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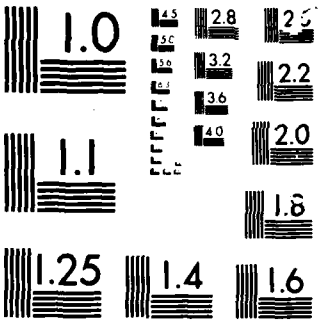

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MODIFICATION OF WR 2721 OF ACUTE AND CHRONIC EFFECTS OF RADIATION INJURY TO THE LUNG

ANNUAL REPORT

LYN A. THET, M.D.

February 1, 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-82-C-2238

Duke University
Durham, North Carolina 27710

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SUMMARY

The purpose of the work was to study the effect of WR2721, without without corticosteroids, on acute and chronic lung injury caused by a sublethal dose of ionizing radiation. The model used was that of 3000 rads unilateral radiation to the left lung of adult rats. The degree of injury and the amount of protection were to be assessed at 12 weeks, 26 weeks and 52 weeks post-radiation by quantitative ultrastructural examination and physiologic measurements.

We have completed morphometric studies on the animals sacrificed at 12 weeks post-radiation. WR2721 by itself did not confer significant protection at this time point. However, WR2721 plus corticosteroids significantly protected against changes in the interstitium; there was also a trend towards protection of the vascular compartment.

At 26 weeks post-radiation, studies have been completed on 1 animal in each experimental group. The changes in the radiated animal were more severe than at 12 weeks; WR2721 seemed to allow improvement of the changes seen at 12 weeks and WR2721 and corticosteroids had an even more striking protective effect.

FORWARD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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STATEMENT OF THE PROBLEM

Radiation injury to the lung results in acute and chronic effects leading to morbidity and mortality (1-15). WR2721 is the most efficient radioprotective agent currently available but is less effective for the lung than for other organs, at least in terms of preventing mortality (8-10). There have been no quantitative or even qualitative morphological studies regarding the protective effect of WR2721 in the lung; to our knowledge, there have also been no studies of the protective effect or physiological functions other than on breathing frequency (4).

BACKGROUND

(1) Response of the lung to radiation injury

The lung is regarded as a moderately radio-sensitive organ with a slow renewal population of cells, that is, only a small fraction of the cells are multiplying at any one time (15). Nongenetic early damage probably occurs in the endothelial and epithelial cells, and the late effects are thought to be related to genetic damage in the endothelial and/or Type II cells (15). However, the specific details are probably very complex and are not well understood since no quantitative data on the numbers of cells and the morphological changes in different cellular and extracellular compartments is available. For example, because the absolute number of cells has not been measured, it is not clear whether the endothelial cells or the Type II epithelial cells are the main site of initial injury.

The response of the lung to injury is generally divided into 3 phases. The early changes (0-2 months) are thought to be due to the acute damaging effects of radiation causing swelling, damage and increased capillary permeability leading to an exudative reaction (16,17). Fragmentation of connective tissue may also take place (18). Intermediate changes (2 and 9 months) are thought to occur when genetically damaged cells reach mitosis, resulting in anaphase arrest or non-viable daughter cells (15). The destruction of basement membrane may also result in greater disorganization of lung tissue architecture (19). The late phase (after 9 months) is associated with the development of fibrosis (20,21).

(2) Effect of WR2721 on radiation injury to the lung

WR2721 or S-2-(3-aminopropyl amino) ethylphosphorothioic acid is the most efficient radio-protective drug now available (8,10). The proposed mechanism of protection is that the sulfhydryl groups on the compound help repair the damage caused by radiation-generated free radicals (10). The amount of protection avoided to the lung is relatively less than that conferred to other organs. In experimental studies in mice using mortality rate as an indicator, WR2721 is reported to confer a protective effect of 1.2 to 1.8 times (8). In a very recent abstract (4), breathing rate and lethality were used as an index of lung damage; the protective factor was 1.2 to 1.3 times in the early (3 to 5 months) phase, and 1.4 to 1.5 in the late (more than 7 months) phases of fibrosis. These data suggest that WR2721 offers more protection against late fibrosis than early pneumonitis in mouse lung. To our knowledge there have been no morphological and functional studies done to assess the efficacy of WR2721 in modifying the effects of radiation injury to the lung. Since WR2721 does not totally protect against lung damage, it is reasonable to hypothesize

that different cellular compartments may be affected to varying degrees. This can only be detected by quantitative ultrastructural study. It also seems reasonable to hypothesize that, if WR2721 is more efficacious in preventing the late effects of radiation injury than the early effects, combined use of WR2721 with an agent (such as corticosteroids) which will reduce mortality in the early stages, should result in improved protection.

(3) Effect of corticosteroids on radiation injury to the lung

We are not aware of any controlled clinical trials in humans. However, in at least one noncontrolled study, prednisone administered soon after the onset of radiation pneumonitis was associated with a dramatic clinical response (22). The use of corticosteroids has been studied in rats and mice given thoracic irradiation and in several studies, significantly reduced the mortality as well as changes in lung compliance (5,6). In the study by Moss and associates (6), the changes in lung compliance observed in radiated control rats was almost completely absent in rats given cortisone continuously from the time of thoracic irradiation. The changes were also somewhat less in those given cortisone at the time of onset of radiation changes in the lungs, i.e. about 4 weeks after irradiation. Phillips and associates studied pulmonary lethality in irradiated mice (5); prednisolone was given from 7 days before to 60 days after irradiation and significantly reduced mortality. When prednisolone was given from 100 to 160 days after irradiation, i.e. during the time that most of the untreated mice died, it almost completely prevented deaths during the period of treatment. However, as soon as therapy was stopped, there were a large number of deaths. In one study where a lower dose of steroids was given and 3000 rads of radiation was administered as a single dose to one hemithorax, the mortality rate was not reduced (23). Corticosteroids have also been found to have some effect on early changes in surfactant after irradiation (7). Thus in summary, although there have been no morphological studies or controlled clinical trials, corticosteroids when administered early in the time period after irradiation seemed to prevent the development of radiation pneumonitis or the functional and lethal effects of it.

APPROACH TO PROBLEM

We will study each of 4 different experimental groups at 3 different time points; there will thus be 12 different sub-groups.

Experimental Groups	Time Post-Radiation		
	12 weeks	6 months	12 months
1. Controls	+	+	+
2. Radiated	+	+	+
3. WR2721 + Radiation	+	+	+
4. WR2721 + Radiation + Corticosteroids	+	+	+

In each sub-group, 3 different types of measurement will be performed:
(a) Morphometry of the lung ultrastructure.
(b) Measurement of lung elastic recoil.
(c) Measurement of the pattern of ventilation.

RESULTS

Table 1 displays the morphometric findings for the 4 different groups at 12 weeks after unilateral irradiation with 3000 rads.

Table 2 displays partial morphometric data for 1 animals each in the 3 experimental groups. Because of the long time required to perform ultrastructural morphometric analysis, the first groups of animals were all used for that purpose.

DISCUSSION OF RESULTS

At 12 weeks, the results suggest that WR 2721 by itself does not confer statistically significant protection from radiation to any of the compartments of the lung as a whole. However, there are trends that suggest that the number of capillary endothelial cells, the capillary lumen (or blood) volume, and the capillary and epithelial surface areas may be somewhat more normal than in the group receiving radiation alone.

In the radiated animals treated with both WR2721 and corticosteroids, the trend towards protection of the vascular compartment is also present at 12 weeks. In addition, the total volume of interstitial cells and the mean thickness of the interstitium are both significantly less than in the radiated animals. Also, although the total volume of lung interstitial matrix in the radiation + WR2721 + steroid animals is not statistically significantly different from that in the radiated animals, it is 17% less in mean value and perhaps more importantly is only 16.8% (58 mm^3/344 mm^3) of total lung tissue volume (excluding air) versus 26% (79 mm^3/303 mm^3) in the animals receiving radiation alone.

At 26 weeks post-radiation, although we have only completed full measurements on only 1 animal from each experimental group, there seems to be a definite protective effect associated with WR2721 and an even more striking effect associated with the combined use of WR2721 and corticosteroids. This is noticeable especially in the volume and thickness of the interstitium and in the preservation of epithelial and capillary surface areas. Qualitatively, the radiated lungs appeared to be an almost solid mass of cells and tissue whereas the lungs from radiated animals treated with radioprotectants appeared to be aerated and had recognizable topography.

It is intriguing that the protective effects are more significant at 26 weeks post-radiation than at 12 weeks. The data needs to be confirmed in more animals but suggest that remodeling and repair takes place much more effectively in the radioprotectant treated animals. This may explain previous observations in the literature (4) that the protective effect of WR2721 seems more at the later stages of the process.

Table 1 Changes in cell number and total volumes in the left lung of rats 12 weeks after unilateral irradiation

	Controls	Radiated	WR2721+ Radiation	Corticosteroids+ WR2721+Radiation
n	7	7	7	7
Total No. of Cells/ Left Lung ($x10^6$)				
Type 1 epithelial cells	28.2±2.7	14.4±2.0*	12.5±1.9*	17.4±2.7*
Type 2 epithelial cells	40.0±4.9	12.1±1.9*	10.6±2.4*	16.4±3.6*
Interstitial cells	75.2±7.7	94.1±11.9	140.9±23.3*+	73.0±13.1
Endothelial cells	111.4±9.8	40.8±5.3*	55.0±11.7*	57.8±7.2*
Alveolar macrophages	7.8±1.4	8.8±1.6	16.1±3.3*+	6.5±1.5
Absolute Volume/ Left Lung (in mm^3)				
Type 1 epithelial cells	37±3	23±5*	29±4*	21±2*
Type 2 epithelial cells	24±6	16±3*	29±4	14±4*
Interstitial cells	62±18	51±8	87±17	29±12+
Interstitial matrix	84±14	79±15	89±19	58±8
Endothelium	57±9	27±7*	33±7*	26±3+*
Alveolar macrophages	11±2	16±4	23±7*	8±2+
Capillary lumen	354±30	91±21*	225±75*	178±26*
Surface Area/ Left Lung (cm^2)				
Type 1 epithelium	1848±126	859±208*	1110±214*	1040±149*
Type 2 epithelium	75±17	55±18	56±17	37±9
Endothelium	2094±263	810±221*	1121±248*	1000±134*
Arthmetic Mean Thickness (μm)				
Epithelium	0.284±0.030	0.474±0.070*	0.469±0.040*	0.379±0.080
Interstitium	0.610±0.040	2.364±0.680*	1.892±0.510*	1.016±0.201+*
Endothelium	0.272±0.009	0.413±0.130	0.311±0.020	0.265±0.018

$\bar{X}$ ± SEM.
* P <0.05 vs control groups.
+ P <0.05 vs radiated group.

Statistical comparisons by Wilcoxon-Wilcox non-parametric method of multiple comparisons between groups.

Table 2 Morphometric findings in the left lung 26 weeks after irradiation with 3000 rads

	Controls	Radiated	WR2721+ Radiation	Corticosteroids+ WR2721+Radiation
n	-	1	1	1
Absolute Volume/ Left Lung (in mm^3)				
Type 1 epithelial cells	-	0.2	38.6	37.4
Type 2 epithelial cells	-	15.2	66.3	38.4
Interstitial cells	-	169.8	103.1	38.8
Interstitial matrix	-	217.8	185.6	72.3
Endothelium	-	13.9	49.8	43.1
Alveolar macrophages	-	4.0	15.3	8.9
Capillary lumen	-	7.6	33.3	127.2
Surface Area/ Left Lung (cm^2)				
Type 1 epithelium	-	1.5	1226.0	1901.0
Type 2 epithelium	-	58.6	116.1	97.1
Endothelium	-	53.3	505.4	1148.0
Arthmetic Mean Thickness (µm)				
Epithelium	-	2.566	0.781	0.379
Interstitium	-	68.329	3.125	0.706
Endothelium	-	2.601	0.986	0.376

OTHER ACCOMPLISHMENTS

(1) We have irradiated and sacrificed the following number of rats in each group:

Unilateral radiation:	10 rats sacrificed at 12 weeks 8 rats sacrificed at 26 weeks
Radiation+WR2721:	10 rats sacrificed at 12 weeks 8 rats sacrificed at 26 weeks
Radiation+WR2721+steroids:	8 rats sacrificed at 12 weeks 8 rats sacrificed at 26 weeks
Controls:	8 rats sacrificed at 12 weeks 8 rats sacrificed at 26 weeks

(2) Post-irradiation duration:

Between 40 and 50 weeks:	10 Controls 17 Radiated 15 Radiated+WR2721 11 Radiated+WR2721+steroids
Between 2 and 15 weeks:	24 Controls 52 Radiated 49 Radiated+WR2721 47 Radiated+WR2721+steroids

(3) Processing of tissue:

12 weeks post-irradiation: All the animals sacrificed at this time point here had their lungs fixed and cubes of tissue randomly selected, dehydrated and embedded for electron microscopy. Tissue has been thin-sectioned, placed in grides and stained.

26 weeks post-irradiation: All the tissue has been processed up to the embedding stage. Three lungs in each of the 3 experimental groups have been sectioned and stained.

PROBLEMS ENCOUNTERED

(1) High mortality with bilateral irradiation

In our preliminary data submitted in the original proposal, we had studied rats 3 and 6 weeks after 3000 rads had been delivered bilaterally to both lungs. Although there was virtually no mortality at these timepoints, by 12 weeks after irradiation over 90% of the rats had died. This would have made the original protocol almost impossible to achieve logistically and the survivors would have represented a very biased sample. We tried reducing the dose of radiation delivered bilaterally from 3000 to 2500 rads. Again, the survival after 12 weeks was very poor, only about 15%. After these failures, we began irradiating rats unilaterally with 3000 rads — which had the advantage of maintaining the original dose of radiation and which still allowed us

to perform the proposed study. There was a 12-week post-radiation survivial of over 90% in these rats. This was comparable to the 100% survival rate achieved with rats by Watanabe et al (20) and the 88% survival achieved with mice by Adamson et al (21) using 3000 rads delivered unilaterally tc the lungs. Because our COTR, Colonel Davidson, was out of the country at the time, we addressed a formal request for change of protocol on March 8, 1983, to Dr. Howard Noyes in the Office of Research Management at WRAIR. Subsequently, we received permission from the US Army Medical Research and Development Command in Fort Detrick to proceed with the change in protocol. The new protocol has been effective and we will still be able to complete the proposed studies within the time and budget originally proposed.

(2) Problems with toxicity of WR2721

Initially, the batch of WR2721 received in 1981 was used in a dose of 500 mg/kg I.P. We found that 90% of the animals which received WR2721 + radiation died within 24 hours; this amounted to 12 rats. We, therefore, took the same batch of WR2721 and injected the same dose to non-irradiated rats. Again, all died. We requested a new batch of WR2721 and after telephone consultation with Captain Korte at Walter Reed, made the following changes:

(a) Phosphate-buffered saline instead of saline was used as solvent.
(b) The solution was made up at the last possible moment before injection.
(c) Decreased the dose to 400 mg/kg I.P.

These changes plus perhaps the change in drug batch worked; no immediate deaths have subsequently occurred, although the rats receiving WR2721 still seem very subdued in comparison to saline-injected controls.

(3) Reduction of changes in breathing pattern following unilateral irradiation as opposed to bilateral irradiation

In some preliminary experiments with rats which were between 12 and 26 weeks post-unilateral irradiation, the changes in tidal volumes and rates were less than what had previously been seen after bilateral irradiation. This is very likely related to the fact that only 35% of the total lung volume is contributed by the left lung; therefore, the main mass of the normally functioning lung is unaffected by radiation even though radiation-induced injury and the protective effects of WR2721 and steroids, if any, in the left lung would still be representive of effects on lung tissue in general.

FUTURE PLANS

A major part of our effort will be to complete the morphometric studies on the 4 groups of animals at 26 weeks post-irradiation. As animals are available, we will also be beginning the physiologic studies.

REFERENCES

1. Thet, LA, U, R and Crapo, JD. Morphometry of ultrastructural changes in the lung due to radiation injury. Am Rev Respir Dis 125(4 pt. 2):229, 1982.

2. Shrivastava, PN, Hans, L and Concannon, JP. Changes in pulmonary compliance and production of fibrosis in x-irradiated lungs of rats. Radiology 112:429-440, 1974.

3. Travis, EL, Down, JD, Holmes, SJ and Hobson, B. Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. Radiat Res 84:133-143, 1980.

4. Trave, EL and Fowler, JF. Protection against late and early damage in irradiated mouse lungs by WR2721 (abst.). Proceedings of conference in Chemical Modification: Radiation adn cytotoxic drugs (held in Key Biscayne, FL, September 1981). p 83.

5. Phillips, TL, Wharam, MD and Margolis, LW. Modification of radiation injury to normal tissues by chemotherapeutic agents. Cancer 35:1678-1684, 1975.

6. Moss, WT, Haddy, FJ and Sweany, SK. Some factors altering the severity of acute radiation pneumonitis; variation with cortisone, heparin and antibiotics. Radiology 75:50-54, 1960.

7. Gross, NJ. Radiation pneumonitis in mice. Some effects of corticosteroids on mortality and pulmonary physiology. J Clin Invest 66:504-510, 1980.

8. Yuhas, JM, Spellman, JM and Culo, F. The role of WR2721 in radiotherapy and/or chemotherapy. Cancer Clin Trials 3:211-216, 1980.

9. Kligerman, MM, Shaw, MT, Slavik, M and Yuhas, JM. Phase I clinical studies with WR2721. Cancer Clin Trails 3:217-221, 1980.

10. Phillips, JM. Sensitizers and protectors in clinical oncology. Seminars in Oncology 8:65-82, 1981.

11. Crapo, JD, Barry, BE, Foscue, HA and Shelburne, J. Structural and biochemical changes in rat lungs occurring during exposure to lethal and adaptive doses of oxygen. Amer Rev Respir Dis 122:123-143, 1980.

12. Crapo, JD, Peters-Golden, M, Marsh-Salin, J and Shelburne, JD. Pathologic changes in the lungs of oxygen-adapted rats. A morphometric analysis. Lab Invest 39:640-653, 1978.

13. Thet, LA, Wrobel, DJ, Crapo, JD and Shelburne, JD. Morphologic aspects of the protection by endotoxin against acute and chronic oxygen-induced lung injury in adult rats. Lab Invest 4:448-457, 1983.

14. Woody, D, Woody, E and Crapo, JD. Determination of the mean caliper diameter of lung nuclei by a method which is independent of shape assumptions. J Microsc 118:421-427, 1980.

15. Gross, NJ. Pulmonary effects of radiation therapy. Ann Intern Med 86:81-92, 1977.

16. Phillips, TL and Margolis, L. Radiation pathology and the clinical response of lung and esophagus. Front Radiation Ther Onc 6:254-273, 1972.

17. Phillips, TL and Wyatt, JP. Radiation fibrosis. In: Pulmonary Diseases and Disorders. ed. AP Fishman, p 658-674. McGraw Hill, Inc., New York, 1980.

18. Bailey, AJ. Effect of ionizing radiation on connective tissue components. Int Rev Conn Tissue Res 4:233-281, 1968.

19. Vracko, R. Significance of basal lamina for regeneration of injured lung. Virchows Arch (Pathol Anat) 355:264-274, 1972.

20. Watanabe, S, Watanabe, K, Ohishi, T, Aiba, M and Kageyama, K. Mast cells in the rat alveolar septa undergoing fibrosis after ionizing irradiation. Ultrastructural and histochemical studies. Lab Invest 31: 555-567, 1974.

21. Adamson, IYR, Bowden, DH and Wyatt, JP. A pathway to pulmonary fibrosis: an ultrastructural study of mouse and rat following radiation to the whole body and hemithorax. Am J Pathol 58:481-498, 1970.

22. Castellino, RA, Glatstein, E and Turbow, M et al. Latent radiation injury of lungs on heart activated by steroid withdrawal. Ann Intern Med 80:593-599, 1974.

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