THE CORRELATION OF THE PRESENCE OF HYALINE MEMBRANES IN RADIATION PNEUMONITIS TO TIME AND RADIATION DOSE

[1]Jan ÖSTERREICHER, [1]Jiřina VÁVROVÁ, [2]Jan NOŽIČKA, [1]Pavel PETÝREK, [1]Jiří KNÍŽEK
[1]Purkyně Military Medical Academy, Hradec Králové
[2]Fingerland´s Institute of Pathology, Hradec Králové

Summary

This study monitors the presence of hyaline membranes in (C57Bl/6xDBA/2)F_1 mice irradiated locally with gamma radiation of ^{60}Co in the area of the thorax with absorbed doses of 14, 16 and 18 Gy and in a time interval of 18, 24, 30 and 36 weeks after the irradiation of the histologically examined mice. Hyaline membranes were not noted in mice irradiated with the lowest dose of 14 Gy of gamma radiation nor in all the animals examined 18 weeks after the irradiation. We can see from the results that hyaline membranes are observed in the lungs only after higher doses of radiation and in time intervals of 24, 30 and 36 weeks after the irradiation as the other signs of RP.

KEY WORDS: Radiation pneumonitis; Hyaline membranes; Histology; Mice.

Introduction

Radiation pneumonitis (RP) is a separate nosologic unit, which is classified as ARDS (Adult Respiratory Distress Syndrome), sometimes known as diffuse alveolar damage (7). The phase of the development of RP corresponds to the exudative phase of ARDS. It appears after the internal irradiation of the thorax with ionizing radiation in absorbed doses higher than 8 Gy (16). At present, RP is one of the most frequent life-limiting factors after the bone-marrow syndrome of acute irradiation illness is cured. RP is usually manifested from the 2nd to the 6th month after the irradiation (16). Travis (15) describes maximum histologically observable changes in the 24th week after irradiation.

The inception of oxygen radicals, which have in the final effect a harmful influence on cells, is one of the mechanisms that lead to the damage of pulmonary tissue after irradiation of the lungs with ionizing radiation. In some aspects, we can compare the mechanisms of pulmonary damage due to ionizing radiation with pulmonary damage after a 16-hour application of 95% O_2 (1). Pulmonary tissue responds to the presence of oxygen radicals among other things by producing hyaline membranes. The production of these is considered a non-specific response (8). The presence of these membranes is specified as a criterion for the diagnosis of pulmonary oxygen toxicity. (13). But the presence of hyaline membranes is not specific to pulmonary oxygen toxicity. Such membranes may be found in other

disease processes or conditions, such as fat embolism, drowning viral pneumonia, sepsis, and paraquat poisoning or in any other causes of ARDS. Fajardo (2), in the development of radiation pneumonitis, describes interstitial edema in particular. He notes the presence of hyaline membranes in the lungs affected by RP as occasional.

Hyaline membranes covering the alveoli are formed mainly from plasmatic proteins, fibrins and detritus. Immunohistochemical and immunofluorescent staining of hyaline membranes demonstrates fibrinogen, fibronectin and complement. Due to the importance of this nosologic unit in the development of RP, the presence of hyaline membranes was monitored by means of histologic examination.

Material and methods

Experimental Animals and Irradiation

Male (C57Bl/6xDBA/2)F1 mice aged 8-12 weeks and with a weight of 24-34 g were used for the experiment. The experimental animals were 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 which received 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 the irradiation. The laboratory animals were given a DOS-2B diet and 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 before irradiation by means of a solution composed of 1 portion of Rometa R (Spofa, Praha), and 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 carried out 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% (14).

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 Mallory´s stain (to determine hyaline membranes), and Gramm´s stain (to evaluate bacterial infection).

Results

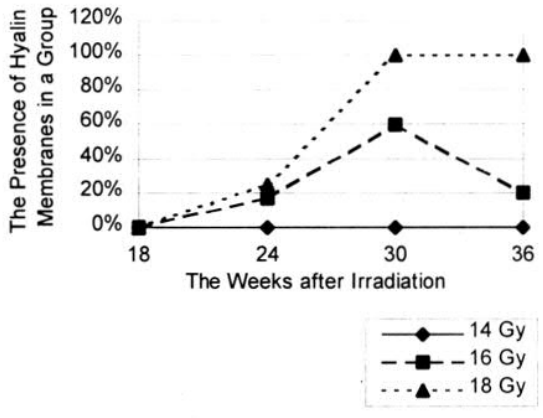
Within a time interval of 24 weeks after the irradiation, 2 mice died. They were both irradiated with the highest dose of 18 Gy. In the group that survived 30 weeks after the irradiation, 3 mice out of 6 irradiated with a dose of 18 Gy, and 1 mouse irradiated with a dose of 16 Gy, died. Within a time interval of 36 weeks, 2 mice irradiated with a dose of 18 Gy, 1 mouse irradiated with a dose of 16 Gy, and 1 mouse irradiated with a dose 14 Gy, died.

During the histologic examination of the lungs of laboratory animals means Gramm´s stain, no infectious agents were found. In the control, non-irradiated group, no presence of hyaline membranes was found, nor was the presence of hyaline membranes diagnosed in irradiated animals (14-18 Gy) 18 weeks after the irradiation. In other time intervals (24, 30 and 36 weeks after the irradiation), hyaline membranes were not diagnosed in animals irradiated with the lowest dose of 14 Gy.

In mice irradiated with a dose of 16 Gy, in a time interval of 24 weeks after the irradiation, the presence of hyaline membranes was noted in one animal out of 6, which is 16.67%. Among 4 surviving animals irradiated with a dose of 18 Gy, this phenomenon was also noted in one case, which is 25%.

3 out of 5 laboratory animals, which is 60%, irradiated with a dose of 16 Gy and all three mice (100%) irradiated with a dose of 18 Gy showed the presence of hyaline membranes 30 weeks after the irradiation.

In a time interval of 36 weeks after the irradiation with a dose of 16 Gy, the presence of hyaline membranes was noted in one animal out of 5 examined, which is 20%. In 4 surviving animals irradiated with a dose of 18 Gy, the monitored phenomenon was present in all the cases, which is 100%.



Graph 1 The Correlation of the Presence of Hyaline Membranes to Time and Dose

Discussion

Our results show that in monitored time intervals of 24, 30 and 36 weeks after the irradiation, the extent of the presence of hyaline membranes in pulmonary tissue rises with an increasing dose of irradiation.

It is a well-known fact (2) that the choice of species of animal for irradiation is an important factor. This factor can influence the presence or absence of hyaline membranes. Absence of hyaline membranes was particularly noted in rabbits or laboratory rats and was explained as due to a different plasmatic concentration of an activator of plasminogen from that noted in mice or dogs, which are the species that show the presence of hyaline membranes after local irradiation of the lungs. In addition, the presence of hyaline membranes was noted in mice only as occasional. In our study, however, we noted up to 100% presence of this phenomenon in mice irradiated with a dose of 18 Gy and examined 30 and 36 weeks after the irradiation. It is possible that this state also depends on the selection of murine strain.

The period, in which hyaline membranes are present, is also influenced by the time interval of manifestation of RP. In $(C57Bl/6xDBA/2)F_1$ mice, the histologic signs of RP were noted in time intervals from the 24^{th} week after the irradiation (10). On the contrary, Travis (15) describes the acute pulmonary changes in the case of local irradiation of the thorax of CBA mice. Histologic manifestation of RP culminates from the 16^{th} to the 24^{th} week after the irradiation. From the 30^{th} week after the irradiation, however no RP was noted. The presence of hyaline membranes in $(C57Bl/6xDBA/2)F_1$ mice 30 weeks after the irradiation, which are histologic sign of RP, is an important finding. The possible cause of the phenomenon observed in this study is that the inception of RP in individuals of $(C57Bl/6xDBA/2)F_1$ strain as tested by us appears later and after higher doses of radiation than in CBA mice.

We may therefore assume that alveolar edema of the lungs and the subsequent formation of hyaline membranes in the development phase of RP is a sign of a rather higher intensity of progression of the pathological process.

The mechanism of the inception of hyaline membranes has not been revealed up to now. During examination of respiratory distress syndrome in newborn babies, the speculations were published that the damage to alveolar epithelium and endothelium of capillaries in alveolar walls is a primary point in the pathogenesis of hyaline membranes. Such a disorder of epithelial--endothelial integrity leads to the development of intraalveolar edema. The proteins contained in it take part in the inhibition of surfactant activity (4). After the subsequent parcial absorption of edema, deposits of eosinophilous blood proteins, which are the basis for the inception of hyaline membranes, are formed in the alveoli. The above mentioned mechanism can amplify accumulations of leucocytes in the pulmonary tissue and the damage to type II pneumocytes, producing a secondary surfactant deficiency (7). In addition, it was found (6) that epithelial-endothelial damage is associated with TNF induction.

During local irradiation of the thorax with ionizing radiation, disorders of the epithelial-endothelial integrity (2), the development of intraalveolar edema (15), accumulation of leucocytes, mainly of neutrophil granulocytes in the pulmonary tissue, and damage to type II pneumocytes production were described. An increased TNF level after the local irradiation of the thorax (12) was also determined.

It is apparent from these findings that the quantitative changes of alveolar epithelium need to be monitored, which will be the objective of our further study.

Conclusion

The knowledge gained from our work shows that the presence of hyaline membranes monitored by means of histologic examination of mice irradiated locally in the area of the thorax is dependent, above all, on the amount of the absorbed dose of ionizing radiation, in the time interval in which the animals were examined, and on the strain of the laboratory animals.

References

1. DAVIS, WB., et al.: Pulmonary oxygen toxicity: Early reversible changes in human alveolar structures induced by hyperoxia. N. Engl. J. Med., 1983, vol. 309, p. 878-883.
2. FAJARDO, LF.: Pathology of radiation injury. Masson Publishing USA, 285.
3. FAJARDO, LF. - ACKERMAN, S.: Scanning electron microscopy of irradiated lung. Unpublished.
4. FUCHIMUKAI, T., et al.: Arteficial surfactant inhibited by proteins. J. Appl. Physiol., 1987, vol. 62, p. 429-437.
5. FUKUDA, Y., et al.: The role of intra-alveolar fibrosis in the process of pulmonary structural remodelling in patients with diffuse alveolar damage. Am. J. Pathol., 1987, vol. 126, p. 171-182.
6. GIBSON, RL., et al.: Group B streptococcus induces tumor necrosis factor in neonatal piglets. Am. Rev. Respir. Dis., 1991, vol. 143, p. 598-604.
7. HASLETON, PS. (ed.): Spencer´s pathology of the lung. 5^{th} ed. New York, McGraw-Hill,1283.
8. KATZENSTEIN, A. - BLOOR, CM. - LIEBOW, AA.: Diffuse alveolar damage, the role of oxygen, shock related factors. Am. J. Pathol., 1976, vol. 105, p. 210-222.
9. NASH, G. - FOLEY, FD. - LANGLINLAS, PC.: Pulmonary interstitial edema and hyaline membranes in adult burn patients: Electron microscopic observations. Hum. Pathol., 1974, vol. 5., p. 149-160.
10. ÖSTERREICHER, J. - VÁVROVÁ, J. - NOŽIČKA, J. - PETÝREK, P.: Histologic expression of radiation pneumonitis

on a murine model. Voj. zdrav. Listy, 1998, vol. 67, no. 3, p. 75-78.
11. PHILLIPS, TL.: Radiation fibrosis. In: FISHMAN, AP. (ed.): Pulmonary diseases and disorders. 2nd ed. New York, McGraw-Hill, 1988, p. 773-792.
12. RUBIN, P., et al.: A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int. J. Radiat. Oncol. Biol. Phys., 1995, vol. 33, p. 99-109.
13. SEVITT, S.: Diffuse and focal oxygen pneumonitis: A preliminary study on the treshold of pulmonary oxygen toxicity in man. J. Clin. Pathol., 1974, vol. 74, p. 21-30.
14. SKOPEC, F.: The method for the irradiation of the head area in rats. Sborník věd. prací VLVDÚ JEP Hradec Králové, 1987, sv. 101, s. 169-175.
15. Travis, EL.: The sequence of histological changes in mouse lungs after single doses of X-rays. Int. J. Radiat. Oncol. Biol. Phys., 1980, vol. 6, p. 345-347.
16. VÁVROVÁ, J. - PETÝREK, P.: Pulmonary radiation toxicity. Voj. zdrav. Listy, 1991, roč. 60, č. 1, s. 33-37.
17. VÁVROVÁ, J. - PETÝREK, P. - REŠL, M.: The radio-protective effect of calcium channel blockers in combination with cytokines. Hradec Králové, VLA JEP, 1995, 92 s.

Correspondence: Npor. MUDr. Jan Österreicher
Vojenská lékařská akademie J. E. Purkyně
Třebešská 1575
500 01 Hradec Králové

Received 14. 11. 1997