

The structural changes of the rat's lung induced by intraperitoneal injection of 5-fluorouracil

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Abstract

Objective: To record the main structural changes in the rat's lung induced by administration of 5-fluorouracil.

Methods: The case-control study was conducted at College of Medicine, Mosul, Iraq, from December 2012 to June 2013. Two groups of 6 rats each were used. The experimental group was given 20mg of 5-fluorouracil in 2ml normal saline per kg body weight by intraperitoneal injection for 7 consecutive days, while the other group was given 2ml normal saline per kg body weight intraperitoneally for 7 days and served as the control group. Specimens of lung tissue of the two groups were taken and prepared for light microscopic examination.

Result: Structural changes were found in the experimental (5-fluorouracil) group compared to the controls, including abnormal alveolar duct, sac, and terminal bronchioles with emphysematous changes in most of the alveoli in addition to peribronchiolitis, perivascularitis, inflammatory cells infiltration and interstitial fibrosis.

Conclusion: 5-fluorouracil has toxic effects on the lung tissue resulting in emphysema and interstitial fibrosis.

Keywords: 5-fluorouracil, Rat, Lung, Emphesema, Interstitial fibrosis. (JPMA 64: 734; 2014)

Introduction

Chemotherapy involves the use of chemical agents to combat the neoplastic growth which affects some tissues and organs, but it does not distinguish between the neoplastic and normal cells as it eliminates the fast-growing cancer cells and other healthy cells in the body, including those lining digestive and respiratory tracts.¹

A potent antimetabolite is 5-fluorouracil (5-FU) that acts during the S phase of the cell cycle. It is activated by thymidine phosphorylase enzyme into fluorodeoxyuridylate (5-fluoro-2'deoxyuridine-5'monophosphate, or 5-FdUMP) which inhibits thymidylate synthase, thus preventing deoxyribonucleic acid (DNA) synthesis.² The concentration of thymidine phosphorylase is 3-10 folds higher in tumour cells compared to its concentration in the healthy tissues, and this can enable selective drug activation of 5-FU at the tumour site with limitation of systemic toxicity.³

The 5-FU is metabolised through the liver and has a half-life of about 10 minutes.⁴ The common clinical side effects of 5-FU include myelosuppression, diarrhoea, vomiting and mucositis. However, in the last decade, cardiotoxicity and neurotoxicity have also been reported.⁵

The trade names of 5-FU are Efudex, Carac, Fluroplex, and it is widely used alone or as a combined protocol in the treatment of various malignancies, including

gastrointestinal, breast, head and neck, basal cell carcinoma of skin (as a cream) and in ophthalmic surgery.⁶

Since there are no literature available concerning the effects of 5-FU on the respiratory system, particularly on the lungs, the present study was, therefore, carried out to record any changes in the structure of the lungs during its use in the treatment of some malignancies.

Material and Methods

The case-control study was conducted at the animal house of the College of Medicine, University of Mosul, Iraq from December 2012 to June 2013 as part of a Ph.D thesis, and comprised 12 healthy adult female Wistar albino rats of about 3 months age and 200-250gm weight. The animals were kept at room temperature of about 25°C and all animals were allowed free access to laboratory pellet foods and tap water drink.

The animals were randomly distributed into 2 equal groups. In Group I, each animal was given 2ml/kg body weight/day of normal saline by intraperitoneal injection for 7 consecutive days. This served as the control group.

In Group II, each animal was given 5-FU 20mg in 2ml normal saline (as a carrier solution) per kg body weight per day by intraperitoneal injection once daily for 7 consecutive days. This served as the experimental group.

One day after the last injection of the two groups, all the animals were dissected under light ether to extract the two lungs from each animal. The extracted lungs were then fixed in 10% neutral buffered formalin solution for

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about 48 hours. Small pieces of about 2-3mm in size were obtained from each lung and paraffin blocks were prepared for light microscopic examination. All sections were processed according to Luna⁷ and Dury's methods.⁸

Serial sections of about 5 microns in thickness were collected from each paraffin block using Reichert's Rotatory Microtome. The tissue sections were stained with Harris Haematoxylin and Eosin (H&E) stain and Masson's Trichrome stain. All the stained sections were examined using Compound PhotoMicroscope and some micrographs were taken from some sections using BEI Photonice microscope.

Results

In the control group, normal alveolar duct, alveolar spaces

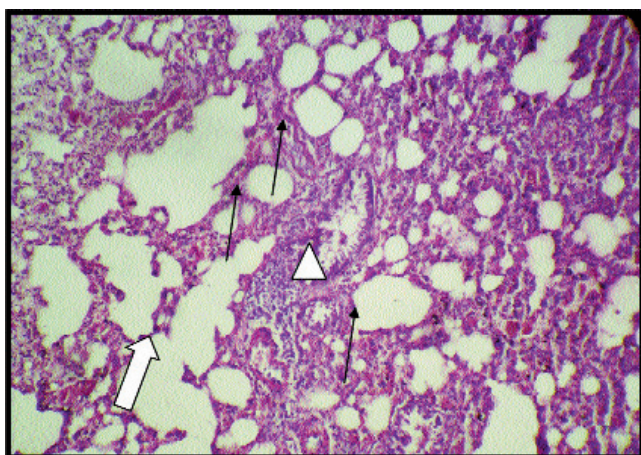


Figure-1: Photomicrograph of rat's lung of the control group showing the alveolar duct (white arrow) alveolar spaces (black arrows) and terminal bronchiole (arrow head) (Haematoxylin and Eosin stain X100).

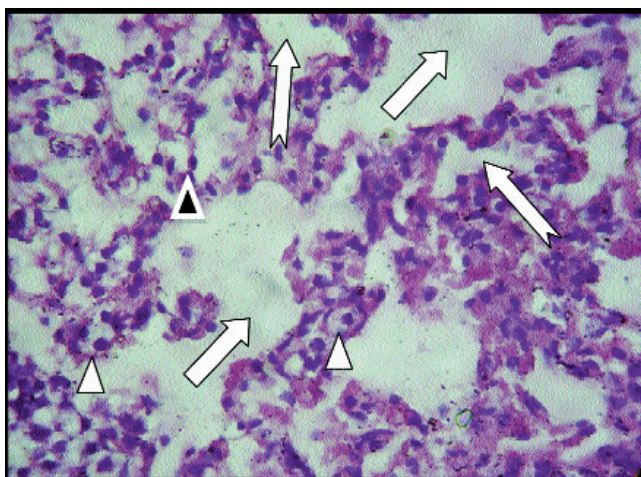


Figure-2: Photomicrograph of rat's lung of the control group showing the thin walled alveolar space (serrated white arrows), alveolar duct (white arrows) and pneumocyte type I (black arrow head) and pneumocyte type II (white arrow head) in the wall of alveoli (Haematoxylin and Eosin stain X 400).

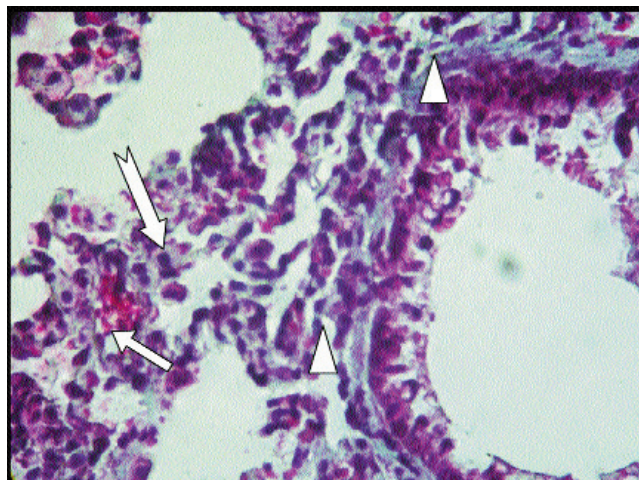


Figure-3: Photomicrograph of rat's lung of the control group showing few collagen fibre in the wall of alveolar spaces (serrated white arrow), in the wall of pulmonary capillary (white arrow) and in the wall of terminal bronchiole (arrow heads) (Masson's Trichrome stain X 400).

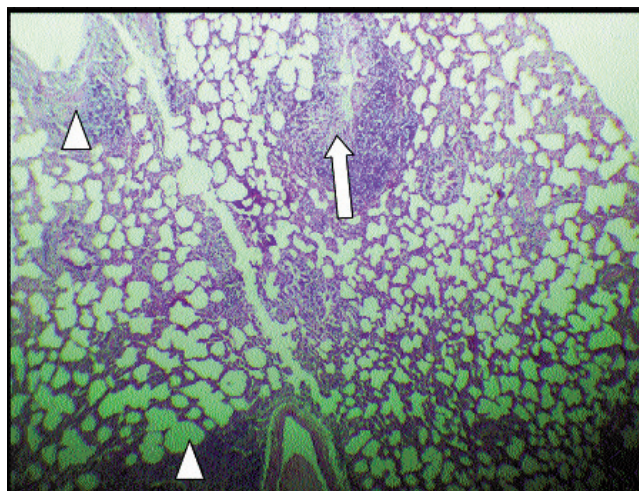


Figure-4: Photomicrograph of rat's lung of the experimental group receiving intraperitoneal injection of 5-FU (20mg/kg) showing thickening in the wall of terminal bronchiole (white arrow) and in the wall of pulmonary vessels (arrow heads) due to lymphocytic infiltration (Haematoxylin and Eosin stain X 40).

and terminal bronchioles with regular size and shape of the alveoli were found (Figure-1). Besides, thin interalveolar septae between the adjacent alveoli was lined by spindle shaped pneumocytes type I and rounded shaped pneumocytes type II (Figure-2). Few collagen fibers in the wall of alveolar spaces, in the wall of pulmonary capillary and in the wall of terminal bronchiole was seen on using Masson's Trichrome stain (Figure-3).

Among the cases, abnormal terminal bronchioles and

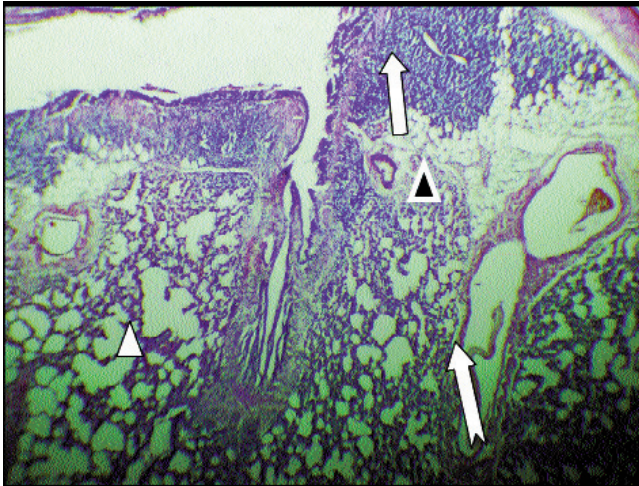


Figure-5: Photomicrograph of rat's lung of the experimental group showing emphysematous dilatation of alveoli (arrow head) and lymphocytic infiltration (white arrow) with congestion within the pulmonary vessels (serrated arrow head) and adipocytic infiltration within the pulmonary lobule (black arrow head) (Haematoxylin and Eosin stain X 40).

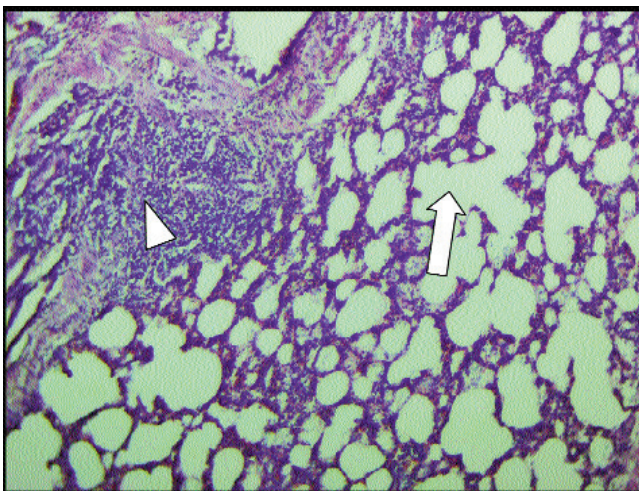


Figure-6: Photomicrograph of rat's lung of the experimental group showing emphysematous dilatation of alveoli (white arrow) with hyperplasia of bronchus associated lymphatic tissue (BALT) (arrow head) (Haematoxylin and Eosin stain X 100).

pulmonary vessels showing features of peribronchiolitis and perivascularitis appeared as thickening in the wall of terminal bronchiole and in the wall of pulmonary vessels due to lymphocytic infiltration (Figure-4). There was congestion within the pulmonary vessels with adipocytic infiltration within the pulmonary lobule (Figure-5).

Also seen were abnormal alveolar spaces with rupture and fusion of some adjacent alveoli causing emphysematous dilatation (Figure-5 and 6) with

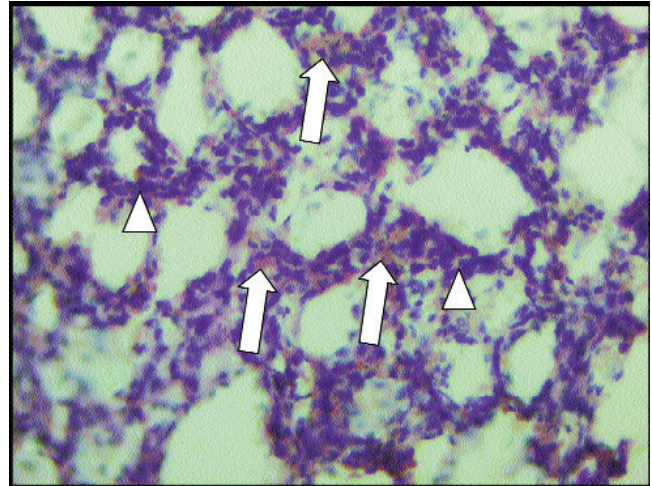


Figure-7: Photomicrograph of rat's lung of the experimental group showing thickening in the wall of alveoli with haemorrhage and congestion of capillary bed (white arrows) and mononuclear cell infiltration in the wall of alveoli (arrow heads) (Haematoxylin and Eosin stain X 400).

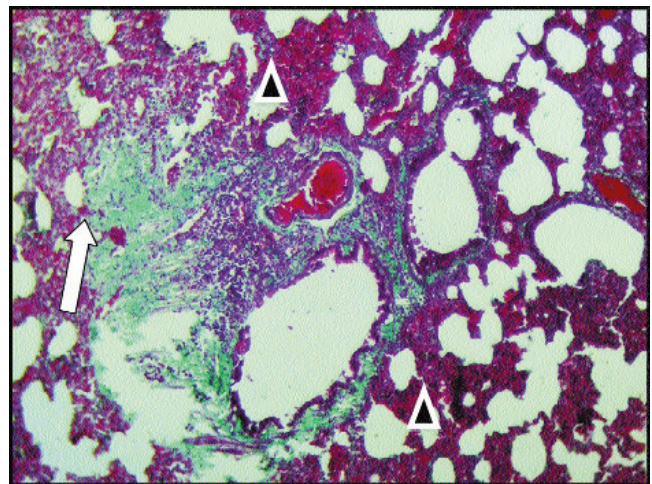


Figure-8: Photomicrograph of rat's lung of the experimental group showing a interstitial fibrosis and accumulation of collagen fibres in between the alveoli (white arrow) with congestion and fibrosis of capillary bed causing thickening of interalveolar septa (arrow head) (Masson's Trichrome stain X 100).

hyperplasia of bronchus associated lymphatic tissue (BALT), forming lymphoid follicles or aggregates.

Besides, there was thickening of interalveolar septae with haemorrhage and congestion of the capillary bed and mononuclear inflammatory cells infiltration both within and in the wall of alveoli (Figure-7). Interstitial fibrosis and accumulation of collagen fibres in between the alveoli with congestion and fibrosis of capillary bed causing thickening of interalveolar septae was also present (Figure-8).

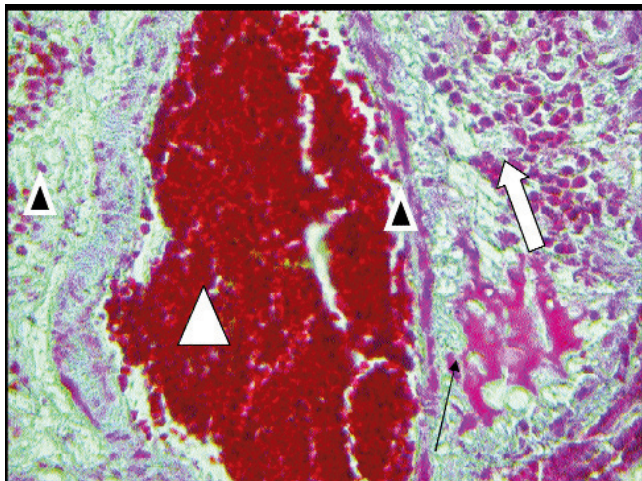


Figure-9: Photomicrograph of rat's lung of the experimental group showing congestion (arrow head) and fibrosis around the pulmonary vessel (black arrow heads) with mononuclear cell infiltration (white arrow) and formation of blood clot in the perivascular area (black arrow) (Masson's Trichrome stain X 400).

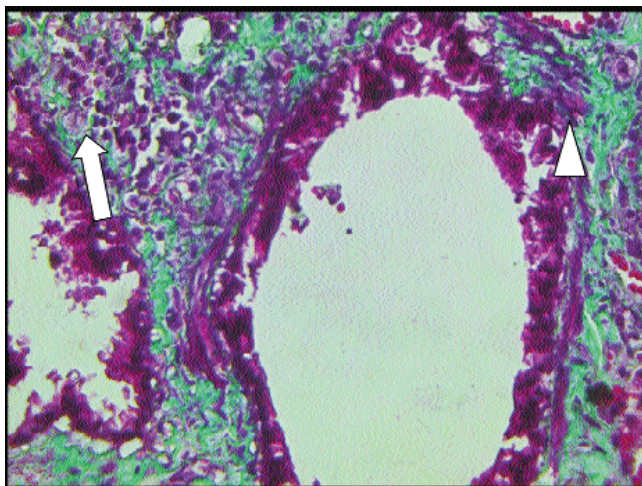


Figure-10: Photomicrograph of rat's lung of the experimental group showing fibrosis (arrow head) with mononuclear cells infiltration (white arrow) around the terminal bronchiole (Masson's Trichrome stain X 400).

Congestion and fibrosis around the pulmonary vessels with blood clot formation and mononuclear cell infiltration in the perivascular area was also seen (Figure-9). And, finally, there were increased collagen fibres due to hyperproliferation of fibroblasts in addition to mononuclear cell infiltration around the terminal bronchioles (Figure-10).

Discussion

The drug 5-FU is usually given with a carrier in case of intraperitoneal injection. The best carrier which can be

used in case of gastrointestinal tumours is HAS-Steri solution (neotype 6% hydroxyethyl starch), as this carrier will delay the absorption of 5-FU from the peritoneal cavity. This exposes the metastatic cells in the peritoneal cavity to 5-FU for a longer time before being absorbed by the peritoneum.⁹ Despite this fact we used the normal saline solution as a carrier for 5-FU as we wanted to get quicker absorption from the peritoneum and to achieve a higher level of toxicity of the drug as we were dealing with experimental model study, and not with the treatment of malignancies.

The occurrence of various structural changes in the lung tissue induced by 5-FU in our study as emphysema, inflammatory cell infiltration and interstitial fibrosis were nearly similar to what has been reported on the effects of rifampicin and isoniazide on the lung tissue.^{10,11} There are two types of alveolar cells, type I and type II pneumocytes. The latter produces surfactant whose main function is to lower the surface tension and also to protect the lung from different pathogens.¹² It seems that probably 5-FU impaired the alveolar surface tension due to the inadequate production of surfactant caused by the cellular damage of pneumocytes type II induced by the drug and thus most of the alveoli ruptured and communicated with the neighbouring alveoli, leading to emphysema. To the best of our knowledge, no previous explanation for the occurrence of emphysema induced by some drugs has been given by any study.

The major amount of connective tissue has a peribronchial location along with severe inflammatory cell infiltration around the terminal bronchioles (Figure-8) of the current study were nearly similar to what has been noted in the experimental lung fibrosis induced by bleomycin.^{13,14} This finding could be attributed to degradation induced by the drug, which, in turn, initiated inflammation with inflammatory cell infiltration.

One of the main causes in the pathophysiology and pathogenesis of broncho-pulmonary dysplasia and lung fibrosis is reactive oxidative species (ROS).^{15,16} It is well known that fibroblasts are responsible for synthesis and secretion of extracellular protein and collagen fibres.^{17,18} In our opinion hyperactivity of fibroblasts induced by 5-FU will lead to accumulation of collagen fibres and extracellular matrix in the interstitial spaces which will be converted into fibrous tissue later on.

Some previous studies noted that in normal lung, pneumocytes type II have been shown to secrete prostaglandin E₂, which can act to suppress fibroblast activity and growth.^{19,20} Thus, in cases of exposure to 5-FU, prostaglandin E₂ secretion is reduced, which, in turn,

leads to overproduction of fibroblasts and interstitial fibrosis. In addition, the fibroproliferative process may be the potential target for the treatment of pulmonary fibrosis in the future.²¹

Conclusion

The use of 5 FU in the treatment of some tumours has toxic effect on the structure of the lung, causing emphysema and lung fibrosis probably due to alteration in the surfactant production and reduction in the secretion of prostaglandin E2 and resulting in overproduction of fibroblasts.

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