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Effect of Cyclophosphamide on The Microanatomy of Lungs of Albino Rats

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ABSTRACT

Background: Cyclophosphamide is one of the many drugs used for the treatment of many neoplastic diseases and diseases associated with altered immunity. Organs like heart, reproductive organs, urinary bladder and lungs are effected when higher doses of cyclophosphamide are used for longer durations.Aim:our aim is thus to see the effects of cyclophosphamide on lungs on chronic exposure Methods: Sixty eight Albino rats were taken to study the effects of cyclophosphamide on the microanatomy of lungs. The animals were divided into three groups, group A (control group) of 20 animals, were fed with routine diet, group B (low dose group) of 24 animals, were given cyclophosphamide at the dose of 0.5 mg/100gms in addition to the routine diet and group C (high dose group) of 24 animals were given high dose of cyclophosphamide at the dose of 0.7gm/100gms of weight of animals in addition to the routine diet. The animals were sacrificed at intervals of 3, 6, 9 and 12 weeks, 5 microns sections of the tissues were prepared and stained with Haematoxylin and Eosin stain and the stained slides are observed under light microscopy.Results: Microscopic changes were seen in the lungs of drug treated animals. The microscopic changes started appearing after third week of drug administration. The changes were in the form of damage to endothelium of pulmonary vasculature with areas of fresh haemorrhage, damage to alveolar epithelium and interstitial fibrosis. Conclusion: Cyclophosphamide induces histological changes in the form of damage to endothelium of pulmonary vasculature, damage to alveolar epithelium and interstitial fibrosis. Thus patients receiving cyclophosphamide need to be continuously monitored for pulmonary dysfunction.

KEYWORDS : Cyclophosphamide, interstitial fibrosis, pulmonary vasculature, endothelium.

INTRODUCTION.

Cyclophosphamide also known as cytophosphane is an alkylating agent of nitrogen mustard specifically the oxazophosphorine group. In 1959 it became the eighth anticancer agent to be approved by FDA1. Cyclophosphamide is used either as a single agent or in combination with other chemotherapeutic agents in the treatment of various neoplastic diseases like lymphomas, chronic lymphocytic leukemia, breast cancers and solid tumors2,3. Because of its immunosuppressive properties, cyclophosphamide is used in the treatment of certain autoimmune diseases either as a sole agent or in combination with glucocorticoids. Cyclophosphamide is given orally or parenterally. Orally it is given in the form of tablets or solutions while parenterally IV route is preferred. Cyclophosphamide is metabolized in liver by hepatic cytochrome 450 to an active metabolite 4-hydroxycyclophosphamide which has a tautomer aldophosphamide. These two metabolites are in steady state with each other4. Aldophosphamide cleaves spontaneously to generate phosphoramide mustard and acrolein. The action of cyclophosphamide is due to phosphoramide mustard. It acts by forming DNA cross links both between and within DNA strands at guanine N-7 position. This is irreversible and leads to cell apotosis5.

Cyclophosphamide causes many side effects like hemorrhagic cystitis, hepatic venooclusive syndrome, pulmonary fibrosis, gastrointestinal bleeding, irreversible azospermia, alopecia etc6. Metabolites of cyclophosphamide are excreted primarily in urine in an unchanged form, so drug dosage needs to be appropriately adjusted in the setting of renal dysfunction7. Because of its wide use in the treatment of various neoplastic diseases and diseases associated with altered immunity, cyclophosphamide is known to cause many side effects and the target organs being liver, urinary bladder, lungs and male reproductive organs. Because of its adverse effects on lungs on the lungs the present study is aimed to see the effects of cyclophosphamide on the microanatomy of lungs of albino rats and to correlate these findings in human beings.

METHODS

The present study was undertaken in the department of anatomy Govt medical college ,srinagar.Sixty eight albino rats weighing on average 100 grams were taken from the animal house of our college after approval from institutional animal ethics committee. The animals were divided in three groups: group A (control group) of 20 rats were fed with routine diet and water, group B (low dose group) of 24 albino rats were given cyclophosphamide at the dose of 0.5mg/100gms of weight of rat besides the routine diet and group C (high dose group) of 24 albino rats were given high dose of cyclophosphamide at the dose of 0.7mg/100gms of weight of rat besides the routine normal

diet. The drug was given by mixing it with pellets of flour. The animals were kept in different cages labeled as (A), (B) and (C) under uniform husbandry conditions.

Dose of the drug: The dose of the drug for the rats was calculated from the daily therapeutic dose for human beings which is 5mg/kg body weight. The dose of the drug for albino rats was calculated as 0.5mg/100gms weight of albino rats as low dose group and 0.7mg/100gms weight of albino rats as high dose and the process of drug administration continued for 12 weeks.

The animals were sacrificed in intervals 3, 6, 9 and 12 weeks to study the effects of the drug. 5 rats from group A and 6 rats each from group B and C were taken in each sitting. After anaesthetizing the animals with chloroform, a midline thoracic incision was given. Lungs were identified and removed and were put in between blocking papers. The tissues were processed by using standard histological techniques. 5 t0 7 micrometer thick sections of the tissues were made, stained with haematoxylin and eosin and observed under compound light microscope. The observations were recorded.

RESULTS

The microscopic findings in the tissues of the control group showed no apparent changes. The pulmonary vascular endothelium should normal pattern. There was no change in the intrapulmonary conducting system and the alveolar architecture was maintained throughout.

Cyclophosphamide treated group: Histological changes started appearing as early as 3 weeks in group C animals and 6 weeks in group B animals. There was damage to alveolar epithelium with loss of alveolar architecture. Alveolar spaces were swollen with edema. Interstitial fibrosis was also seen with areas of fresh haemorrhage. Damage to pulmonary vascular endothelium was seen in the lung tissues of group C animals after 12th week of drug administration.(FIG.1,2,3)

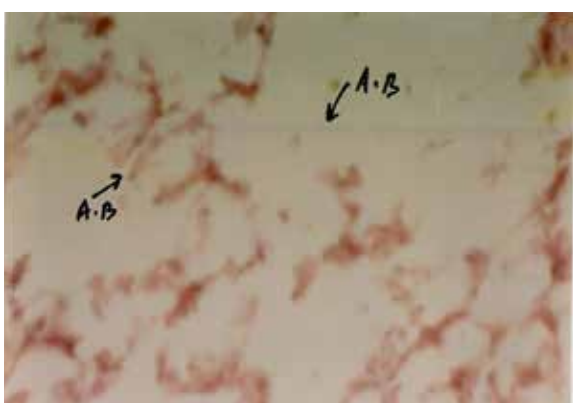

Fig1.Photomicrograph of lungs of group C animals at 6 weeks showing diffuse alveolar damage with alveolar breakage.

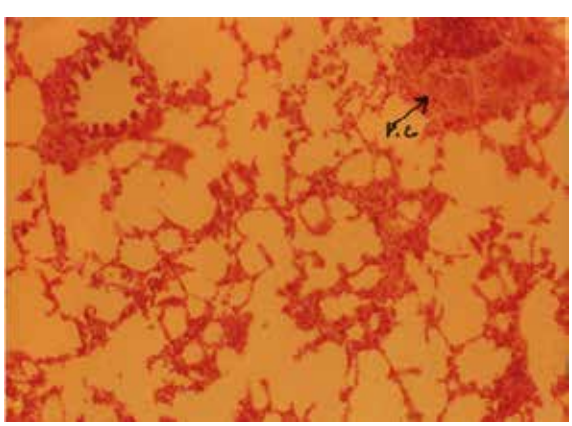

Fig 2: photomicrograph of lungs of group C animals at 9 weeks showing vascular congestion (V.C)

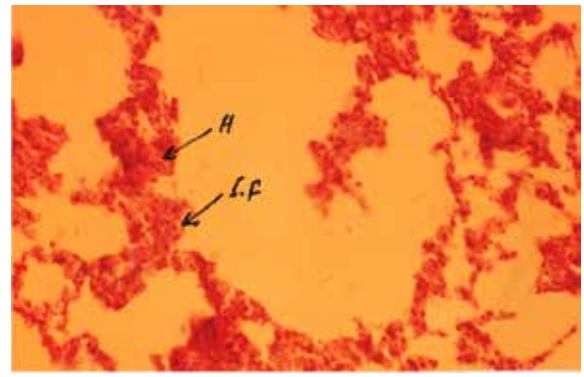

Fig 3: photomicrograph of lungs of group C at 12 weeks showing interstitial fibrosis (I.F) with areas of hemorrhage

DISCUSSION

Cyclophosphamide is routinely used for treatment of various neoplastic diseases and diseases associated with altered immunity. Not much work has been done on the effect of cyclophosphamide on the lungs of animals; however Narayanan etal (1993)8studied the effects of cyclophosphamide on the lungs of rats. They observed that cyclophosphamide caused endothelial damage to the pulmonary vasculature with areas of fresh haemorrhage. They also observed damage to the alveolar epithelium with loss of alveolar architecture. The findings observed in the present study are similar to the earlier workers. D L Kachel eta l (1994)9while working on toxic effects of cyclophosphamide on bovine lung observed damage to the endothelium of pulmonary vasculature with areas of fresh haemorrhage. In the present study the damage to the endothelium of pulmonary vasculature with areas of haemorrhage correlate with the findings of earlier workers.

Young etal (1995)10 while observing the lungs of cyclophosphamide treated patients found that there was significant toxicity in the form of endothelial swelling , lymphocyte infiltration and interstitial fibrosis . In the present study the observation of pulmonary edema, lymphocyte infiltration and interstitial fibrosis correlate with the findings of these earlier workers.

The pulmonary side effects of cyclophosphamide are less common. Clinical features of toxicity include dyspnea, fever, cough, new parenchymal infiltrates, gas exchange abnormalities on pulmonary function tests and pleural thickenings on chest radiographs11..The parent drug is non toxic to lungs but it is metabolized in the liver and to a lesser extent in the lungs to the cytotoxic metabolites 4-hydroxycyclophosphamide,phosphoramide mustard and acrolein .Effects on lung tissue varies in its ability locally metabolized cyclophosphamide and susceptibility to cyclophosphamide induced lung fibrosis in humans may depend upon genetic differences in local pulmonary drug metabolism, genetic susceptibility is further supported by the absence of a clear dose response relationship for development of lung toxicity in humans.

CONCLUSION

Confounding variables like concomitant use of other cytotoxic drugs, opportunistic infections, ,diffuse pulmonary infiltrates, radiation pneumonitis and oxygen toxicity make cyclophosphamide induced drug toxicity difficult to recognize. However from the present study it is concluded that patients receiving cyclophosphamide as a single agent or in combination with other chemotherapeutic agents be screened for lung injury particularly when they present with clinical features of dyspnea, fever, cough and gas exchange abnormalities.

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