THE EFFECT OF IONIZING RADIATION ON THE MICROFLORA AND COURSE OF EXPERIMENTAL INFECTED WOUNDS

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The action of ionizing radiation (x-rays, γ-rays etc.) on the body has been studied in research by V. Ya. Aleksandrov, P. D. Gorizontov, P. N. Kiselev, B. N. Mogil'nitskii and other workers [1, 3, 5, 6].

Ionizing radiation produces lesions in several systems and organs, and interferes with their functions. Among these are the functions responsible for the defense of the body against microorganisms.

We accordingly considered that it would be useful to investigate the microflora and the character of the inflammatory reaction during total irradiation of animals with x-rays.

The study of this problem is of practical as well as theoretical interest. During x-ray therapy and in operations on irradiated tissues it is essential to know what course will be followed by the processes of inflammation and regeneration of the wound in cases where the contamination by microorganisms is only very slight.

EXPERIMENTAL METHOD

Experiments were carried out on 96 white rats and 138 white mice, by L. G. Peretts's method [8], which we used in our previous research [2].

A small incision was made in aseptic conditions in a shaved area of the skin of the dorsal surface of the animal, and by means of a palette-knife the skin, together with the subcutaneous cellular tissue, was separated from the muscles. A known volume of a suspension of microorganisms was introduced from a graduated pipette into the skin pocket thus formed.

For infection of the wound we used strains of Staphylococcus haemolyticus, Bacillus proteus vulgaris and Bacterium pyocyaneum. The largest of experiments was performed with the hemolytic staphylococcus.

As a preliminary step we determined the minimal dose of microorganisms for the experimental animals to cause a local inflammatory reaction with a favorable issue.

The animals were irradiated by means of an RUM-3 apparatus, the total dose for the rats being 500 r and for the mice 240 r (sublethal doses). The animals in group I were first inflicted with the infected wound, and 24 hours afterwards were subjected to total irradiation. The animals in group II were irradiated first, and the infected wound was inflicted 24 hours later. The animals of group III acted as controls, i.e., wounds were inflicted but no irradiation was carried out.

The results of the experiments on white mice were assessed by counting the numbers of dying and surviving animals, by the postmortem findings and by bacteriological cultures from the wound and internal organs, and in the white rats, in addition, on the basis of cytological investigation of wound impressions [4].

In order to study the course of the wound, bacteriological and cytological investigations were made at definite intervals of time (after 24 hours and 3, 7 and 12 days), for which purpose 3 rats in each group were killed at the times indicated.

The microflora was examined by means of subcultures of the wound contents and internal organs in meat-peptone broth and on blood agar, followed by study of the cultural, morphological, plasma-coagulating and hemolytic properties of the microorganisms thus isolated.

EXPERIMENTAL RESULTS

It will be seen from the data in Fig. 1, a that only 10% of the animals in the unirradiated (control) group died. At postmortem, a slight enlargement of the spleen and slight swelling of the intestine were found. The original organism — the hemolytic staphylococcus — was found only in the region of the subcutaneous pocket.

In the first days after x-ray irradiation of white mice with a recently infected wound (Fig. 1, b), the majority of animals died (70%). At postmortem, considerable enlargement of the liver, kidneys and spleen, and edema of the lungs were found.

Inoculation of nutrient media with material from the wound, the heart, liver, spleen and kidneys gave an abundant growth of Staph. haemolyticus with a positive plasma-coagulation reaction and a well-marked zone of hemolysis on blood agar. In films from meat-peptone broth, cocci of various sizes and arrangements, and small Gram-positive and Gram-negative bacilli were seen in addition.

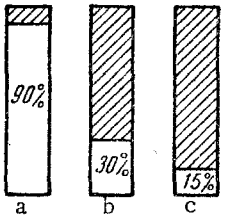

Fig. 1. The effect of total irradiation with x-rays on white mice with infected wounds. a) Control (infection of wound without irradiation); b) infection of wound, followed 24 hours later by irradiation; c) irradiation, followed 24 hours later by infection of the wound.

In the experiments in which mice were irradiated first (Fig. 1, c) the percentage of dying animals was even higher (85%). At postmortem, besides the changes mentioned, hemorrhagic inflammation of the intestine was found. The original organism was isolated from the wound and the internal organs, giving a luxuriant growth on blood agar with a wide zone of hemolysis. Other bacteria were also found, with predominance of bacillary forms.

In the unirradiated white rats, at the site of introduction of the organism causing wound infection, after 24 hours insignificant signs of inflammation were seen, taking the form of the appearance of slight swelling in the region of the subcutaneous pocket and infiltration of the tissues. From the wound contents a culture of the original hemolytic staphylococcus was isolated, with a positive plasma-coagulation reaction (after 6 hours) and a small zone of hemolysis on blood agar. In wound impressions neutrophils were predominant, with active phagocytosis, and only solitary freely situated staphylococci were to be seen.

Three days later the inflammatory focus remained small in size and was encapsulated. Inoculation of nutrient media gave feeble growth of Staph. haemolyticus. After seven days, no signs whatever of inflammation were seen at the site of the incision and introduction of the organisms. Cultures taken from the region of the subcutaneous pocket showed no growth of microorganisms. In wound impressions a small number of phagocytosed neutrophils, basophilic plasma cells and profibroblasts, with solitary plasma cells were seen (Fig. 2, a). After 12 days neither macroscopic nor microscopic signs of inflammation were found. The general condition of the animals in the period of observation was good; cultures taken from their internal organs gave no growth ofthe original organism.

A different picture of inflammation with predominance of destructive processes and generalization of the local suppurative focus was observed in the irradiated animals.

In animals with preliminary infection of the wound and subsequent irradiation, swelling was observed after 24 hours in the region of the subcutaneous pocket; on incision the tissues were edematous and slightly infiltrated with pus. Inoculation of blood agar with material from the wound gave growth of Staph. haemolyticus with a well-marked zone of hemolysis. At postmortem, the internal organs were without visible changes. The original organism — the hemolytic staphylococcus — was isolated from the liver. In wound impressions a large number of destroyed neutrophils and neutrophils with incomplete phagocytosis were seen, and also a considerable number of free staphylococci.

After three days the signs of inflammation were more prominent. At postmortem, slight enlargement of the liver, spleen and lymphatic glands was found. Culture of the wound contents gave growth of the hemolytic staphylococcus with a wide zone of hemolysis on blood agar. The original strain of staphylococcus was isolated from the liver and spleen. In wound impressions degenerated forms of neutrophils were predominant and an abundance of freely arranged staphylococci was observed, together with solitary neutrophils in the act of phagocytosis.

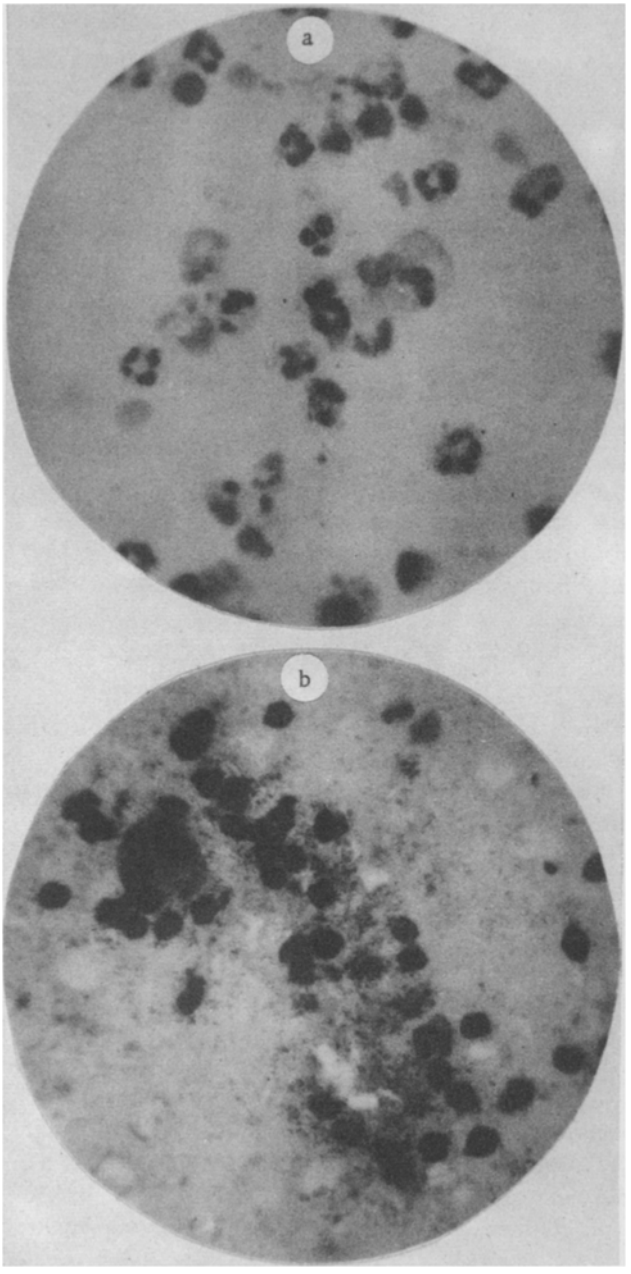

Fig. 2. Impression of the contents of an infected wound in unirradiated (a) and irradiated (b) animals.

After 7 days widespread necrosis of the skin with ulcer formation was found in the region of the subcutaneous pocket. At postmortem, considerable enlargement of the liver and spleen was seen and metastatic foci of suppuration were present in the peritoneal cavity. A hemolytic staphylococcus was isolated from the wound contents which showed a positive plasma-coagulation reaction after 2 hours. Besides the original organism, representatives of the ordinary intestinal microflora were isolated from the liver and spleen.

After 12 days the surviving animals (78%) showed a picture of sluggish inflammation with necrosis of tissues and ulceration at the site of introduction of the microorganisms. At postmortem, the internal organs were edematous; the liver and spleen were enlarged. Necrotic areas were observed on the surface of the liver. A hemolytic staphylococcus with clearly marked pathogenic properties was isolated from the wound. The original organism and intestinal microorganisms were also isolated from the heart, liver and spleen.

On cytological examination of the wound impressions a small number of cells was observed with predominance of degenerative forms of neutrophils; phagocytosis was either incomplete or absent altogether. Besides neutrophils, solitary Unna's cells were found, together with a vast number of freely distributed staphylococci.

The course of the wound process was of an analogous character, although with more pronounced signs of depression of the local reactive processes and with the abundant development of microorganisms not only in the wound but also in the internal organs, was observed in those animals irradiated in the first place.

In the animals of this group, only 24 hours after the moment of infection of the wound, necrotic processes were observed to predominate over inflammatory. Cultures from the wound and from the liver and spleen gave an abundant growth of the hemolytic staphylococcus with clearly marked signs of pathogenicity – a sharp and wide zone of hemolysis on blood agar and a positive plasma-coagulation reaction developing after two hours. In wound impressions destroyed forms of neutrophils predominated and there was a considerable number of freely distributed staphylococci.

On the third day extensive necrosis of the skin with ulcer formation was observed in the region of the subcutaneous pocket. Cultures from the wound and organs, and the cytology of the wound impressions were evidence of the active development of the microflora and of the supression of phagocytosis.

After 7 days the course of the wound process was even more severe, with the development of secondary foci of suppurative inflammation in the peritoneal cavity. In wound impressions degenerated forms of neutrophils and an abundance of freely distributed staphylococci were found (Fig. 2, b). After 12 days the majority of the animals had died with signs of a clearly marked bacteriemia.

In all the irradiated animals, especially those irradiated before infection of the wound, the general condition was found to deteriorate, the animals losing weight and often refusing their food.

Analogous results were obtained in the irradiated animals in which B. pyocyaneus and B. proteus were used as the agent of wound infection.

The investigations thus showed that irradiation with x-rays of animals with local foci of suppuration causes generalization of the process and bacteriemia. This is in full agreement with the reports in the literature of a depression of the barrier functions of the tissues (especially of the lymphatic glands) and a fall in the bactericidal power of the blood in irradiated animals.

In all the experiments the irradiated animals showed a rapid increase in the number of microorganisms whose pathogenic properties were more pronounced than those of the original strain (increase in the plasma-coagulating and hemolytic properties).

During irradiation the inflammatory process was inhibited, whereas in the unirradiated animals a vigorous inflammatory process with active phagocytosis was observed. The phagocytic function of the neutrophils was suppressed in the irradiated animals, which created favorable conditions for the development of an abundant wound microflora.

The experiments showed that the intensity of the process of wound infection also depends on the time of irradiation. For instance, in the animals in which irradiation followed the infliction of an infected wound, it resulted in a less severe course of the wound than in the animals irradiated beforehand.

Presumably the healthy, unirradiated animal, in the first hours, mobilizes all its defensive mechanisms to combat the microorganisms, destroys them at the site of administration and prevents them from penetrating from the wound into the internal organs. Preliminary irradiation suppresses these defensive properties of the tissues, creating conditions in the wound in which proliferation of microorganisms takes place unhindered, so that they invade the blood and internal organs.

SUMMARY

Ionizing radiation affects a number of systems, organs and their functions (among them the functions providing the antimicrobic protection of the body).

Experiments conducted on 96 white rats and 138 white mice demonstrated that x-ray irradiation of the animals with local suppurative focus causes an acute depression of phagocytosis and pronounced increase of the number of microbes in the wound with increase of their pathogenic properties. This promotes the generalization of the process, bacteriemia and causes the death of the animals.

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