

EFFECTS OF METHOTREXATE AND TOTAL BODY IRRADIATION ON MOUSE LUNGS

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ABSTRACT

Separate and combined effects of methotrexate and total body irradiation were studied on normal lungs of mouse. Forty female albino mice weighting 251030 gms were divided Into five groups including that of controls. On gross inspection, 70% of lungs of combined therapy group showed signs of congestion, 10% edematous changes and 20% blackish mottling. Microscopic examination revealed marked histopathological changes In the lungs of combined therapy group and milder changes in the methotrexate (Group III) and total body Irradiation (Group IV) groups. These findings confirm the enhanced effects of combination of radiations and methotrexate (JPMA 40:134, 1990).

INTRODUCTION

Surgery, radiations, and chemotherapy are the three recognised forms of cancer treatment. These methods are either used singly or in combination. Radiation pneumonitis is an inflammatory reaction in which there is first a predominantly exudative phase. This reaction may either resolve completely without leaving any radiographically detected changes or may progress to a chronic or delayed late phase ending up with fibrosis. However, on pathologic basis, radiation pneumonitis cannot be sharply divided in to acute or chronic phases, since one may merge subtly or mix with the other and complete or partial resolution may occur in either early or late periods of the process. Pulmonary fibrosis secondary to radiation, alveolar fibrosis and perivascular and peribronchial scarring, and the dependence of lung changes on the radiation dose was first described in 1962¹. These workers^{1,2} studied experimental pneumonitis due to radiation and concluded that radiation dose, time and probably hypersensitivity responses appear to influence radiation pneumonitis. Later experiments on mice showed that radiation pneumonitis was due both to direct radiation damage to parenchymal cells and to indirect damage secondary to injury to the vascular connective tissue³. The effect of fractionated irradiation on lungs with special emphasis on slow repair was also observed by these workers. Van den Brenk et al⁴ suggested that the primary damage might be due to. the alveolar epithelial cells, some of which are vacuolated and others are not. Both acute and chronic pneumonitis have been reported in patients treated with methotrexate. Acute pneumonitis in a patient receiving chemotherapy is difficult to distinguish from infective causes, especially those due to pneumocytis carinii. Acute pulmonary disease in patients receiving methotrexate for chronic skin diseases has also been reported⁵. The degree to which cancer chemotherapeutic agents are merely additive or sensitizing is not clear from the clinical data. Most reported experimental work seems to indicate that actinomycin-D and adriamycin maybe true radiation sensitizers and the remainder are probably working in an additive mode⁶. Damaging effects of the concomitant use of methotrexate and radiotherapy have also been reported⁷. This study reports the effect of combined and separate therapy of methotrexate and total body irradiation on normal lung.

MATERIALS AND METHODS

Forty, female albino mice, weighing 25-30gms were given gram seeds and plain drinking water as diet. They were exposed to light for fourteen hours a day by an automatic set up. The temperature of the experimental room was maintained at 24- 25°C by an airconditioning plant. The bedding of the cages was changed at least once a day. Five to ten animals were placed in each cage.

Grouping of the animals

The experimental animals were divided into five groups and each animal was marked using an ear punch. Experimental Technique The grouping of animals and the schedule of dosage is shown in table-I.

TABLE I. Grouping of Animals and dose schedule.

Group	No. of animals (40)	Type of Therapy	Dosage/animal
I.	5 (controls)	—	—
II.	5 (Sham Group)	Physiological saline.	0.1 ml, seven doses over a period of four weeks (26 days)
III.	10	Total body irradiation	200 rads of ⁶⁰Co daily or initial 3 days of the experiment.
IV.	10	Methotrexate Sodium	1. mg, in 0.1 ml. of solution, seven doses over a period of four weeks, approx (26 days)
V.	10	Methotrexate and total body irradiation.	A combination of the above mentioned doses on the same dates and times.

- Group I animals did not receive any medication or irradiation and served as controls for all.
- Group II animals were given 0.1ml of physiological saline by intraperitoneal injections and were used as sham group for Group III animals.
- Methotrexate Sodium, 1 mg, 0.1 ml of solution was TABLE I. Grouping of Animals and dose schedule. given by intraperitoneal injections to group III and group V animals. The first injection was given at 10.30 A.M. on the first day of the experiment. A total of seven doses were administered, over a period of 26 days.
- Total body irradiation was given to group IV and group V animals on the first day of the experiment at 12.30P.M.. A single dose of 200 rads was given as total body irradiation and three such doses were given for the first three consecutive days at the start of the experiment. A total of 600 rads of ⁶⁰Co were given to each animal as fractionated total body irradiation.

RESULTS

The experiment was designed in such a way that all animals except two control groups and group IV animals (Total body irradiation group) were sacrificed on day 26 of the experiment. However, some animals died before this day and were autopsied. The lungs of all the animals were preserved in 10% formaline after the gross inspection, sectioning was done after paraffm embedding. Microscopic fmdings are shown in table II.

TABLE II. Histopathological findings in the mouse lungs.

Group	Type of Therapy	Alveolar architecture	Material in the lumen of alveoli.	Alveolar Septs	Generalised edema	Generalised congestion	Pneumonic changes	Lung Infarction
I.	—	Intact in 100% cases	—	normal	—	—	—	—
II.	Normal saline	-do-	—	normal	—	—	—	—
III.	Methotrexate	-do-	—	moderate congestion in 50% & edema in 10% cases	—	—	—	—
IV.	Total body irradiation	-do-	—	Mild congestion in 50% & edema in 20% cases	—	—	—	—
V.	Combined therapy	Intact in 80% cases	Inflammatory exudate in 20% & fluid in 10% cases	Congested to variable degree in 70% cases edema in 10% cases	Severe in 10% cases	Congestion of variable degree in 70% cases	in 20% cases	in 10% cases

has been successfully used in the treatment of leukaemias, choriocarcinoma and other malignancies. Toxic effects of methotrexate on lungs have been reported by Nesbit et al⁵. Now-a-days, a majot approach to improve the cure rates in cancer treatment involves the combination of chemotherapy and radiotherapy. The study of combined effects on normal tissues is important because the normal tissues are also exposed to the risk of damage alongwith the diseased tissue during the administration of these two modalities of cancer treatment. The present study showed that animals receiving combined therapy (group V), showed marked changes in lungs with 70% of the animals showing congestion of variable degree and 10% marked edema. Severe pneumonic changes were seen in 20% of animals. On the other hand animals receiving methotrexate alone (group III animals) showed moderate congestion in 50% and edema in 10%. Similarly the lungs of animals of total body irradiation group (group IV) showed mild congestion in half and edematous changes in 20% animals. These findings proved the potentiating effects of combined therapy in normal lungs of mouse. The pulmonary reactions due to methotrexate mentioned in the clinical studies^{5,8} resembled disseminated.

DISCUSSION

Adverse effects of radiations on human as well as experimental animals have been known for a long time. As lungs are one of the common sites for metastasis of malignant tumours, effects of radiation on this organ have invoked special interest amongst the research workers. The success of cancer chemotherapy in palliation, remission and now even cure in certain haematologic and other malignancies has led to the rapid introduction of the anticancer agents into the wide spectrum of cancer therapeutic agents. Methotrexate, a folic acid analogue progressive infection. Such reactions were not seen in our study, because duratian of methotrexate administration was too short. Moreover, we used the drug on normal healthy mice and the aforementioned clinical studies showed the adverse effects, in diseased human beings, who had greater risks of secondary infections. Development of radiation pneumonitis has been observed by various workers, but with much higher dose than 600 rads as is used

in the present study. Jennings and Arden¹ observed development of radiation pneumonitis by delivering 3000 rads to the thorax of rats with shielding the remainder of the body. The present study indicates the potentiating effects of methotrexate and total body irradiation, but does not give any information about the dosage of the drug which should be considered toxic in case of human beings, but on the basis of the present study, it is suggested that the concomitant use of Methotrexate and radiation in humans should be avoided.

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