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Abstract

The growth inhibitory effect of the fatty acids (OX) from the liver of X-irradiated rabbits on the solid type of Ehrlich ascites tumors has been observed both in gross and histologic observations. OX substance, a fraction of fatty acids extracted from the liver of X-irradiated rabbits has actually been found to inhibit the tumor growth by the local injection, resulting in the disappearance of the tumor after 12 injections for onemonth period, 2.4 ml of 2.5% emulsion in total dosage. Histologic observations reveal degeneration and necrosis of tumor cells, whereas in the control animals always active proliferation of tumor cells can be observed.

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HISTOLOGIC OBSERVATION ON THE TUMOR TISSUE AFFECTED BY FATTY ACIDS FROM THE LIVER OF X-RAY IRRADIATED RABBITS (OX)

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As has been reported[1-4], some powerful hemolytic substances are found in the organs of X-ray irradiated rabbits. These are extracted with ethanol and have been identified to be phospholipids[9] and unsaturated fatty acids. Later on, it has been proved that the latter has such an antitumor activity, as has been demonstrated by animal experiments and clinical application on human cancers, e. g. Brown Pearce tumors of rabbits have disappeared by repeated and massive doses of OX intravenously or squamous cell carcinoma of human beings painted with OX, DNA synthesis has been depressed in the cells[7,10], Ehrlich ascites tumor cells, by being exposed to OX, as has been revealed by the observations both *in vivo* and *in vitro*. Of course, the cells exposed to OX have shown abnormal mitosis and degeneration similar to those in the case of spermatogonia of rabbits treated by OX injection[1,8]. These observations are of a considerable importance in tumor cell biology, but OX has shown a large variety of antitumor activity depending upon the materials used and the method employed in extracting fatty acids. This time we have obtained quite good samples thanks to the aid of Dr. Hagitani and Toshiba Pharmaceutical Company, and tried to observe precisely the histologic changes which may be induced in solid tumors by injecting OX.

MATERIALS AND METHODS

About 50 male mice, Strong A strain, were used in total. Ehrlich ascites tumor cells were transplanted into subcutaneous tissue of the abdominal wall of these animals. The tumor cells used for transplantation were the ascitic tumor cells harvested 5 days after the intraperitoneal inoculation in a mouse of Strong A strain. The ascites were diluted with Hank's solution to have 20 million cells per 1 ml of the solution. Each animal was injected with 0.1 ml of the cell sus-

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pension, 2 million cells. After about one week the tumors about 0.7—0.9 cm in diameter appeared at the site of the inoculation, and the animals bearing the tumors of almost the same size were used, and those having too big or too small tumors or those developing an ascites tumor were discarded. Actually 36 animals were used for the observation. They were divided into 3 groups of 12 animals each.

The animals belonging to the first group were injected with the fraction of fatty acids, OX, and those of the second group with fatty acids extracted from non-irradiated rabbits and those of the third group with 0.5% Tween 80. Each of these agents were injected in the dosage of 0.2 ml per animal at a time into tissue just under the tumor from the subcutaneous tissue both on the right and left sides of tumor in the divided dose of 0.1 ml each. The injections were given once a day for the first three days and then once every two days for 30 days, 11 injections in the longest observation.

The fatty acids injected were consisted of 2.5% colloidal solution prepared by mixing 2.5 ml of fatty acid, 97 ml of water and 0.5 ml of Tween 80 in a Waring blender. The fatty acids were donated by Toshiba Pharmaceutical Company.

During the experiments the size of tumor and body weight were recorded. The size of tumors was measured on its short (S) and long (L) diameters with a calibrator and recorded in the value of $S \times L$. Every three animals in respective group were sacrificed at an interval of one week and histologic observations were carried on the sections stained by routine hematoxylin-eosin staining.

OBSERVATIONS

During the first week of experiment, not any marked difference in the growth of tumors was found among those of the first, second and third groups. Two weeks later, however, there occurred a marked suppression of the growth in the tumors treated with OX, three weeks later the tumors became very small and 4 week later they actually disappeared (Plate 1, Fig. 5), while the tumors of those animals belonging to the second and third groups continued to grow larger by degrees (Fig. 1, Plate 2, Fig. 10, Plate 3, Fig. 15)

Generally the Ehrlich tumor tissues grown in the subcutaneous tissue showed more or less irregular necrotic areas, which appeared markedly in the central regions of the tumors as clearly seen under microscope. Therefore, the histologic observations and comparison among the tumors from each group were made on the margin of the tumor where one might always see the active of tumor cells infiltrating into the surrounding healthy tissues.

In the cases of tumors of the animals treated with the fatty acids from non-irradiated animals and of those injected with 0.5% Tween 80 active growth of

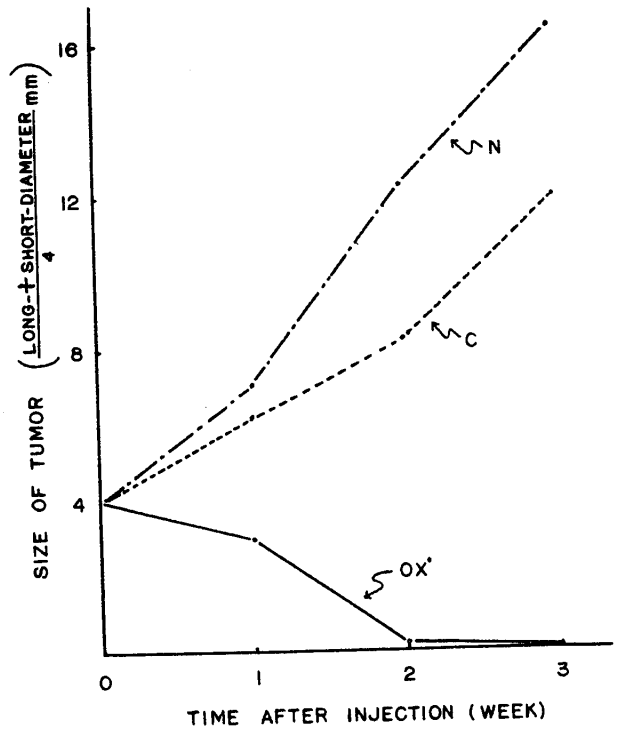

Fig. 1. Development of the Ehrlich ascitis tumors inoculated into the subcutaneous tissue of abdominal wall. N: injected with fatty acid from non-irradiated rabbits. C: injected with 0.5% Tween 80. OX: injected with OX.

the tumor cells was invariably detected. The growth of the tumors was faster in the former than in the latter, but not any difference in histologic pictures could be found between these two groups. In the animals treated with OX, however, some degenerative changes of tumor cells were found even in the cells lying in the peripheral area of the tumor. That is, two weeks after the first injection the tumor cells infiltrating into the adjacent connective tissues and muscles showed a nuclear pycnosis, karyolysis and cytolytic changes (Plate 1, Fig. 1). After 3 weeks the cell damage led to the tissue necrosis in which one could see only the necrotic mass demarcated from the adjacent normal tissue with the regenerating granulation tissue (Fig. 2). In some parts the demarcation was accompanied with a marked proliferation of fibrotic tissues (Figs. 3, 4). In the animals belonging to the other groups an active infiltration of tumor cells into the normal tissues was always observed (Plate 2, Figs. 6—9, Plate 3, Figs. 11—14).

After 4 weeks in the animals treated with OX the necrotic masses changed

to a dry black mass which exfoliated from the subcutaneous tissue and histologically only the scar-formation could be demonstrated. No tumor cells were found in any tissues, but in the tumors of the control animals of both groups, ones treated with fatty acids from non-irradiated animals and others left without any treatment, the tumor cells had grown, showing almost the same picture as that seen at first, though there appeared some inflammatory cells in the tissues around the tumor.

COMMENTS

The experiments proved that the fatty acid fraction extracted from the X-ray irradiated rabbits has a marked, inhibitory effect on the development of the solid type of Ehrlich ascites tumors while the fatty acids from normal animals rather accelerated the tumor development. These effects can also be observed *in vitro* on the same strain cells as has been reported elsewhere[5]. This marked difference in the biological activity between the fatty acids from the animals irradiated and non-irradiated with X-rays will reflect the chemical and structural changes in fatty acide or contamination of some substances in a small amount induced by X-ray irradiation[6]. Gas-chromatographic analysis of the fatty acids proved the production of not any specific substance but only showed an increase in unsaturated fatty acids suggesting that some desaturation will be induced by X-ray irradiation, e. g. in the line of X-ray irradiated animal the saturated fatty acids decrease while there occurs an increase in unsaturated fatty acids, i. e. palmito-oleic, oleic, linolic and linolenic acids[6]. *In vitro* tests show that all these fatty acids both from irradiated and non-irradiated rabbits have a cytolytic activity[6,10]. However, a marked difference in the antitumor activity between these fatty acids of the irradiated and non-irradiated animals seems to suggest a possible change in the position of the double bonds from the general one. The fact that an abnormally rapid increase in peroxide formation can be seen in the fatty acids from the irradiated animals and the amount of peroxide reaches a very high level, suggests also some structural differences between these fatty acids[11].

Histologic changes seem to be general degenerative ones, but not any specific change. Atypical mitosis of specific type as was the case with germ cells affected by OX has not been seen. Only from this experiment it is yet uncertain to what degree the formation of OX after irradiation is responsible to the damage of the tumor cells observable subsequent to X-ray irradiation.

SUMMARY

The growth inhibitory effect of the fatty acids (OX) from the liver of X-irradiated rabbits on the solid type of Ehrlich ascites tumors has been observed

both in gross and histologic observations.

OX substance, a fraction of fatty acids extracted from the liver of X-irradiated rabbits has actually been found to inhibit the tumor growth by the local injection, resulting in the disappearance of the tumor after 12 injections for one-month period, 2.4 ml of 2.5 % emulsion in total dosage. Histologic observations reveal degeneration and necrosis of tumor cells, whereas in the control animals always active proliferation of tumor cells can be observed.

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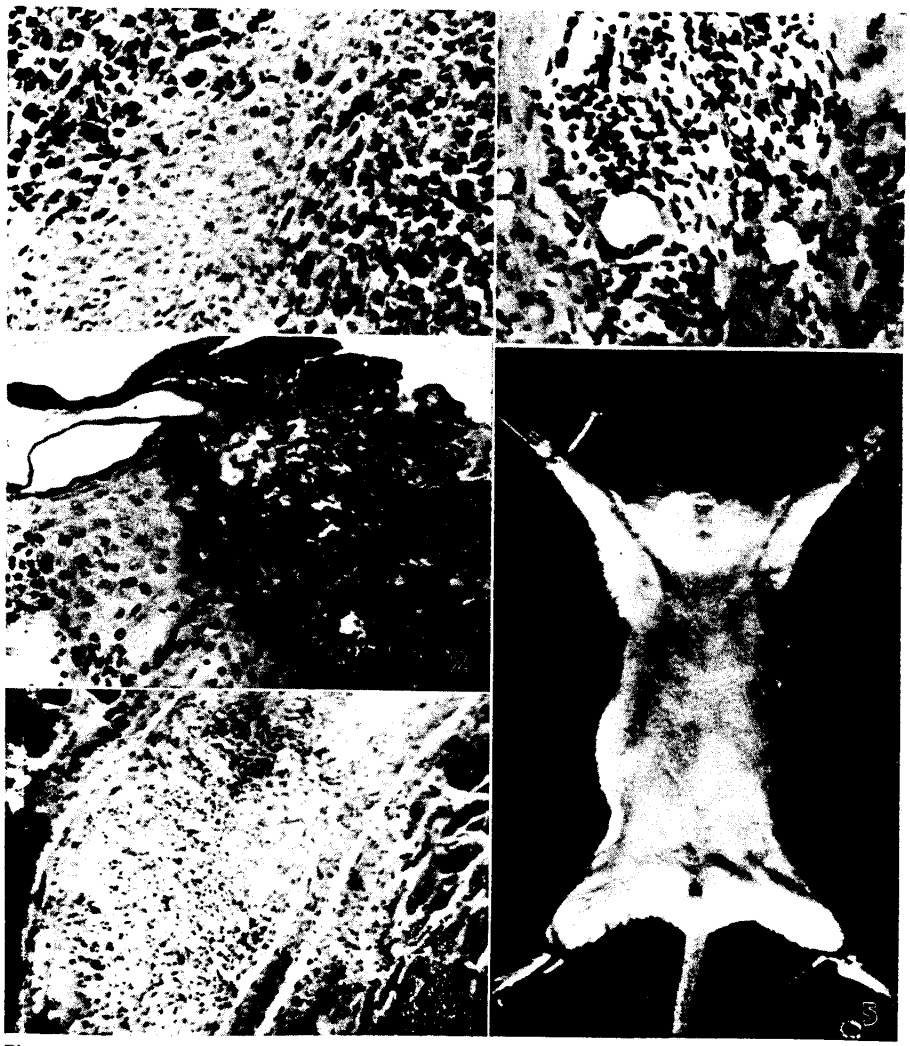

Plate 1. Changes of the tumor tissues (Ehrlich ascites tumor cells growing in subcutaneous tissue of a mouse) exposed to the unsaturated fatty acid fraction from irradiated rabbits by injecting OX in the adjacent area of the tumor.

Fig. 1. Tumor cells seen in the peripheral growing area one week after the injection, 6 injections of 0.2 ml of 1% solution. A marked nuclear picnosis can be seen.

Fig. 2. After 2 weeks, 9 injections. The tumor tissue comes necrotic and just about to peel off (on the right).

Fig. 3. Three weeks later and after 12 injections. The connective tissue, which was the bed of the necrotized tumor, shows the infiltration of some inflammatory cells but no tumor cells.

Fig. 4. An enlarged picture of Fig. 3.

Fig. 5. Showing the animal which had the tumor on its abdominal wall was cured completely by OX injection, after 9 injections in 3 weeks.

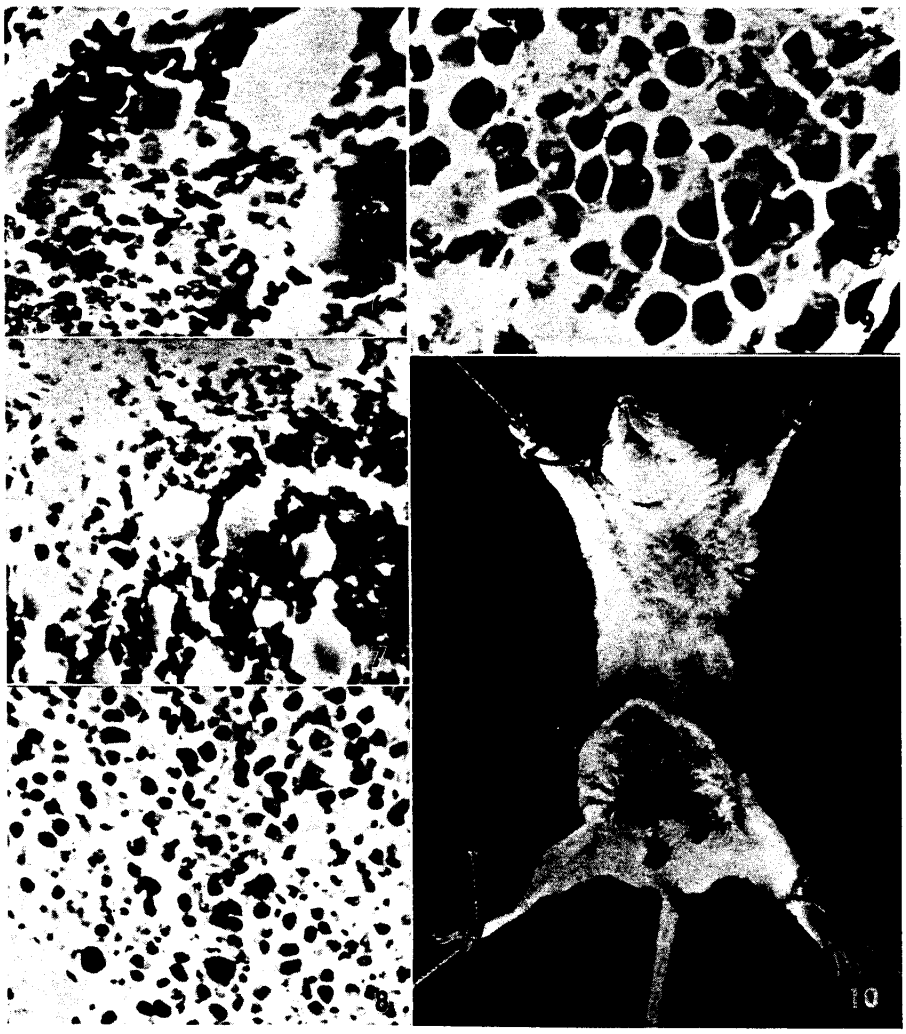

Plate 2. Changes of tumor tissues after the treatment with the fatty acid fraction from the normal rabbit liver as in the case in Plate 1.

Fig. 6. Showing tumor cells in the peripheral area of the tumor tissue after 6 injections in one week. No degenerative change of the tumor cells can be seen.

Fig. 7. After 2 weeks, 9 injections.

Fig. 8. After 12 injections in 3 weeks.

Fig. 9. An enlarged picture of Fig. 8, showing the active proliferation of the tumor cells.

Fig. 10. Well developed tumor after 12 treatments in 3 weeks.

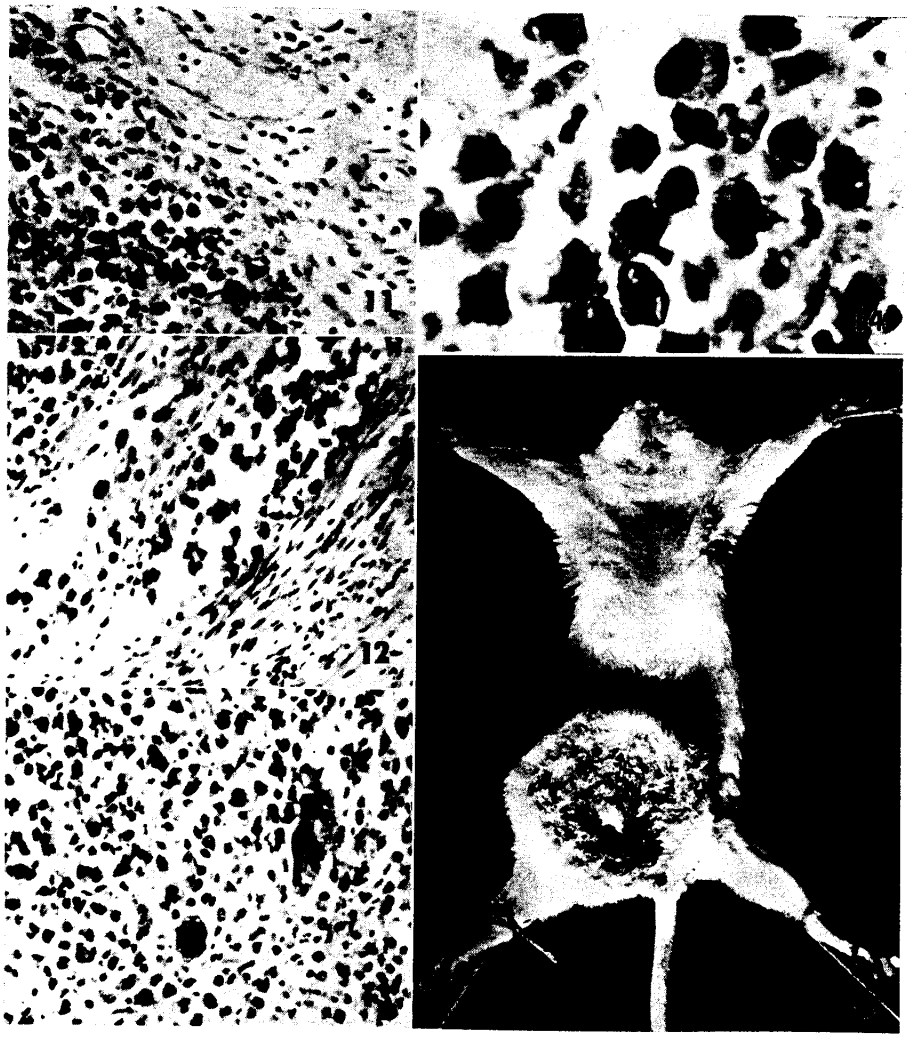

Plate 3. Tumor tissues of the control animal.

Figs. 11. 12, 13, 14, 15 correspond to those 2, 3, 4, 5 of Plate 1 or 6, 7, 8, 9, 10 in Plate 2, respectively.