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EFFECT OF THE PREOPERATIVE IRRADIATION AND SUCUTANEOUSLY CANCER TISSUE IMPLANTATION ON THE ANTITUMOR ANTIBODY LEVELS REPORT I.

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癌の術前照射並に摘出癌組織の治療的移植の抗癌抗体価に及ぼす影響

（第 1 報）

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血清中の抗癌体価は術前照射並に摘出癌組織の治療的移値によつて昂められ，術後照射によつても昂める事が出来る．

之によつて"癌の放射線治療は癌細胞破壊療法と癌免疫療法より成り立つている"と云う私の仮説を実証した．超遠心法によつて自家抗原は自癌組織の可溶性成分に存在している事を確認したが，この事実は将来の免疫学的治療に重要な示唆を与えている．

Summary

Antibody titer in the serum were raised serologically by the preoperative irradiation and it further could be raised and sustained by therapeutic implantation of the extirpated cancer tissue. Also, it could be raised by the post-operative irradiation.

The super-centrifuge method has determined the antigen of auto-cancer tissue is being existed in the soluble part but not in the insoluble part, and this fact gave us an important indication to the future treatment.

Introduction

I made the following conclusion for radiotherapy based on my past many years' clinical experiences to the cancer treatment. That is, the cancer cells can be destroyed by the first effect, and in the secondary effect, the destroyed products of the cells, so-called the Caspari's necrohormones, have turned to antigen which induces the auto-immunity for individual and the tumor could further be destroyed immunologically by them.

With this hypothesis, I tried to adjust the necrohormones by breaking down a portion of tumor according to " Small Irradiation Field (4×4 cm.) " method.

My latest method to the cancer treatment is to raise the antitumor antibody titer by preoperative irradiation for the safety of surgical operation and to sustain this titer by implanting a portion of the extirpated cancer tissue (5-10 gr.).

This method was already introduced in the Hiroshima Medical Journal and Nippon Acta Radiologica under the title of "Study on the Pre-and Post-operative Irradiation to Cancer".

However, my hypothesis could not be said as fully satisfactory unless the antitumor antibody were proved in the indvidual body of cancer patient even though it was clinically corroborated. Therefore, I had contemplated to prove that the antibody titer could be raised against antigen, which was produced from the cancer tissue by radiotherapy, and furthermore, I tried to prove that this titer could not be raised only by therapeutic implantation but it sustains the rise as well.

Materials and Methods

Patient: The patients were diagnosed as the stomach cancer or the cancer of other organs. They underwent the preoperative irradiation by my method and were succeeded the radical operation.

Preparation of Tumor Tissue:— Tumor tissue was obtained from each patient at biopsy or surgery. A portion of cancer tissue (5-10 gr.) was removed aseptically and immediately preserved in a frozen state under 30°C. below zero.

Approx. 0.5-1 gr. of the tumor tissue was taken out from the gynaecological patients (2 cases) by the biopsy prior to irradiation.

Preparation of Tumor Homogenate:—A portion of the preserved frozen cancer tissue (1 gr.) was taken out and minced, to which add 10 cc. of 0.14M NaCl. Then, it was placed in a glass container and set on the homogenizer and destroyed by cooling with the dry ice.

Making the Antigen:—Above homogenate was centrifuged at 3,000 r.p.m. for five minutes and the supernatant serum was obtained. This solution is called 'A' solution. Next, add 10 cc of H_2O (pH 7.2) to the remnants of 'A' solution and stir for thirty minutes on a magnetic stirrer, and the supernatant serum was obtained after five minutes centrifuged at 3,000 r.p.m. This solution is called 'B' solution. These 'A and 'B' solutions were used as the auto-antigen for immunity test.

Above work was done in a refrigerating room under the temperature of 0°C. at the Department of Biochemistry of Hiroshima Medical School, directed by Prof. Dr. Kazuno and Ass't Prof. Okuda.

Obtaining the Sera of Patient:—Approx. 5-6 cc. of blood was withdrawn from the vein of patient in accordance with a strict schedule, then the serum was isolated and preserved in a freezer of 0° C. and was used as occasion demanded.

Immune Test:—To re-examine the methods of Graham and Graham, Finney, Byers and Wilson, I had tried the following methods:—

(a). Ring precipitation test

(b). Complement fixation test

(c). Tannic acid treated sheep erythrocyte agglutination test

These tests were conducted at Serological Testing Room of Yamasaki Dispensary, (Director Dr. Yamasaki M.D) Inarimachi, Hiroshima.

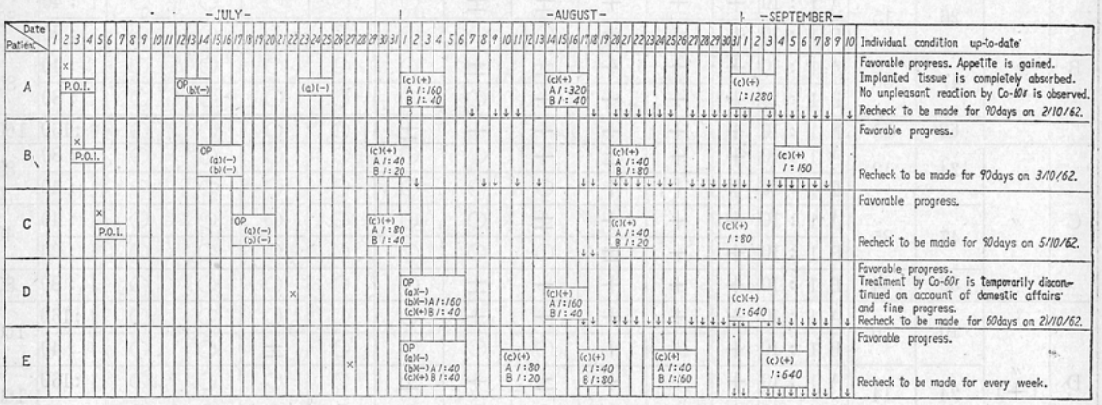
I used the 'A' solution as the antigen for the above method (a), used 'B' solution for the method (b), and 'A' and 'B' solutions were used for the method (c). Normal and other cancer patients' sera were used for the serological controls.

Table 1, shows that a schedule for the four cases of stomach cancer patients and one case of intestinal cancer patient whom conducted with the preoperative irradiation and their radical operation were succeeded.

In Table 1, (a) represents the ring precipitation test, (b) represents the complement fixation test, and (c) represents the tannic acid treated sheep erythrocyte agglutination test.

Table 1

Table 1. Schedule of Our Experiment and Its Results.



Remarks:

X—Preoperative irradiation
Op—Operation
(a)—Ring precipitation test
(b)—Complement fixation test
(c)—Tannic acid treated sheep erythrocyte agglutination test

— Patients —

A—'A' Solution	A—Mr. Higuchi (51)	B—Mrs. Kanemoto (62)
B—'B' Solution	C—Mr. Mitani (56)	D—Mr. Watayoshi (54)
	E—Mrs. Okada (42)	

The result of both (a) and (b) tests showed negative, and I found some unsatisfaction in the preparation upon experiment. So, I immediately discontinued the above two methods and the method (c) was practised.

Its result is shown in Table 2.

Table 2

As it is apparent from this Table, the titer is shown rise in each case by the preoperative irradiation. The titer rise of the patients A and D whom underwent the therapeutic implantation showed the titer level which could not be seen in the patients B, C, and E after operation.

Table 2 Effect of Preoperative Irradiation and Subcutaneousy Cancer Tissue Implantation on the Antitumor Antibody Levels.

Patient	diagnosis and Implantation	After Preoperative Irradiation	After Operation	S.D. / A.D.	1 / 1	2 / 2	3 / 4	4 / 8	5 / 16	6 / 32	7 / 64	8 / 128	9 / 256	10 / 512	Titer	Results
A	stomachcancer BII Adenocarzinom (+)	31	21	A 1 : 10	+	+	+	±	+	○	○	○	○	○	1 :160	160
				B 1 : 10	+	+	+	±	±	○	○	○	○	○	1 : 40	
		44	35	A 1 : 10	+	+	+	+	+	+	±	○	○	○	1 :320	320
				B 1 : 10	+	+	+	±	○	○	○	○	○	○	1 : 40	
		60	51	B 1 : 10	+	+	±	+	+	+	±	+	○	○	1:1280	1280
B	〃	26	15	A 1 : 10	+	+	+	±	±	○	○	○	○	○	1 : 40	80
				B 1 : 10	+	+	±	○	○	○	○	○	○	○	1 : 20	
		47	36	A 1 : 10	+	+	+	±	±	○	○	○	○	○	1 : 40	80
				B 1 : 10	+	+	+	+	○	○	○	○	○	○	1 : 80	
		62	51	B 1 : 10	+	+	+	+	+	±	○	○	○	○	1 :160	160
C	〃	24	12	A 1 : 10	+	+	±	+	○	○	○	○	○	○	1 : 80	80
				B 1 : 10	+	+	+	○	○	○	○	○	○	○	1 : 40	
		47	35	A 1 : 10	+	+	+	±	○	○	○	○	○	○	1 : 40	40
				B 1 : 10	+	+	±	○	○	○	○	○	○	○	1 : 20	
		56	44	B 1 : 10	+	+	+	+	±	○	○	○	○	○	1 : 80	80
D	〃 (+)	10	0	A 1 : 10	+	+	+	+	+	○	○	○	○	○	1 :160	160
				B 1 : 10	+	+	+	○	○	○	○	±	○	○	1 : 40	
		24	14	A 1 : 10	+	+	+	±	+	±	○	○	○	○	1 :160	160
				B 1 : 10	+	±	+	○	○	○	○	○	○	○	1 : 40	
		41	31	B 1 : 10	+	+	+	±	±	+	+	±	±	○	1 :640	640
E	Sigmodium cancer Adenocarzinom	5	0	A 1 : 10	+	+	+	±	○	○	○	○	○	○	1 : 40	40
				B 1 : 10	+	+	+	○	○	○	○	○	○	○	1 : 40	
		15	10	A 1 : 10	+	+	+	+	±	○	○	○	○	○	1 : 80	80
				B 1 : 10	+	+	○	○	○	○	○	○	○	○	1 : 20	
		22	17	A 1 : 10	+	+	+	○	○	○	○	○	○	○	1 : 40	80
				B 1 : 10	+	+	±	+	±	○	○	○	○	○	1 : 80	
		29	24	A 1 : 10	+	+	+	±	○	○	○	○	○	○	1 : 40	160
				B 1 : 10	+	+	+	+	+	○	○	○	○	○	1 :160	
		39	34	B 1 : 10	+	+	+	±	±	+	+	±	○	○	1 :640	640

Remarks:- S.D.-Serum Dilution, A.D-Antigen Dilution, A-'A' Antigen Solution, B-'B' Antigen Salution

In each above patient, it was unable to recognize due to inadequacy of the site of cancer whether they have the antibody or not against auto-cancer tissue before the preoperative irradiation.

Accordingly, if we try to select any suitable case, then we should select either from the gynaecological patients or from the patients with skin cancer which will be easy to conduct the biopsy or surgery. In this respect, the stomach and sigmoid cancer is unsuitable.

To recognize whether the antibody is existed or not in the cancer patients, I especially selected the cervix cancer among the gynaecological diseases and the following results were obtained by the biopsies. Afterwards, this patient was conducted the therapy by the radium and X-ray, and I am now intending to see the further process for this patient how the antitumor antibody titer will be raised after the completion of full dose.

Table 3, below, indicates the titer of two cervix cancer patients without any treatment which showed as 1:4, 1:20 and the latter was observed the necrose in the tumor.

Table 3 Cervix Cancer Without Treatment (Necrose (—))

Patient F	1	2	3	4	5	6	7	8	9	10	Titer
Serum Dilution	1	2	4	8	16	32	64	128	256	512	
A	+	+	+	±	○	○	○	○	○	○	1 : 4
A	○	○	○	○	○	○	○	○	○	○	neg

Antigen Dilution 1 : 1 Titer A—1 : 4, B—neg

Cervix Cancer Without Treatment (Necrose (+))

Patient H	1	2	3	4	5	6	7	8	9	10	Titer
Serum Dilution	1	2	4	8	16	32	64	128	256	512	
A	+	+	±	○	○	○	○	○	○	○	1 : 20
B	+	+	○	○	○	○	○	○	○	○	1 : 20

Antigen Dilution 1 : 10 Titer A—1 : 20, B—1 : 20

As it is apparent from the above Table, the antibody titer of the cancer patient without irradiation indicated 1:4 or 1:20 which apparently showed the difference from the irradiated group.

Next, the result was evaluated as the controls, as is shown in Table 4, and the antigen of patient E was used for the serum of stomach cancer patient J.

Antigen of patient B was used to the serum of the 6-month pregnant woman.

As the serological controls, several combinations were conducted by using the

Table 4 Controls

Patient	Sex	Age	Diagnosis	1	2	3	4	5	6	7	8	9	10	Antibody Titer
J	M	56	Stomach Cancer											
Antiserum Dilution				1	2	4	8	16	32	64	128	256	512	
Patient Antigen A				+	+	±	±	○	○	○	○	○	○	1 : 20
〃 B				○	○	○	○	○	○	○	○	○	○	Neg.

Antigen dilution 1 : 10

Controls For 6-Month Pregnant Woman

Patient	Sex	Age	Diagnosis	1	2	3	4	5	6	7	8	9	10	Antibody Titer
K	F	30	Pregnancy											
Antiserum Dilution				1	2	4	8	16	32	64	128	256	512	
Patient Antigen A				+	+	±	±	○	○	○	○	○	○	1 : 40
〃 B				+	+	±	○	○	○	○	○	○	○	1 : 20

Antigen dilution 1 : 10

tanned cells in literature, but I used the tanned cells with antigen (E and B patient) at my control test. Therefore, I considered that the titer rise such as 1 : 20 or 1 : 40, as shown in the above Table, was observed. However, this fact might also suggest that there is some common antigen in the antigens which I used at my control test. Anyway, this titer is entirely incomparable from the serum titer of irradiated patients.

The doubt that everyone might have when the above results were looked through will be that there is some difference of the antibody titer in 'A' and 'B' solutions against same serum.

To solve this question, we should determine whether the antigen is soluble or insoluble to the saline.

To separate 'A' and 'B' solutions from the homogenate of cancer tissue, I centrifuged at 3,000 r.p.m. for five minutes according to the literature. However, if the separation by this centrifuge method is insufficient, then it is not probable to consider that there exist the antigen both in 'A' and 'B'.

To prove this, I conducted the following experiment. That is, the homogenate of the patient E was separated by the supercentrifuge method (40,000 r.p.m.) and the antigen was obtained.

As it is apparent from the Table 5, the antigen is soluble which mainly exist in 'B' solution but a little in the 'A' solution.

The reason that the 'A' and 'B' solutions were used for the agglutination test was due to the fear ot maldistribution to either one of them, however, it was cleared by this test that the higher titer can be used as the result of the test in the past.

I try to make some review on the results of Graham and Graham and Finney

Table 5. Ultra Centrifuge Test
After completion of pre-operative irradiation: 27 days
After operation: 21 days
Titer 1 week before this test: A 1 : 40, B 1 : 160 at 3,000 r.p.m. 5′
'A' Dilution: 40,000 r.p.m. centvifuged.
'B' Dilution: The remnants of 'A' extracted at HO.

Patient E	1	2	3	4	5	6	7	8	9	10	Titer
Serum Dilution	1	2	4	8	16	32	64	128	256	512	
A	±	±	+	±	+	○	○	○	○	○	1 : 80
B	+	±	+	+	+	+	+	○	○	○	1 : 640

Antigen Dilution: 1 : 10 Titer A 1 : 80, B 1 : 640

et. al. which are noted in the literatures.

It is described that "A standard ring type precipitation test was employed to measure the antibody titer in Group I. The antigen consisted of a saline extract of the patient's homogenized tumor. The patient's serum was used as the antiserum" Finney et. al. used the 'A' solution for this test, however, Graham and Graham used the 'B' solution for the complement fixation test and he reported that the remarkable antibody titer within from 1:16 to 1:128 were observed in the twelve cases out of forty-eight patients.

Finney had attempted the complement fixation test, but he stated that "This test was found to be of little value because of the anti-complementary action of the majority of the tissues tested".

I consider that there is some point to recheck on their experiments since I have determined that the antigen is being existed in the 'B' solution based on the result of my agglutination test, so I am now arranging to try to test the aforementioned (a) and (b) methods simultaneously with the agglutination test.

Conclusion

With my experiment, it was recognized that the autoimmunity for cancer was induced in the individual body by radiotherapy because I found the antitumor antibody in the patient's serum after radiotherapy. Therefore, my hypothesis, that is the radiotherapy is constituted with the destruction treatment of cancer cells and the cancer immunity treatment, was proved as correct. I convince that a problem of biological amplification in radiobiology was solved by this auto-immunity after irradiation.

Accordingly, the anti-cancer state could be accomplished by the preoperative irradiation, the safety for surgical operation could be raised, and the improvement for the treatment could be expected with the rise of antitumor antibody titer by therapeutical implantation of the extirpated cancer tissue and also by the postoperative irradiation.

It is of great advantage in making the future auto-vaccine since the antigen of the auto-immunity is being existed in the soluble site of cancer cell components.

From the results of this experiment, I contemplated the betterment of the irradiation methods; expect the further development for the therapeutical use of the cancer tissue; and also I intend to urge my study what should 1 do to rise the titer and sustain it as long as possible.

Since the number of cases are yet few, I will report by accumulating the cases one by one as well as to develop my study.

Acknowledgement

Upon a conclusion of this manuscript, I wish to express my deepest appreciation to Professor Kazuno and Assistant Professor Okuda of the Department of Biochemistry of Hiroshima Medical School and Dr. Masao Yamasaki, M.D., Director of Yamasaki Dispensary, who gave me the enthusiastic assistance and direction throughout this study. Also I wish to express a heartfelt thanks to Dr. Takeuchi, M.D., the Director of Takeuchi Hospital and Dr. Ueda, M.D., the Director of Ueda Hospital, who offered me the cancer tissues and patients' sera for accomplishing my experiment.

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