcutaneous injection of living stphylococcus and that of penicillin successively and two of three animals, which had been given 1 cc of a mixed solution of a 10% bovine serum and crystalline penicillin G containing 1,000 u/cc once a day for five days were observed to react positively to 1~100 u/cc penicillin solution. However, no desensitization phenomenon was observed, which may be in agreement with the findings by MCCLOSKY and SMITH.

From these findings it may be inferred that the penicillin may act as hapten in sensitization.

PENICILLIN-ALLERGY

——from the point of view of dermatology——

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From clinical experience (76 cases) and experiment, we obtained the following results.

Skin manifestations of penicillin-allergy

1) Erythema (measles-like, scarlatina-like lesion, unspecific papules etc.) 2) Urticaria 3) serum-sickness-like syndrome 4) angioneurotic edema 5) dysidrosis-like eruption, penicillids 6) bullous dermatitis 7) exfoliative dermatitis 8) pruritus 9) contact-dermatitis 10) inflammation of mucous membrane from contact 11) Erythema exsudatum multiforme 12) Erythema nodosum 13) fixed drug-eruption 14) purpura 15) Arthus-phenomenon 16) Shwartzman-phenomenon 17) light-sensitive-dermatosis 18) disseminated lupus erythematosus 19) periarteritis nodosa 20) Herpes zoster, Herpes simplex 21) other lesions (erythema migrans, gangren etc.) 22) granulomata formation (deep foreign body granulomata, universal granulomata) 23) Herxheimer reaction 24) symbiotic imbalance (moniliasis) 25) anaphylaxis

Etiologic factors

1) allergic constitution

2) underlying factors (dysfunction of adrenocortical hormon, disturbance of autonomic nervous sys-

tem, Leber dysfunction etc.)

Mechanismus of penicillin reaction

1) allergy (parallergy)
2) biotropisme
3) pharmacologic effect of penicillin
4) symbiotic imbalance, ecologic relationships
5) auto-sensitive-dermatitis
6) Herxheimer-reaction
7) toxic effect

EXPERIENCE OF PENICILLIN ANAPHYLACTIC SHOCK

YASUSHI UEDA

During the past few years the author experienced 10 cases of penicillin anaphylactic shock (one case showing shock symptoms died following penicillin injection). An outline of the clinical findings will be reported here.

The 10 cases consisted of 3 males and 7 females. Their ages varied from 15 to 52. The drugs used were procaine penicillin (oil solution 6 cases), procaine penicillin (aqueous solution 2 cases), penicillin G(aqueous solution 1 case), and Bicillin (1 case).

Although the majority of the cases showed shock symptoms within 5 minutes, one fatal case showed shock symptoms 10 hours after injection.

Anaphylactic shock symptoms were of the typical type, and serious cases showes speech disorder, incontinence and unconsciousness.

Nine cases (90%) have had penicillin injections previously. Six of the nine cases have had no exprience of side effects while two did. Two cases had a history of allergic diseases.

Eight (one fatal) of the 10 cases showed serious conditions, 1 moderate and 1 mild.

The autopsy findings of the one fatal case are as follows :

1) Cross findings :
   a) Hemorrhage from the distal transversal colon to the proximal descending colon.
   b) Hemorrhage of the sigmoid.
   c) Hemorrhage of the left adrenal gland.
   d) Bilateral hemorrhage of the ovarries.
   e) Parenchymatous hemorrhage, congestion and edema of the lungs.
2) Microscopical findings :
   a) Transversal colon: Marked dilatation of blood vessels, edema and hemorrhage. Fresh fibrinous thrombosis of small and middle sized vessels. In some of the small arteries, picture of endothelial desquamation, proliferation and new thrombosis.
   b) Hemorrhage of the adrenal, lungs and ovaries.

INTRAABDOMINAL ADHESION FROM THE STANDPOINT OF LOCAL ADMINISTRATION OF PENICILLIN

(Presented specially at the third annual meeting of Japan Chemotherapy Society)

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Direct administration of penicillin in the cavities of human body may result in adhesion and various difficulties, which are often encountered in the abdominal cavity and subarchnoid space. Understanding and agreement have been established concerning the local use in the subarachnoid space, The present report, therefore, reviews the relation between intraabdominal administration of penicillin and adhesion in the abdominal cavity in which many clinicians are most interested.

At the 48th annual meeting of Japan Surgical Society in May, 1948, we reported that local administration of penicillin or marphanil is very effective to experimental peritonitis of rabbits, though the latter is more reasonable as far as resultant adhesion is concerned. Thereafter a few opinions regarding this problem have been presented. Some belive that intraabdominal use of water-soluble penicillin results in adhesion. Others have objection against it. But these opinions are not fundamentally different, because adhesion in the abdominal cavity is likely to be produced by a certain kind of stimulus with the action of administerd penicillin, whereas penicillin administration in the normal abdominal cavity itself does not induce adhesion. Of course, in cases of laparotomy operative procedures are more or less applied in the abdominal cavity which may often be inflammatory in various degrees. Consequently, it is the nucleus of this problem whether intraabdominal administration of penicillin in such instances is followed by adhesion or not.

Statistical studies by Dr. SAITO, Surgical Deparrtment of Nippon Medical School, still show high percentage of post-operative ileus in cases both throughout Japan and in his department. What is more, a greater part of such postoperative ileus is of adhesive nature. It is a matter worthy of retrospection for surgeons that the fact still remains when signs and symptoms, for the most part, became slighter and postoperative infection became less than before the war. Of course many reasons may be considered about that, among which local use of antibiotic substances, especially local administaration of penicillin is the first to be discussed. Therefore, we followed up 31 patients since 1948 who received laparotomy and reoperation, thereafter, on the diagnosis of adhesive ileus in our clinic. No particular relation between adhesive ileus and local use of penicillin was recognized. What could be found was that most of the operators were young and freshmen of our clinic that the operati-

ons required considerably long time.

Based on the clinic fact above mentioned, we cerformed the experimental studies of rabbits as follows.

By rubbing a certain portion of rabbit-colon with gauze, cotton gloves or rubber gloves, adhesion was clearly demonstrated in case handled with the former two after ten days, while little or no adhesion was found in cases manipulated with the latter even when autogeneous blood was instilled.

Immediately after the intraabdominal procedures above mentioned, penicillin solution was used intraabdominally The results were examined after a few days. The cases which were rubbed with rubber gloves and locally adminitered 10,000 u/cc of penicillin showed slight adhesion, while all the cases rubbed with cotton gloves revealed severe adhesion. In the latter, the cases administered 10,000 u/cc of penicillin produced severer adhesion than those treated by 1,000 u/cc of penicillin in the same amount. These findings were also ascertained in histopathological changes. There was no particular relation between pH of penicillin solution and adhesion.

Various changes in the abdominal cavity were also observed ten days after smearing over the intestinal wall with a certain amount of intestinal contents, concurrently with penicillin administration in the abdominal cavity.

The results are as follows :

When a small amount of 100～500 u/cc of penicicillin solution was used,the inflammation remained marked and adhesion was severe in the abdominal cavity. By application of 5,000～10,000 u/cc of penicillin solution or more concentrated solution, inflammation remarkably subsided, although adhesion was found to be considerably marked. Conclusively, a fairly large amount of intraabdominal administration of 1,000～2,000 u/cc was not only followed by less adhesion, but also effective to the inflammation itself. In such cases of experimental peritonitis, intramuscular injection of penicillin in a large amount i.e. 10,000～50,000 u made both intraabdominal inflammation and adhesion slight. Also in the experimental peritonitis due to mixed solution of *Staphylococcus* and *Bacillus coli*, best results were obtained by administrating 5 cc of 2,000 u/cc solution in the abdominal cavity. The local use of penicillin in the normal abdominal cavity induced no adhesion at all if intraabdominal procedures were not applied.

Now we are studying the problem of adhesion from the view point of the permeability of the peritoneal capillary.

Judging from precipitation of heterogenous protein and coloring reaction tested with a dye, the severer the inflammation, the higher is the permeability of capillary.

On the contrary, it is inclined to decrease as inflammation becomes slighter by penicillin administration. But reasonable concentration and dosage to such inflammation have not been determined in these experiments.

According to these clinical and experimental studies, it is most necessary to wear rubber gloves in laparotomy with careful intraabdominal procedures to prevent excessive manipulation. It is also reasonable to administer an appropriate amount of penicillin solution in low concentration *i. e.* 1,000～2,000 u/cc or to inject it in a large amount in order to reduce inflammation and prevent adhesion as much as possible.

STUDIES ON ANTITUMOR SUBSTANCES PRODUCED BY ACTINOMYCETES

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About four years ago, when I undertook the study of searching antitumor substances in culture broths of actinomycetes, it was the principal problem whether there would exist antitumor substances among products of actinomycetes. Studies in recent three years, however, have confirmed that actinomycetes are the richest source of substances exhibiting antitumor activity. Following substances have been reported to exhibit antitumor activities: actinomycin (ROKUJO and other, 1949 ; STOCK and others, 1953), actinomycin C (HACKMANN, 1953), sarkomycin (UMEZAWA and others, 1953), cycloheximide (STOCK and others, 1953), oxytetracycline (STOCK and others, 1953), puromycin (TROY and others, 1953), azaserine (STOCK, EHRLICH and others, 1954), carzinophilin (HATA and others, 1954), No. 289 substance, caryomycin and actinoleukin (UMEZAWA and others, 1953).

The rapid progress in this field also depends upon the recent development of experimental mouse tumors. Four years ago, YOSHIDA sarcoma of rat was only available in this country. YAMAMOTO, one of my colleagues, studies the course of EHRLICH carcinoma inoculated to several kinds of highly inbred mice in this country, and now this mouse tumor is most widely used for the screening of antitumor substances. Moreover, according to YAMAMOTO and SOUTHAM's contributions, mouse sarcoma 180 became also available.

Thus, with the first success of the screening studies made by my group, and with the aid of the experimental mouse tumor, the studies of antitumor substances are becoming the chief subject of antibiotic researchers in this country.

In this report, I show the experimental results on sarkomycin, actinomycin, and screening of an-

titumor substances which have been made by my group chiefly in the recent year.

On Sarkomycin

1. Chemical studies :

Sarkomycin has been obtained by the solvent process. It is extracted from the culture liquid to ethyl acetate at pH 3.0, and after evaporation of the solvent it is dissolved in water, and again it is transferred into ethyl acetate and it is concentrated to syrup by vacuum evaporation. Further purification from this syrup was studied. Carbon chromatography with ethyl acetate was found to be suitable for the purification. Active fractions obtained by the carbon chromatography were collected and evaporated under vacuum. The syrup thus ob tained was dissolved in water and extracted with chloroform. Then, it was found that there were at least two kinds of sarkomycins, one was transferred into the solvent and another left in the aqueous layer. The latter was studied by 60 tubes, countercurrent distribution with ethyl acetatetartarate buffer of pH 4.0. The weight of the residue, milliliter of 1/100 N-NaOH necessary for neutralization, anthrone value (sarkomycins gave positive anthrone reaction) and antibacterial activity were examined in each tube. Then, sarkomycin distributed around the tube No. 38. It was named sarkomycin A. The part which was transferred into chloroform was also studied by the countercurrent distribution. Two peaks were found, one around the tube No. 43, and another around the tube No. 55. The former was named sarkomycin B and the latter C.

Sarkomycin A, B and C were colorless liquids. $[\alpha]_D^{20}$; A, -57° ; B, -47°; (C, -33°). Anal. : A: C, 55.07; H, 6.22; B:C, 54.19; H, 5.94; C:C, 59:47; H, 6.94 ; (N, 0.94). No nitrogen. A and B had no maxima in ultraviolet absorption. They has only strong end absorptions. C had a maximum at 27 mμ. Equivalent found by titration: A, 167-182; B, 131-171. They all gave red color with anthrone reagent and black precipitates with Tollens reagents.

When the solvent was lightly removed by vacuum evaporation, they had the following potencies : A, 3.6 mg*/mg, B, 3.5mg*/mg; C, 1.2mg*/mg. In the case when the solvent was forcedly removed, A was 2.4 mg*/mg, B 1.9 mg*/mg and C 0.58 mg*/mg. Minimum effective daily dose by the intraperitoneal injection for the inhibition of ascites type of EHRLICH carcinoma was 1.25 mg*/mouse in the case of A, and 2.5 mg* in the case of B. The antitumor effect of C was weaker than that of B. However, they all induced the desquamation of HeLa cells in tissue culture when they were added to the medium at 30-60 mcg*/ml.

From purified sarkomycin A and B their 2,4-dinitrophenyl hydrozones were prepared. The hydrogenated product of B was obtained by catalytic hydrogenation, but it lost both antibacterial and antitumor activities.

During the purification studies crystalline substance was obtained. Colorless crystals. M. P. : 169 C. $C_8H_{10}O_4$. U. V. : a maximum at 292 mμ, E (1%, 1 cm) = 2.9. It had one carboxyl, one carbonyl (by 2,4-dinitrophenylhydrozone), and one hydroxyl (infrared spectrum). Daily intraperitoneal injections of 2.5 mg inhibited only the ascites increase. This substance was considered to be related with sarkomycin.

In the previous report I described the existence of sulfur in crude sarkomycin. The analytical data of sarkomycin purified by carbon chromatography indicated the existence of sulfur at 1.46%. It should be determined in future, whether there is kinds of sarkomycin containing sulfur.

2. Effect of sarkomycin on Ehrlich carcinoma of mice:

In order to avoid the fractuation of results, we studied the experimental conditions of ascites type of EHRLICH carcinoma. A highly indred mice ddN was used. Since the salt content of feed was found to influence the course of the ascites increase, instead of water, physiological saline was supplied to the mice during the experimental period. In the case when the tumor cells obtained from the mice 10~15 days after the inocula tion were inoculated, the ascites increase was slower than that in mice inoculated with tumor cells obtained 7~9 days after the inoculation. Therefore, in all experiments the cells obtained 7~9 days after the inoculation were used. In mice of various ages ascites tumor was found to grow, but ascites increase was most marked in the mice weighing about 20 g and grown for about 5 weeks after the birth. Therefore, mice of this age were used. In general, one million tumor cells were inoculated. For quantitative determination of the antitumor effect of sarkomycin, sorkomycin was intraperitoneally injected to mice in 6 hours after the inoculation, and thereafter it was daily injected. Minimum effective daily dose of sarkomycin used for clinical experiments was 1.25~2.5 mg*/mouse.

The summarized result of 51 experiments testing sarkomycin syrup indicated that 62.5% of the treated mice survived 30 days after the inoculation, while all untreated mice died in 12—24 days (98% of mice died in 12—20 days). The summarized result of 77 experiments testing sarkomycin sodium obtained by freeze-drying indicated that 64% of the treated mice survived 30 days after the inoculation, while all untreated mice died in 11~28 days (98% of mice

died in 11~21 days). The body weight decrease of mice injected with sarkomycin syrup was very slight, but that of mice injected with freeze-dried sarkomycin sodium was marked. It suggested that toxic impurities increased during the freeze-drying.

It was reported that the intravenous injection of sarkomycin to ascites type of EHRLICH carcinoma was as effective as the intraperitoneal injection. Also, it was found that, if the intraperitoneal injections of sarkomycin was started in 72 hours after the inoculation, it inhibited the ascites increase and prolonged the survival period. The daily intraperitoneal injection of sarkomycin which was started 24 hours after the subcutaneous inoculation of tumor cells made the size increase of the subcutaneous tumor slower. After 9~15days' treatment, the mean weight of the solid tumors of mice treated was about a half of that of mice untreated.

3. The effect of sarkomycin on other experimental animal tumors:

YAMAMOTO recently confirmed that the daily intraperitoneal injections of sarkomycin which were started 24 hours after the inoculation inhibited the ascites increase of mice which had been intraperitoneally inoculated with one million cells of mouse sarcoma 180 and prolonged the survival period.

According to OBOSHI and others, sarkomyin induced destructions of cells of HIROSAKI sarcoma of rat, but not prolonged the survival period. However, sarkomycin damaged the cells of USUBUCHI sarcoma of rat and prolonged the survival period. Nitrogen mustard N oxide prolonged the survival period of rats bearing HIROSAKI sarcoma, but not USUBUCHI sarcoma. It was unfortunate that the results reported by STOCK and others fractuated among the samples of sarkomycin tested. They failed to get positive results with one sample, but they got the following results with another sample: sarcoma 180 of fluid form ?; mammary adenocarcinoma E 0771 −; BASCHFORD carcinoma 63 ±; EHRLICH carcinoma solid form, ±, EHRLICH carcinoma fluid form, ±; Krebs 2, fluid, ±; carcinoma 1025, ±; Mecca lyphosarcoma;−; RC carcinoma, −; Walker carcinosarcoma 256, ±; FLEXNER-JOBLING rat carcinoma, −.

4. Chronic toxicity of sarkomycin to animals:

It has been reported that LD_{50} was 1,000-1,800 mg*/kg by *i.v.* to mice and 520-800 mg*/kg by s.c. to mice. Daily intraperitoneal injections of 200 mg*/kg to monkey did not cause ay toxicities. It was found also that daily intraperitoneal injections of 10 mg* (per mouse) for thirty days did not cause any changes in organs except light peritonitis, when the mice were killed and histologically studied. The same was found in the case of daily intraperitoneal injection of 40 mg* to rats for thirty days. It is the chracteristic of sarkomycin that it has no chronic toxicity.

5. The influence of sulfhydryl compounds on sarkomycin:

Cystein lowered the antibacterial effect and toxicity of sarkomycin, but not lowered the anti-HeLa cell effect. Glutathione lowered the toxicity but not the antibacterial effect and anii-HeLa cell effect. It will be possible to find sulfhydryl compound to be useful for the purpose to lower the toxicity of sarkomycin. There is a possibility that such substance is contained in the culture liquid of sarkomycin-producing stretomyces. Such a substance is also considered to be present in blood. The addition of whole blood lowered the antibacterial effect and the toxicity, but it did not influence the antitumor effect of sarkomycin on ascites type of EHRLICH carcinoma.

6. Antitumor effects of synthetic antitumor substances:

YAMAMOTO and YAMAOKA reported experiments testing nitrogen mustard N oxide, TSPA and 6-mercaptopurine. Nitrogen mustard N oxide was effective to YOSHIDA sarcoma of rat to which sarkomycin was almost inactive, but inactive to EHRLICH carcinoma. TSPA inhibited the growth of the subcutaneous solid tumor of EHRLICH carcinoma, but in the case of the ascites type it only inhibited the ascites increase, but it did not prolong the survival period. 6-Mercaptopurine inhibited the ascites increase of mice bearing EHRLICH carcinoma and prolonged the survival period. If 6-mercaptopurine and sarkomycin are compared, the frequency of the occurences of subcutaneous tumors, which grew at the site of the injection for the inoculation of the survived mice, was lower in the case of 6-mercaptopurine than in the case of sarkomycin. It will be interesting to study the effect of the combination of sarkomycin and 6-mercaptopurine, since sulfhydryl compounds lowered the toxicity of sarkomycin. We made an experiment with azaserine. Azaserine was effective to the subcutaneously inoculated EHRLICH carcinoma, but it did not prolong the survival period of mice bearing EHRLICH carcinoma.

On Actinomycin

Actinomycin which was prepared by our group had the following characters: M.P., 248-250C(dec.); maxima at 244mμ and 445mμ ; $[\alpha]_D^{10}$, −323°(C, 0.2 in ethanol); acid hydrolysis : threonine, sarkosine, proline, valine, N-methylvaline; one spot by ring daper chromatography; produced by *S. flavus*.

LD_{50} to mice was 0.68mg/kg by *i.v.* and 0.65mg /kg by *i.p.* to mice ; 0.59 mg/kg by *i.v.* and 0.53 mg/kg by *i.p.* to rats. The tolerable daily dose, when the daily intraperitoneal injection to mice was continued for 30 days, was 12.5mcg/kg for 20 days

to three dogs did not cause toxic signs. But daily intravenous injections of 8mcg/kg for 10 days caused albuminuria and light decrease of liver functions. Daily intravenous injection of 16 mcg/kg for 5 days caused the death of all dogs. It suggested that the safety daily dose might be 2~4 mcg/kg.

Daily intraperitoneal injections of 0.125~0.25 mcg to mice inhibited the ascites increase and prolonged the survival period of mice bearing EHRLICH carcinoma, when the treatment was started in 24 hours after the inoculation. Also, daily intraperitoneal injections of 0.25 mcg to mice inhibited the growth of the subcutaneously inoculated EHRLICH carcinoma. It prolonged also the survival period of rat bearing YOSHIDA sarcoma. It caused the desquamation of HeLa cells when it was added at 0.1 mcg/ml. Actinomycin, though it is very toxic, is an interesting antitumor substance, for it exhibited antitumor effects to all kinds of experimental animal tumors ever tested.

On Screening of Antitumor Substances

I can present here four screening methods. (1) YOSHIDA sarcoma of rat is used. Three or four days after the inoculation, the smear of ascites is examined, and the growth of tumors is confirmed. Then, 1 ml of the culture liquid is intraperitoneally injected, and 2, 4, 6, 12, 24 hours later the smear of ascites is made, and the effect on tumor cells is microscopically studied. Thereafter, daily 1 ml of the culture liquid is intraperitoneally injected. We used this method three years ago. Among 262 streptomyces tested, the cell effect was positive in 35 strains, the culture liquids of 4 strains prolonged the survival period. (2) Ascites type of EHRLICH carcinoma is used. The culture liquid is daily injected to mice to which tumor cells were inoculated, and the influence on ascites increase and the survival period is examined. 1–5 % of soil actinomycetes gave positive result. But it is 0.4 % which produced constantly the antitumor substances. The production of other strains fractuated, and it was most time-consuming work to get the stable subcultures. The classification of the strains which produced antitumor substances have been studied. (3) EHRLICH carcinoma or mouse sarcoma 180 is used. Tumor cells are inoculated subcutaneously, and the culture liquid is intraperitoneally daily injected, and the increase of the size is examined.

It is considered that the different methods of the screening may give different kinds of antitumor substances. Cycloheximide (actidione) was reported by STOCK and other to inhibit the growth of the subcutaneous tumor of mouse sarcoma 180. But I found that its daily injection to mice bearing EHRLICH carcinoma inhibited the ascites increase during the injection period, but it did not prolong the survival period. That is, it gave negative result to the ascites type of EHRLICH carcinoma. During the screenning we met the same things. The strains, culture liquid of which inhibited only the ascites increase of mice bearing EHRLICH carcinoma, have been removed from the further studies. As already described, TSPA and azaserine were effective on the subcutaneous solid tumor, but not on ascites type of EHRLICH carcinoma.

Recently we tested the culture liquid of soil actinomycetes for the destruction or the inhibition of HeLa cells in the tissue culture. The result was not completely parallel with that obtained by using ascites type of EHRLICH carcinoma. However, this method was useful for determination of the concentration of antitumor substances in the extracts.

Conclusion

Sarkomycin can be said the first antitumor substance found by the systematic screening of soil actinomycetes. Further systematic screening has cleared the existence of many antitumor substances in actinomycetes cultures. It is important not only from the biologically fundamental point, but also from the possibility of their usefulness for cancer chemotherapy.

STUDIES OF PENICILLIN ALLERGY

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1. Incidence of penicillin allergy

According to various investigators penicillin allergy occurs in 2–5 % of all patients receiving penicillin and remarkably higher in patients with dermatological diseases. In our clinic of internal medicine various types of penicillin allergy were observed in 2.9 % of patients which received penicillin therapy from Jan. 1954 to Mar. 1955.

Incidence of allergy in the employees of the penicillin factories was also studied. Occurrence of penicillin allergy in total 1833 employees of 11 factories was 11.34 %, which is higher than in hospital. The occurrence was highest in the employees preparing drugs, who are much exposed to penicillin by skin contact or inhalation, while that in business section and tank culture section is 10 % and 8.36 % respectively. The occurrence in persons having allergic histories is remarkably higher (20.13 %) than those who have no history of allergic disease.

2. Penicillin anaphylaxis

Since 1950 more than ten cases of penicillin anaphylaxis were reported in Japan. The author has sent the questionaries of anaphylaxis to many doctors in various parts of Japan. Among 22 cases, in total, of anaplhyaxis, aging from 5 to 25 years old, exclusively female except one male, about two third of cases have histories of allergic diseases

such as bronchial asthma, urticaria, eczema, drug allergy and alimentary allergy.

Anaphylaxis occurs exclusively in those who had had the same antibiotic therapy previously on several occasions. Usually anaphylaxis follows first injection of new course of antibiotic therapy, only one case was induced with streptomycin on 11 th day of continuous therapy.

Classification of antibiotics and preparations is as follows: streptomycin 1, aqueous suspension of procaine penicillin 8, procaine penicillin in oil 7, aqueous solution of crystalline penicillin G 4, penicillin of which name was not answered 2.

Route of administration inducing anaphylaxis was intramuscular in all cases except one who received 25 units of penicillin G by intracutaneous injection for allergy test.

Cardinal symptom of anaphylaxis due to antibiotics was acute circulatory collaps, individually modified by various allergic symptoms, such as angioneurotic edem, urticaria, asthma-like dyspnea, asphyxia, nausea, vomiting, abdominal pain, diarrhea, labour-pain with and without genital hemorhage, etc.

3. Autopsis of fatal penicillin anaphylaxis

3 cases at Medicolegal Institute of Osaka University and 6 cases at Institute of Medical Examination of Tokyo City were autopsied.

Congestions and parenchymal hemorhages in various organs due to acute circulatory collaps were observed in all cases. As the characteristic changes for anaphylaxis presented by several investigators, edema of glottis and upper respiratory tract, pulmonary emphysema, intensive mucoid secretion of bronchi were found in about one half of all cases. These findings were also confirmed histologically.

4. Skin test for diagnosis of penicillin allergy

The author tested patients initially by the scratch test (one drop of penicillin G 500 u/ml in normal saline), for one case of anaphylaxis following intracutaneous test (0.05ml of 500u/ml penicillin G in saline) was experienced.

Intensive positive immediate reaction (15-30min.) to the scratch or intracutaneous test was observed in most of cases having severe immediate type of allergy including anaphylaxis. The positive delayed reaction, i. e. tuberculin type of reaction, was observed rather frequently in the case of delayed type of allergy. Only few cases had positive reactions to intracut aneous test of procaine, extracts of peanut, sesame and camellia japonica, those oils of latter three were used for deposit penicillin.

A certain correlation between penicillin and trichophytin by intracutaneous test was observed in non allergic persons ($r:0.4$, $P<0.05$), but in patients of penicillin allergy. This fact suggests that penicillin allergy would be a drug allergy in nature and would have a crossed allergy to fungi in few exceptional cases.

5. Passitive transfer by PRAUSNITZ-KÜSTNER's method

Sensitizing activity to normal human skin against penicillin was studied with serums of 31 cases of penicillin allergy comparing with normal controls. Statistically significant difference was observed between these two groups. Therefore we could assume the existence of allergic antibody in the patients of penicillin allergy.

6. Leukocytolysis phenomenon by FAVOUR's method

A new method to detect antibody of tuberculin in the patients of tuberculosis was devised by FAVOUR using leukocytolysis phenomenon, which follows antigen antibody reaction upon leukocytes *in vitro*. Leukocytes changes after addition of penicillin to heparinized blood from 37 patients of penicillin allergy are -11.4% (mean value) after one hour's incubation and -15.8% after two hours. Specificity of this phenomenon should be criticized from now ont for slight decreases occurred after addition of streptomycin to the same serums of penicillin allergy (-4.6 % after an hour, -8.6 % after two hours).

7. Experimental allergy with penicillin

Intravenous challenge, SCHULTZ-DALE's phenomenon (isolated intestine), histamin release phenomenon from lung were examined in guinea pigs, previously injected penicillin and procaine penicillin with and without human or horse serum. All results were negative. We could not sensitize guinea pigs with penicillin except extract of broth culture of penicillium which had no crossed antigenicity to penicillin G.

8. Allergic reaction to penicillin O, penicillin BT and degradation derivatives of penicillin

Intracutaneous test of penicillin O, penicillin BT, penicilloic acid, penillic acid and penicillenic acid was carried out in patients of penicillin allergy. Patterns of intensity of reactions to these compounds are different in each individuals. Therefore, determinant groups of penicillin as allergen are supposed to be different in each case. And there is no general principle that penicillin O or penicillin BT is available to hypoallergic purpose unless safety of these derivatives were ascertained in each case.

9. Specific desensitization

Among 6 cases administered penicillin for specific desensitization we have only one successful case, who was highly sensitive to the dose below 100 u and received incresasing doses of penicillin 2-3 times weekly and at last 300,000 u without any allergic reaction aftrer 20 months. The other patients gave up the therapy after several months due to allergic reactions.