Studies on the Static Effect of Solcoseryl on Ehrlich Ascites Cancer Cells

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Ehrlich 腹水癌に対する Solcoseryl の抑制作用の研究

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添田は Ehrlich 腹水癌の癌細胞外液および内液中に病原因子（1961）を発見し，このものゝ電子顕微鏡写真の撮影に成功した．従つて，特異性抗元による免疫療法の研究に着手するに当り，先ず非特異性抗元として，Solcoseryl を用い，Ehrlich 腹水癌に対する抑制作用の研究を行つた．

1） マウスに対する，Ehrlich 腹水癌の感染に要する最少癌細胞数を確かめた．

2） Solcoseryl 20mgを2回マウスの腹腔内に注射し，10^4で攻撃すると，無処置対照群は定型的癌死をとげたが，処置群は8匹中が腹水癌からまぬがれ，健康に生存した．

3） 癌細胞10^6を皮下注射し，固型癌を作つたあと，Solcoseryl 20—40mgのみ皮下注射するよりも，Solcoseryl と Marinamycin（1mg宛）を併用すると，4匹中4匹すべて Tumor の消失が見られ健存した．

4） Solcoseryl 40mgを皮下注射し，26日目に固型癌を作る目的で10^6を皮下注射し，16日目から Solcoseryl の各量5日間を皮下注射すると，4匹中，3匹は固型癌の消失を見健康に生存した．対照群はすべて定型的固型癌死をとげた．

Introduction

Soeda[1] reported on studies of a cancer-inducing factor of Ehrlich's ascites carcinoma at the 34 th. general meeting of Japanese Bacteriological Society on 7th. April, 1961. This factor consists of very minute particles, which were subsequently photographed by the aid of electron microscope by Dr. Kimura et al, IV. Division of Central Research Institute, Hitachi Co., Tokyo (December, 1963). Detail of this finding was published at a clinical meeting held by Teishin General Hospital, Tokyo January, 1964).

We have also reported on a stattic action of SSS[2] (Specific Soluble Substance) on Ehrlich's ascites carcinoma, which is Produced by various species of bacteria and yeasts. We have extracted a polysaccharide fraction from Pseudomonas fluorescens and treated mice with this fraction prior to intraperitoneal implantation of Ehrlich's ascites cancer cells. By this pre-treatment, mice were endowed with resistance to challenge of 10^6 cancer cells.

In the course of serial studies on homologuous immunity against Ehrlich's ascites cancer by means of administration of CIF(Cancer Inducing Factor) as well as on heterologous immunity due to substan-

ces such as SSS, we have tested effect of Solcoseryl[3], a known anti-leucopenic agent, on Ehrlich's ascites cancer cells.

For a quantitative assay of static or immune capacity of CIF or SSS, it is extremely important to select an appropriate challenge dose, namely cancer cell count in an implant. We have carried out several animal experiments to estimate a proper challenge dose. The purpose of this paper is to describe several interesting findings obtained in these experiments.

Experimental Materials

1. Solcoseryl[3] used in our experiments were obtained by courtesy of Tobishi Pharmaceutical K.K. Solcoseryl solution was freeze-dried in our laboratory and 1.0 ml. of this solution was shown to contain 40 mg. of Solcoseryl.

2. Male mice of DDS line weighing from 16 to 22 gm. were used in all experiments.

3. Ehrlich's ascites cancer cells were maintained by serial implantation from mice to mice, and carcinomatous peritoneal fluids of mice were collected 9 to 14 days after implantation for challenge experiments.

To produce solid tumors in animals, 0.1 ml. of peritoneal fluid diluted to contain 10^6 cancer cells was subcutaneously injected in an area on the right side of the back of each mouse.

1. Estimation of a challenge dose (Table 1 and 2)

As shown in Table 1 and 2, carcinomatous peritoneal fluid collected from maintenance mice was serially diluted to contain a cancer-cell count of 10^1 to 10^6 per 0.1 ml, and 0.1 ml of each dilution was intraperitoneally injected to each mouse of 6 resepective groups. All mice developed typical carcinomatous peritonitis and were dead on the respective day as indicated in Tables. Thus it became apparent that such a small dose as ten cancer cells is sufficient to cause a typical pattern of Ehrlich's ascites cancer (EAC). Therefore a dose of one hundred times of 10^2 cancer cells was selected as a challenge dose in the following experiments.

Table 1. Outcome of control mice intra-peritoneally inoculated with variable numbers of Ehrlich's ascites cancer cells

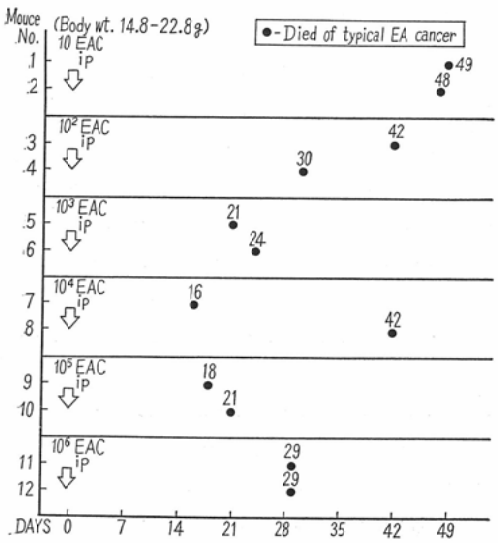
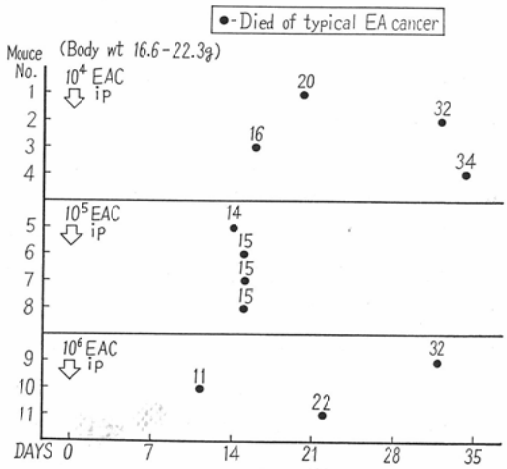


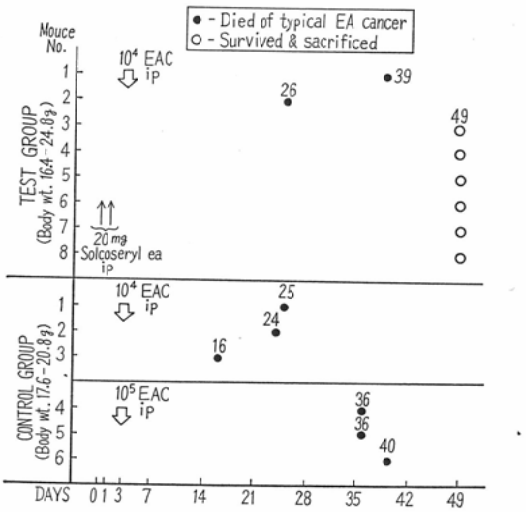
Table 2. Outcome of control mice treated in the same manner as in Table 1.



2. Challenge experiment with 10^4 cancer cells in mice pre-treated with intraperitoneal injection of Solcoseryl (Table 3)

A daily dose of 40 mg of Solcoseryl was intra-peritoneally injected to 8 mice for two days, and on the 3rd. day these mice were intraperitoneally inoculated with 10^4 cancer cells. Two control groups, each of which consisted of three mice, were intraperitoneally inoculated with 10^4 and 10^5 cancer cells respectively, without medication of Solcoseryl. As shown in Table 3, all control mice died from typical ascites cancer within 40 days, while 6 out of 8 mice pre-treated with Solcoseryl remained alive and pathological examination on the 49th. day did not reveal any carcinomatous findings in peritoneal cavities and visceral organs.

Table, 3. Prophylactic effect on Ehrlich's ascites cancer of Solcoseryl intra-peritoneally given to mice one day before inoculation of 10^4 cancer cells (a daily dose of 20 mg for 2 successive days)



3. Challenge experiment with 10^5 cancer cells in mice pre-treated with intraperitoneal injection of Solcoseryl (Table 4)

A daily dose of 20 mg of Solcoseryl was intra-peritoneally injected to 8 mice twice on alternate days, and after 5 days these mice were intra-peritoneally inoculated with 10^5 cancer cells. Six out of 8 mice developed typical patterns of disease in the course between 36th. and 57th. day and died, while remaining two mice survived in healthy condition. Pathological examination on the 63th. day revealed almost no pathological changes and no malingant cells were found in microscopical specimens. Three control mice were all dead of typical ascites cancer within 32 days. Thus two of 8 mice were endowed with some resistance to inoculation of 10^5 cancer cells.

4. Challenge experiment with 10^4 cancen cells in mice pre-treated with subcutaneous injection of Solcoseryl (Table 5)

A daily dose of 20mg. of Solcoseryl was subcutaneously injected to 8 mice twice on alternate days, and on the 5th. day the mice were intra-peritoneally inoculated with 10^4 cancer cells. As showin Table

Table 4. Prophylactic effect on Ehrlich's ascites cancer of Solcoseryl intra-peritoneally given to mice 5 days before inoculation of 10^4 cancer cells (a daily dose of 20 mg twice on the alternate days)

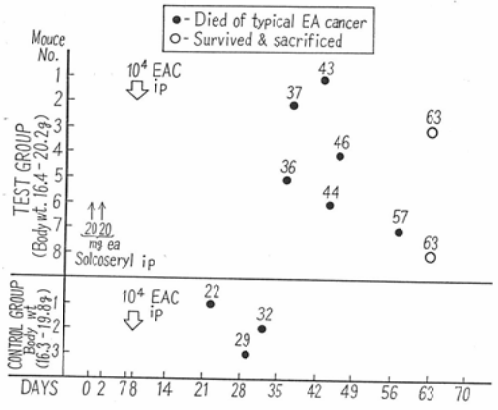
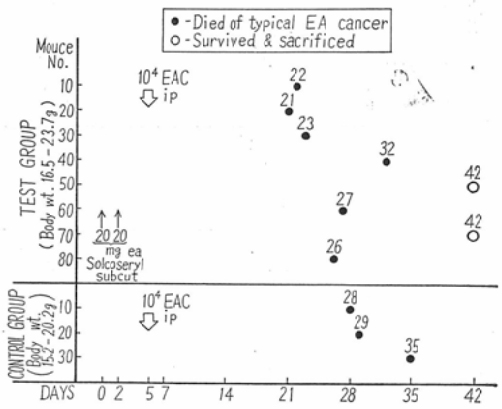


Table 5. Prophylactic effect on Ehrlich's ascites cancer of Solcoseryl subcutaneously given to mice three days prior to inoculation of 10^4 cancer cells (a daily dose of 20 mg twice on the alternate days)



5, three mice in the control group died from typical ascites cancer on the 28th., 29th. and 35th. day respectively. Six out of 8 pretreated mice were dead of typical disease within 32 days, while the other two mice remained in good health until the 42nd day, when pathological examination was performed. No pathological changes were detected.

5. Challenge experiment with 10^5 cancer cells in mice pre-treated with subcutaneous injection of Solcoseryl (Table 6)

Nine mice were divided into three groups. A daily dose of 40mg. of Solcoseryl was subcutaneously injected to 3 mice of the first group for 4 successive days, while respective 20 mg. and 4 mg of Solcoseryl were given to mice of the 2nd. and 3rd. groups in the same manner. On the 13th. day all nine mice were intra-peritoneally inoculated with 10^5 cancer cells. Only one mouse of the first group could escape death of Ehrlich's ascites cancer. Three mice of the control group inoculated with 10^5 cancer cells and the other three mice inoculated with 10^4 cancer cells were all dead of typical ascites cancer.

6. Therapeutic experiment following challenge with Ehrlich's ascites cancer cells (Table 7)

Three mice were inoculated with 10^4 ascites cancer cells and two mice were challenged with 10^5 ascites cancer cells. Thirty minutes after innoculation, 8.0 mg of Solcoseryl was intra-peritoneally administered and a daily dose of 8.0 mg. was injected in the same way for the following four days. Only one mouse challenged with 10^4 cancer cells escaped death of Ehrlich's ascites cancer, while the other mice treated with Solcoseryl and four mice of the control group were all dead of typical ascites cancer. On the 55th. day, pathological examination of the survived mouse was done and no carcinomatous changes were detected in peritoneal cavity and visceral organs. The results are shown in Table 7.

7. Therapeutic experiment of Ehrlich's solid tumor

Peritoneal fluid collected from maintenance mice were adequetly diluted to obtain a density of 10^6 cancer cells per 0.1 ml. Four mice were subcutaneously given 0.1 ml. of this suspension in an area on the right side of the back. Making a start on the 2nd. day, a daily dose of 40 mg. of Solcoseryl was subcutaneously given for 6 successive days and was agina given for two days after a pause of one day. In

Table 6. Prophylactic effect on Ehrlich's ascites cancer of various doses of Solcoseryl subcutaneously given to mice 10 days prior to inoculation of 10^5 cancer cells

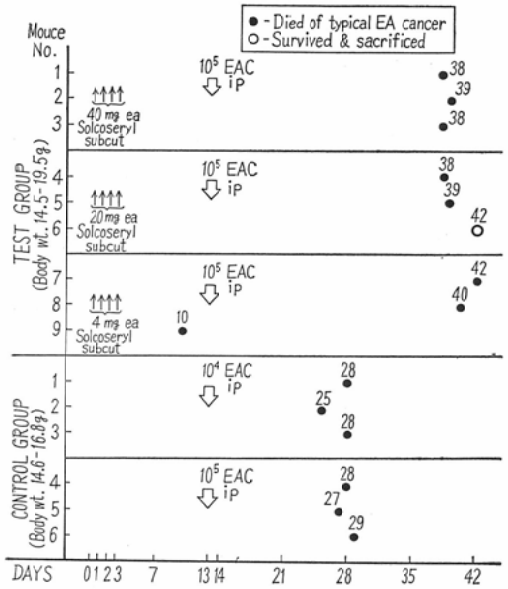
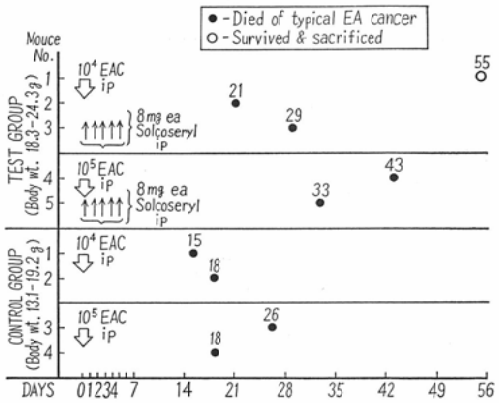


Table 7. Therapeutic effect on Ehrlich's ascites cancer of Solcoseryl intra-peritoneally given to mice immediately after inoculation of 10^4 or 10^5 cancer cells



this experiment, only one mouse could escape death of typical solid tumor. The other mice treated with Solcoseryl and three control mice were all dead of typical solid tumor.

8. Therapeutic experiment of Ehrlich's solid tumor with combined administration of Solcoseryl and Marinamycin (Table 9)

Seven mice were divided into experimental and control groups. Each mouse was subcutaneously given 0.1 ml. of diluted peritoneal fluid containing 10^6 Ehrlich's ascites cancer cells on the right side

Table 8. Therapeutic effect on Ehrlich's solid cancer of Solcoseryl subcutaneously given to mice immediately after subcutaneous inoculation of 10^6 cancer cells

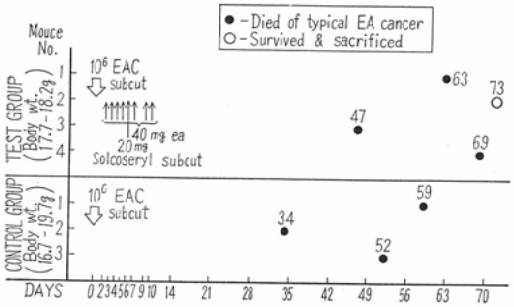
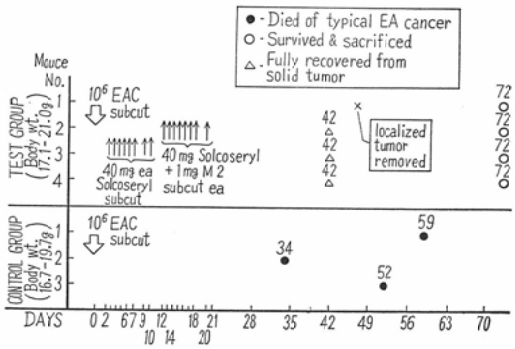


Table 9. Therapeutic effect on Ehrlich's solid cancer of a combined adminsitration of Solcoseryl and Marinamycin to mice immediately after subcutaneous inoculation of 10^5 cancer cells



of the back. Each of 4 mice in the experimental group was subcutaneously given a daily dose of 40 mg. of Solcoseryl for 6 successive days, making a start on the 2nd. day of cancer cell implantation. After a pause of one day, the same dose of Solcoseryl was again injected for 2 days. Because of growth of locally developed tumors, a combined dose of 40mg. Solcoseryl and 1.0 mg. Marinamycin[6][7] was subcutaneously injected for 7 successive days since the 12th. day. Three mice in the control group died from typical Ehrlich's solid tumor on the 34th., 52nd. and 59th. day respectively, but grown tumors in 3 out of 4 experimental mice gradually reduced their size and completely disappeared until the 42nd. day. A grown tumor in another mouse remained almost unchanged in size, but gradually became so movable from the surrounding tissues that it was very easily resected on the 47th. day. Since then no more malignant signs were observed in this case.

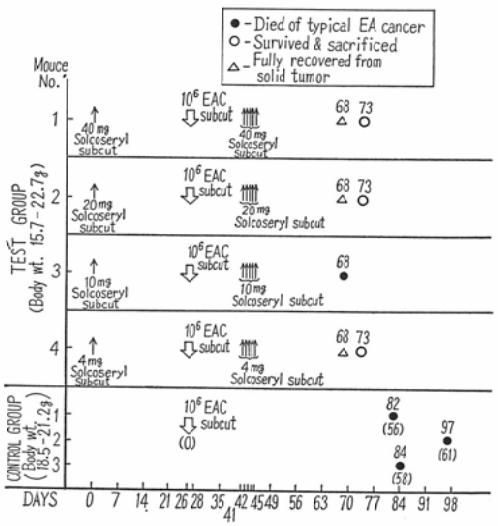
9. Preventive and therapeutic experiment of Ehrlich's solid tumor with administration of Solcoseryl (Table 10)

A single dose of 40, 20, 10 and 4 mg. of Solcoseryl were subcutaneously injected to 4 respective mice, and after 26 days 10^6 ascites cancer cells were subcutaneously inoculated in areas on the right back of those mice. Each mouse was then treated with subcutaneous injection of the same quantity of Solcoseryl as the initial dose for 5 successive days, starting on the 41st. day. As shown in Table 10, three control mice died from typical full-grown solid tumors 56 to 61 days after cancer cell implantation. In the experimental group, the mouse treated with 10 mg. of Solcoseryl was dead of typical solid tumor, but the other 3 mice escaped death of cancer and developed tumors completely disappeared during the course of 68 days.

Conclusion

The mechanism of the action of Solcoseryl on Ehrlich's ascites cancer cells is not yet clear, but it

Trble 10. Prophylactic effect on Ehrlich's solid cancer of different doses of Solcoseryl subcutaneously given to mice 26 days before and 16 days after subcutaneous inoculation of 10^6 cancer cells



seems to have some beneficial effect to inactivate or destroy Ehrlich's cancer cells when a relatively small dose as 10^4 cells are intra-peritoneally inoculated into mice. Solid tumors are also influenced by its administration and tend to localize and to reduce in size, and in some cases they completely disappear when Solcoseryl are repeatedly administered to mice.

(The main results described in this paper were published at 10th. general meeting of Japanese Society of Chemotherapy held on 7th. October, 1963)

Acknowlegement

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