Inhibition of Malignant Cell Growth and Spread by Combined Internal (Topical) and External (Systemic) Irradiations*

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SUMMARY

Growth of Krebs-2 free tumor cells in the ascitic fluid of the mouse (CFW or albino) and their spread and implantation into peritoneal tissues were considerably reduced either by total-body irradiation (external and systemic) with 400 r or by intraperitoneal injection of 0.15 mc. of colloidal radioactive chromic phosphate (internal and topical irradiation). Combined irradiations given in either succession completely prevented (or very nearly so) invasion of the fluid accumulation. Similarly, growth and spread of tumor cell inoculated subcutaneously into the scalp were arrested partially by either method of irradiation and totally by their combination. Analogous phenomena were produced in some normal tissues: in normal mice given 0.15 mc. isotope into the scalp the spleen was enlarged by congestion; in irradiated (400 r) mice, it was slightly shrunk, and considerably shrunk after combined irradiation. It was concluded that the effect of internal (topical) irradiation on tumor cells and on blood vessels was considerably increased by combination with external (systemic) irradiation or vice versa.

We have reported previously (5) that pretreatment of a normal mouse with a small dose (0.01 mc.) of colloidal radiogold (Au198) did increase the severity of pulp destruction in the spleen and its framework shrinkage by high dose (1200 r) of total-body x-radiation. This evidence of combined action of internal and external irradiations on a normal organ suggested the trial, by similar procedures, of their effect on malignant cells growing free in the peritoneal fluid (ascites tumor) or infiltrated into tissues. For that purpose we have modified our previous technic of irradiation as follows: (a) Pure beta-emitting isotopes Y90 and P32 were used for internal irradiation, thus eliminating gamma rays (emitted by Au198) and, in the effect of combined irradiation, involving only two factors—beta and x-rays. (b) X-rays were given in small doses (400 r) well tolerated by mice. (c) The isotope compound was injected into the target area, thus acting only (in the peritoneal cavity) or mainly (in subcutaneous localization) topically (2, 8). (d) The x-rays were given as total-body irradiation which induced systemic effects. The results obtained with a P32-insoluble compound (CrP32O4) prepared in colloidal suspension are reported below.

MATERIALS AND METHODS

Mouse and tumor strains.—CFW mice (Carpworth Farms, New City, N.Y.) and Swiss Albino mice (Albino Farms, Red Bank, N.J.) of 24-27-gm. weight were used—females for ascites tumors and males for subcutaneous growth. Krebs-2 ascites tumor was carried by serial transfers for these experiments.

Biological technics.—Technics of tumor inoculation with requisite cell numbers, of ascitic fluid withdrawal, of tumor cell counts in the fluid, etc., have been described elsewhere (1, 3, 4).

Radioisotopes and x-ray irradiation.—Radioactive phosphorus was supplied by Abbott Laboratories, Oak Ridge, Tennessee, as water-insoluble radioactive chromic phosphate (CrP32O4) in a colloidal suspension. This preparation is used for intracavitary treatment of malignant conditions (2, 6, 11, 12, 13, 14). X-irradiation was performed with a machine used for radiotherapy at the Department of Radiology.

Technics of irradiation.—(a) Injection of the radioisotope: Radioactive suspension was diluted...
with 0.85 per cent NaCl solution to the concentration of 1 mc/ml and injected, in preliminary experiments, in doses of 0.05, 0.1, 0.15, and 0.2 mc.; the dose of 0.15 mc. was found to be the minimal adequate dose, and it was used throughout in experiments reported below. In ascites tumors the isotope was introduced into the peritoneal cavity (6) and in the scalp on the periphery of the inoculated area as described previously (7, 8). (b) X-radiation: A standard dose of 400 r was given throughout in the experiments. Physical factors used—240 kVp, 18 ma.; filter: 0.5 mm. Cu; 1.0 mm. Al; distance from target of x-ray tube to the mid-coronal plane of the animal bodies: 50 cm. The animals were kept isolated from one another in a fixed position within separated narrow compartments of a box.

Pattern of experiments.—(a) Mice with ascites tumors: Five groups, each of twenty mice, were given inoculations intraperitoneally of approximately 10⁴ tumor cells and received the following treatment: Group X—a single external irradiation with the standard dose of 400 r, 24 or 72 hours after inoculation; Group Y—an injection of a standard dose (0.15 mc.) of CrP³₂O⁴⁺⁺ 24–48 hours after inoculation; Group X + Y—irradiation 24 hours after inoculation and isotope injection 24 hours later; Group Y + X—isootope 24 hours after inoculation and x-rays 24 hours later; Group of controls—inoculated but untreated. Ninety-six hours after inoculation, about 0.5 ml. of ascitic fluid (or less if the fluid was scanty) was withdrawn for counts of tumor cell concentration and for smears; wherever a sufficient amount of fluid was available, it was injected, in a dose of 0.8 ml., into a new mouse (biological assay of tumor cell viability) [1]. Failure to induce ascites tumor was interpreted as evidence of decreased viability or absence of transferred tumor cells. Smears of fluid were stained with aceto-orcein (3). Next day all individuals were sacrificed, autopsies were performed, and the amount of peritoneal growth was estimated by the scale given in the legend to Table 1, and recorded. (b) Mice with tumor cells inoculated into the scalp: Following the same procedures of irradiation, mice were divided into five groups as above, but the isotope was injected into the scalp (7, 8), and condition of animals was recorded after 10 and 30 days. The extent of tumor growth reflected both the growth and the spread of inoculated cells; it was estimated by a scale given in the legend to Table 2.

RESULTS

The results obtained with various procedures of irradiation are reviewed comparatively in Tables 1 and 2.

Experimental data recorded on Table 1 indicated that, using our standard doses of irradiation, well below the lethal tolerance level in mice, a single procedure either of external (Series X) or internal (Series Y) irradiation was able to inhibit only partially any phase of tumor cell life history in the peritoneal cavity. On the other hand, a combination of both procedures (Series X + Y and Y + X) abolished completely or almost completely the viability of tumor cells in the fluid and the peritoneal tissue. Thus, the effect of x-radiation on peritoneal tumor cells was considerably increased by intraperitoneally injected isotope and vice versa. The results of free tumor cell counts received confirmation by examination of smears from the fluid (Figs. 1, 2, 3, and 4) and further interpretation by biological assay of tumor cell viability (see "Materials and Methods"). In ten mice of each series possessing sizable amounts of ascitic fluid, 0.3 ml. of their fluid was transferred from each animal (donor) into a new mouse (recipient); in series of controls (Co) all (100 per cent) of recipients developed ascites tumors, in Series X, 60 per cent; in Series Y, 48 per cent; and in Series X + Y and Y + X only one transfer (5 per cent) was successful. All specimens of transferred fluid contained morphologically recognizable tumor cells which have suffered, obviously, attenuation of their functions responsible for their viability. In Series X + Y and Y + X cells were scanty and mostly distorted (Fig. 4). In Series X and Y, the mitotic index of tumor cells was low and their mitotic figures mostly abnormal (Figs. 2 and 3). The capacity of cells to invade the tissue (column 4) and their tendency to grow as tissue implants (column 5) were reduced by various irradiation methods roughly in proportion to attenuation of free tumor cell viability (column 3). Thus, extensive growth of gross implants (Fig. 5) and diffuse invasion of the pancreas (Fig. 9) were common findings in controls, but only limited implantation (Figs. 6 and 7) and low-grade microscopic invasion (Fig. 10) were recorded in Groups X or Y and no tumor nodules in Groups X + Y or Y + X (Fig. 11). In all facets of these experiments complete inhibition of ascites tumors was more frequent in Y + X Series than in X + Y.

Some irradiation doses were used in analogous procedures on tumor cell growth and spread, in subcutaneous localization. Doses of 10⁶–10⁷ ascitic cells were infiltrated into loose connective tissue separating haired scalp from the parietal and occipital bones. The mice were divided into five series, each consisting of twenty animals, and were irradiated by various procedures according to the pattern of experiments outlined in Table 1. The...
TABLE 1
FREE GROWTH AND IMPLANTATION OF KREBS-2 ASCITIC TUMOR CELLS IN THE PERITONEAL CAVITY OF MICE AFTER TOPICAL AND/OR SYSTEMIC IRRADIATION

<table>
<thead>
<tr>
<th>Procedure of irradiation</th>
<th>Series of mice</th>
<th>Free growth in ascitic fluid</th>
<th>Peritoneal implantation of free cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Approximate amt. of fluid</td>
<td>Concentration of free tumor cells (thousands/cu mm)</td>
</tr>
<tr>
<td>Controls (not irradiated)</td>
<td>Co</td>
<td>++++</td>
<td>55.6</td>
</tr>
<tr>
<td>Topical irradiation (injection of 0.15 mc. CrP32O intraperitoneally)</td>
<td>Y</td>
<td>+</td>
<td>33.1</td>
</tr>
<tr>
<td>Systemic irradiation (total-body with 400 r)</td>
<td>X</td>
<td>++</td>
<td>15.4 - 54.2</td>
</tr>
<tr>
<td>Systemic irradiation 24 hours before the isotope injection</td>
<td>X+Y</td>
<td>++ - +++</td>
<td>11.2 - 20.2</td>
</tr>
<tr>
<td>Isotope injection 24 hours before systemic x-radiation</td>
<td>Y+X</td>
<td>0 - +</td>
<td>8.6 - 2.2</td>
</tr>
</tbody>
</table>

Average and variation extremes are given for each series. Sixty mice in each series (three experiments each of twenty mice). Dose of inoculum: 10³-10⁴ tumor cells. For methods of assay, see "Materials and Methods."

Amount of peritoneal fluid: the amount of exudate was estimated as proportionate to the volume of fluid obtained by insertion of a glass capillary in peritoneal wall: +++ = 0.5 ml. or more; +++ = < 0.5 but > 0.1 ml.; + = < 0.1 ml.; 0 = no fluid. The reliability of this estimate was shown by comparison with the amount of exudate measured at autopsy.

Scale of peritoneal implantation: no implant = 0; nodules only = +; small tumor = ++; large tumor = +++.

Scale of visceral tissue (pancreas) invasion: no infiltration = 0; group of tumor cells outside and between pancreas lobules = +; tumor cells widely separating pancreas lobules = ++; perisplenic tissue mostly replaced by tumor encircling pancreas lobules and acini = +++; perisplenic tumor, malignant cells penetrating into pancreatic acini = ++++

TABLE 2
SUBCUTANEOUS GROWTH OF KREBS-2 IMPLANTS IN THE SCALP AFTER TOPICAL OR/AND SYSTEMIC IRRADIATION

<table>
<thead>
<tr>
<th>Series of mice</th>
<th>Procedure of irradiation</th>
<th>Extension of tumor growth in the mouse (averages and variation extremes)</th>
<th>Survival (averages of mice after 30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>Controls (not irradiated)</td>
<td>++++</td>
<td>0/20</td>
</tr>
<tr>
<td>Y</td>
<td>Topical irradiation (injection of 0.15 mc. CrP32O into the scalp)</td>
<td>+++ - +++</td>
<td>13/7</td>
</tr>
<tr>
<td>X</td>
<td>Systemic irradiation (total-body with 400 r)</td>
<td>+++</td>
<td>12/8</td>
</tr>
<tr>
<td>X+Y</td>
<td>Systemic (as above) 24 hours before topical (as above) irradiation</td>
<td>0 - +</td>
<td>18/2</td>
</tr>
<tr>
<td>Y+X</td>
<td>Topical irradiation 24 hours before systemic</td>
<td>0 - +</td>
<td>16/4</td>
</tr>
</tbody>
</table>

Average and variation extremes are given for each series. Sixty mice in each series (three experiments each with twenty mice). Dose of the inoculum: 10⁷ tumor cells.

Extension of implant growth in the scalp was graded as follows: no growth = 0; strictly localized growth on top of scalp reaching orbits without involvement of eyelids = ++; growth covering whole area of neck, orbits, involving eyelids, and ears = +++; growth extension on ears, neck, and all of face = +++.
scheme of experiments with subcutaneous (scalp) growth and their results were presented in Table 2.

The test objects of assay reviewed in Table 2 were the size of the scalp tumor and the survival span of its host. The two phenomena appeared to be closely interrelated: about 60 per cent of controls died within 15 days after inoculation, and the immediate cause of their death was infection of medium-sized tumors breaking through the skin in the inoculated area. Those surviving 15–30 days showed tumor spread far beyond the scalp area (Fig. 14) and died mostly from starvation due to interference of bulky tumor with their capacity to reach and to consume food and water, and, perhaps, with some other body functions. Unlimited growth of some scalp tumors was due, as shown by our scale of tumor size, to their unlimited spread from the scalp onto the face, the neck, and even the back of the mouse; continued growth was interrupted by death which resulted from the size and weight of tumor tissue. However, even in Series X and Y of Table 2 (Fig. 13), no tumor reached large size (3+ or 4+), and a sizable proportion of animals survived more than 80 days.

It was attempted to supplement the results in tumor cells by testing the effect of the same irradiation procedures on the spleen in new mice. Accordingly, doses of 0.05 mc. of radioisotope were injected subcutaneously into three series, each of twenty mice; Series Y received no other treatment; Series X + Y was irradiated with 400 r before the isotope injection, and Series Y + X inversely. Twenty-four hours after the last treatment, the autopsy showed differences in the size of the spleen in different series, as shown in Figure 14. In all mice of Series Y the spleen was considerably enlarged. Microscopic examination revealed high congestion of the organ and marked distension of connective tissue frame and capsule; however, lymphocytopenic centers were conspicuous among the pulpal elements rarefied by congestion of organs. On the contrary, in Series X the lymphocytic elements were reduced in number, the connective tissue bundles were sharply outlined, without congestion of the pulp, and the size of the organ was slightly decreased. It was remarkably shrunk after combined irradiation (Series X + Y and Y + X) and showed nearly complete disappearance of cellular elements with the exception of fibrous tissue and numerous macrophages. Thus, the effect of x-rays on the spleen structure was considerably increased by the isotope stored in the spleen after subcutaneous injection.

DISCUSSION

In ascites tumors each single procedure of irradiation reduced the average amount of fluid, its concentration of tumor cells, and the extent of their implantation by not more than one-third; therefore, complete disappearance of cells and fluid after successive applications of two different procedures was attributed to combination of their effects. Even in the scalp where the isotope was translocated in more significant amounts than from its storage in the peritoneal cavity (14, 15), growth and spread of the inoculum were inhibited completely by combined irradiations.

Hollcroft et al. (9, 10) reported that the combination of successive irradiations from the same source (x-rays) but by different routes (systemic by total-body irradiation and topically by tumor irradiation) produced "synergistic" effect in human malignancies. In our experiments combined irradiations were given not only by different routes but also from different sources: externally by x-rays and internally by beta-emitting isotope. The nature of their effect, either additive or synergistic, is being investigated in further experiments now in progress.

The present investigation is only an initial step in the study of various methods of combined irradiations. The question of the clinical application of the method described here is related to the problem of safety for humans even of small doses of total-body irradiation. Our present research aims to substitute this procedure by another source of systemic irradiation or by regional irradiation.

REFERENCES


Figs. 1-4.—Free tumor cells in the ascitic fluid of irradiated mice and controls, 6 days after inoculation of 10^4 Krebs-2 cells smears, aceto-orcein, X350.

Fig. 1.—Untreated control.

Fig. 2.—Two days after intraperitoneal injection of 0.15 mc. radioactive chronic phosphate (CrP32O4).

Fig. 3.—Twenty-four hours after total-body irradiation with 400 r.

Fig. 4.—Two days after CrP32O4 injection and 24 hours after x-radiation with 400 r.

Figs. 5-9.—Peritoneal cavity of the mice used in Figs. 1-4.

Fig. 5.—Mouse from Fig. 1.

Fig. 6.—Mouse from Fig. 2.

Fig. 7.—Mouse from Fig. 3.

Fig. 8.—Mouse from Fig. 4.

Figs. 9-11.—Infiltration of pancreas with ascitic tumor cells in irradiated mice and controls. H. & E., X300.

Fig. 9.—Untreated control (as in Figs. 1 and 5).

Fig. 10.—Treated with CrP32O4 (as in Figs. 2 and 7).

Fig. 11.—Treated by combined irradiation with CrP32O4 and x-rays (as in Figs. 4 and 8).

Figs. 12-13.—Tumor growth in the scalp 8 days after inoculation with 10^3 Krebs-2 cells.

Fig. 12.—Untreated control.

Fig. 13.—Treated topically with 0.15 mc. CrP32O4 2 days after inoculation.

Figs. 14-15.—Spleens from controls and irradiated mice. From left to right: (a) untreated control; (b) 24 hours after total-body x-radiation; (c) 2 days after injection of 0.05 mc. CrP32O4; (d) after x-radiation followed 2 days later by CrP32O4 injection; (e) 2 days after CrP32O4 injection followed 24 hours later by x-radiation. Slightly enlarged.
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