THE CORRELATION OF INFILTRATION OF NEUTROPHIL GRANULOCYTES AFTER LOCAL IRRADIATION OF THE THORAX TO TIME AND RADIATION DOSE

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Summary

This study monitors the presence of neutrophil granulocytes in the lungs in (C57Bl/6xDBA/2)F1 mice irradiated locally in the area of the thorax with absorbed doses of 14, 16 and 18 Gy of gamma radiation in a time interval of 18, 24, 30 and 36 weeks after irradiation of histologically examined animals. During the histologic examination, an increased amount of neutrophil granulocytes was noted 24 weeks after the irradiation when the extent of inflammatory infiltration of irradiated lungs reached maximum values. In other time intervals, a decreasing trend in the average number of neutrophil granulocytes in the viewing field was found. The values of the number of neutrophils in pulmonary tissue were dependent on the absorbed dose of ionizing radiation in time intervals of 30 and 36 weeks after the irradiation.

KEY WORDS: Radiation pneumonitis, Neutrophil granulocytes, Histology, Mice.

Introduction

At present, radiation pneumonitis (RP) ranks among the diseases generally known as ARDS (Adult Respiratory Distress Syndrome) (4). The pathogenetic mechanisms of RP have not been fully revealed so far. One of the most important mechanisms of the inception and development of RP is the infiltration of pulmonary tissue with neutrophil granulocytes after exposure to the ionizing radiation. Their increased presence has been described especially in an early stage of the development of ARDS (10) as against the increased presence in the absolute number and the percentual content of alveolar macrophages in people surviving ARDS (10).

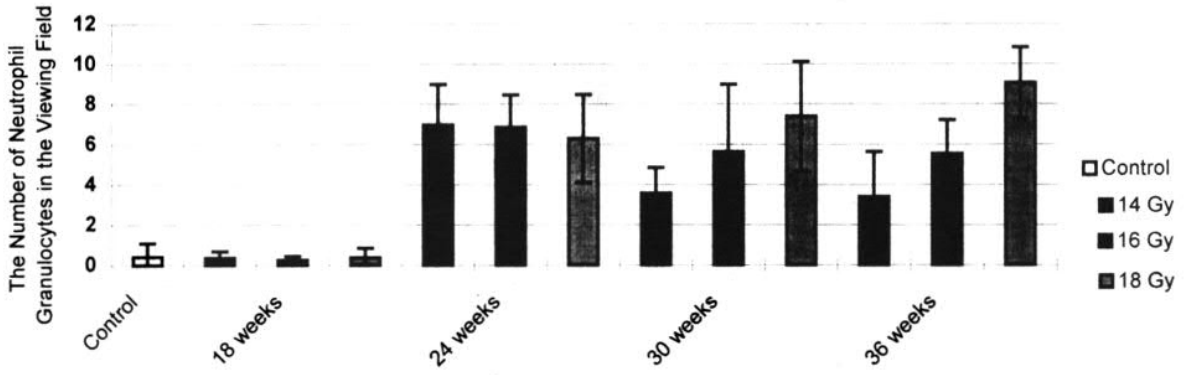
Histopathologic manifestation of RP was noted from the 2nd to the 6th month after irradiation (12). The affection of the lungs by RP seems to be a focal process (11). Interstitial edema of the wall of the alveoli, interstitial infiltration of the wall of the alveoli, lymphocytes and neutrophils, an increase in the number of alveolar macrophages, the presence of hyaline membranes, the hyalinization of arteriol with the appearance of myoepithelial cells, hyperplasia of type II pneumocytes with hyperchromatic nuclei and even intraalveolar edema (2, 4, 5, 11) have all been described as signs of RP.

Due to the importance of this nosologic unit, the monitoring of the infiltration intensity of neutrophil granulocytes was performed by means of histologic examination.

Material and methods

Experimental Animals and Irradiation

Male (C57Bl/6xDBA/2)F_1 mice aged 8-12 weeks and with a weight of 24-34 g were used for the experiment. The experimental animals were



Graph 1 The Infiltration of the Lungs with Neutrophil Granulocytes a 640x Magnification

irradiated locally in the area of the thorax. They were divided into 4 groups: 1. a control, non-irradiated group (6 mice), 2. a group irradiated with a dose of 14 Gy of gamma radiation (24 mice), 3. a group irradiated with a dose of 16 Gy (24 mice), and 4. a group irradiated with 18 Gy of ionizing radiation (24 mice). The animals were killed in batches of 6, at intervals of 18, 24, 30 and 36 weeks after irradiation. The experimental animals were given a DOS-2B diet and they drank tap water ad libitum. The animals were irradiated with gamma radiation using ^{60}Co unit Chisotron Chirana, at a dose rate of 0,3 Gy/min. The target distance was 1 m. The animals were slightly anaesthetized by means of a solution composed of one portion of Rometa R (Spofa, Praha), 3 portions of Narkamon (Léčiva, Praha) and 12 portions of physiological saline. This solution was applied intramuscularly in a 10 ml/kg dose. The local irradiation of the murine thoraxes was performed in a fixating box with a 10 cm thick layer of lead plates to prevent the irradiation of other parts of the body with a dose higher than 2-3 % (9).

Histologic Examination

When the given time intervals had passed the mice were killed by means of cervical dislocation. During the dissection, the removed lungs were fixed in 10% neutral buffered formalin, tissue particles were then embedded into paraffin, and histologic preparations were stained by means of chloracetatesterase stain (to determine neutrophil granulocytes) and Gramm´s stain (to evaluate bacterial infection). Histologic preparations were quantified in the light microscope. 15 randomly selected viewing fields were evaluated in each preparation at a 640x magnification. The results were summarized and set out in a graph 1.

Results

Two mice died within a time interval of 24 weeks after the irradiation. They were both irradiated with the highest dose of 18 Gy. 3 mice out of 6 irradiated with a dose of 18 Gy and also one mouse irradiated with a dose of 16 Gy died in the group that survived 30 weeks after the irradiation. Two mice irradiated with a dose of 18 Gy, one mouse irradiated with a dose of 16 Gy, and one mouse irradiated with a dose of 14 Gy died within a time interval of 36 weeks. No infectious agents were found during the histologic examination of the lungs of any experimental animals by means of Gramm´s stain.

In the control, non-irradiated group, on average 0,42 neutrophil granulocytes were noted in the viewing field.

In a time interval of 18 weeks after the irradiation, 0,37 neutrophils in the viewing field were found in histologically examined mice after irradiation with a dose of 14 Gy, and on average 0,26 neutrophil granulocytes were noted in mice irradiated with a dose of 16 Gy. After irradiation of laboratory animals with a dose of 18 Gy, 0,29 neutrophil granulocytes were noted at a 620x magnification.

24 weeks after the local irradiation of the thorax with a dose of 14 Gy, 6,97 neutrophils were seen in histologically examined mice. In mice irradiated with a dose of 16 Gy, on average 6,83 neutrophils were noted. In mice irradiated with 18 Gy, on average 6,28 neutrophil granulocytes were found in the viewing field.

During the histologic examination of mice in a time interval of 30 weeks after irradiation, 3,56 neutrophils were present in laboratory animals irradiated with a dose of 14 Gy, and on average 5,63 neutrophil granulocytes were found in mice irradiated with 16 Gy. 7,38 neutrophils in the viewing field were noted in the same time interval during the irradiation of the laboratory animals with a dose of 18 Gy.

In a time interval of 36 weeks after local irradiation with 14 Gy in the area of the thorax, on average 3,39 neutrophil granulocytes were determined in histologically examined animals. 5,53 neutrophils were found in the laboratory animals

irradiated with 16 Gy of gamma radiation, and 9,07 neutrophil granulocytes in the viewing field were found in animals irradiated with a dose of 18 Gy.

Discussion

Our results show that the extent of infiltration of the pulmonary tissue with neutrophil granulocytes is significantly increased from a time interval of 24 weeks after the local irradiation of the thorax. The extent of infiltration of the pulmonary tissue with neutrophils was the largest 24 weeks after irradiation and then there was noted a decrease in the number of neutrophil granulocytes in the observed time intervals of 30 and 36 weeks after the irradiation. In addition, a directly proportional significant increase in the number of neutrophils in the lungs was noted with an increasing dose of radiation in time intervals of 30 and 36 weeks after the irradiation. In a time interval of 24 weeks after irradiation, no infiltration of pulmonary tissue with neutrophil granulocytes dependent on a dose was noted.

Large standard deviations of measured values are given by the character of RP. RP is a focal process and neutrophil granulocytes were noted above all in foci of RP and this explains why the number of neutrophils in the affected focus is different from the number of neutrophil granulocytes in the rest of the tissue.

An important factor for the observation of the infiltration of pulmonary tissue with neutrophil granulocytes, which is the period of the development of RP, is the selection of the murine strain under investigation. In $(C57Bl/6xDBA/2)F_1$ mice, the acute phase of RP was seen in the time intervals from the 24^{th} week after the irradiation. On the contrary, Travis (11) describes the acute pulmonary changes in the case of local irradiation of the thorax of CBA mice much earlier. Histologic manifestation of RP culminated from the 16^{th} to the 24^{th} week after irradiation, but from the 30^{th} week after irradiation, RP was not noted. The presence of an increased number of neutrophils in the pulmonary tissue in $(C57Bl/6xDBA/2)F_1$ mice from the 30^{th} week after the irradiation, which is one of the signs of RP, is an important finding. The possible cause of the phenomenon observed in this study is a larger radioresistance in individuals of $(C57Bl/6xDBA/2)F_1$ strain as tested by us. The acute phase of RP appears later and after higher doses of radiation than in CBA mice.

In the pathogenesis of ARDS and also RP, an infiltration of the pulmonary tissue with neutrophil granulocytes is considered the key indicator. In ARDS, an increase in the number of neutrophils in broncho-alveolar lavage was described at an early stage. In the case of pneumonia, it is within the first few hours. (10). For penetration of neutrophil granulocytes over the wall of septal capillaries into alveoli, the linkage to endothelial cells enabled by intercellular adhesion molecules is helpful. Endothelium also produces endothelial leucocyte adhesion molecules. This reaction is enhanced above all by TNF - alfa, and also by Il-1 and a gamma interferon. It was also found that neutrophil granulocytes after penetration into the lungs contain smaller specific granules, which take part in phagocytosis, but the number of primary granules containing myeloperoxidase and proteolytic enzymes remains the same. It is clear from this that during the degranulation of cells, there is a higher risk of damage to the alveoli with proteolytic enzymes (7). It was found that neutrophil granulocytes localized in alveoli show a decreased production of superoxide anion and hydrogen peroxide. This phenomenon is explained by the negative influence of oxidants on the production of these important components for phagocytosis (1).

The function of complement is important for the better understanding of the mechanisms of inception and development of RP and also ARDS. It was found that C5a can activate the penetration of neutrophil granulocytes into the lungs (8). On the contrary, an increased production of C3a during the first hours of the development of ARDS is associated with a delayed incidence of ARDS (8). Both the above mentioned molecules are formed in fibroblasts and type II pneumocytes. In RP, the production and secretion of C5a is an important mechanism mainly in type II pneumocytes. It is a well-known fact (5) that there is a presence of hyperplasia of type II pneumocytes after the irradiation of pulmonary tissue. We may therefore assume that hyperplasia of type II pneumocytesis one of the morphologic bases for the activation of chemotaxis of neutrophil granulocytes into pulmonary tissue after the irradiation with ionizing radiation.

Glauser and Fairman (3), however, state their assumption that neutrophil granulocytes are not a primary mediator for the inception and development of ARDS, but one of many.

The influencing of postradiation pulmonary changes and their deeper investigation in irradiated animals will be the objective of our further work.

Conclusion

The information gained from our work shows that the extent of histologically measured infiltration of pulmonary tissue with neutrophil granulocytes of locally irradiated mice in the area of the thorax is dependent above all on the amount of the absorbed dose of ionizing radiation, on the time interval in which the animals were examined, and also on the strain of the laboratory animals. The measurement of the intensity of infiltration of pulmonary tissue with neutrophil granulocytes can

be used as a quantitative indicator for the histologic evaluation of the effect of radioprotective and therapeutic measures used to combat the inception of RP in a time interval of 30 and 36 weeks after irradiation.

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