Original Research Article

Pulmonary Changes in the Lungs of Wistar Rats Exposed to X-Ray Film Developer Solutions

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ABSTRACT

X-ray film developer solution fumes can be injurious when inhaled or ingested. This experimental study investigated the histological changes in the lungs of wistar rats exposed to varying concentrations of x-ray film developer solutions. Fifteen apparently healthy wistar rats weighing between 209g and 210g were put in three groups A-C of 5rats each. Groups A and B served as the experimental groups while group C was the control group. The rats in the experimental groups were exposed to varying concentrations of the x-ray film developer solution fumes for periods ranging from 15-30days (1st -30th July, 2014) while the rats in the control group were not. At the end of each desired period, a rat from each group was randomly selected, painlessly sacrificed and the lungs harvested and sent to the Medical Laboratory Sciences department of the Nnamdi Azikiwe University for examination and analysis. The findings showed histological changes of tissue injury evidenced by macrophage infiltration, distortion of interstitial tissue architecture, thickening of basement membrane, tissue fibrosis and formation of pleomorphic nuclei which are inflammatory responses indicating cellular injuries. The observed histological changes increased with the concentration of the developer solution and the duration of exposure to the developer solution. When these changes are extrapolated to a 70kg human subject, these histological changes would manifest within twenty-five year of exposure to the developer solution.

Key words: X-ray film developer solution, Wistar rats, Lungs, Histological changes.

INTRODUCTION

The exchange of respiratory gases is a basic essential process of life. In mammals, the respiratory system consists of the nasal cavity, the nasal and oral portion of the pharynx, the larynx, trachea, bronchi and the bronchioles and alveoli in the lungs where gas exchange occurs. The epithelial layer of the cartilaginous airways comprises a diverse array of pseudo stratified columnar cells. The ciliated, serous, Clara and goblet cells are essential to mucociliary defense. The ciliated cells dominate in the trachea-up to 60% of the total number of cells- with 20% of them being mucous secreting goblet cells. By the 50th year of life, the ciliated cells fall to about 15% and with advancing years, the ciliated and goblet cells are replaced by serous and Clara cells.
Inhaled gases/fumes move from the upper airways and reach the alveoli in the lungs. As the gas/fume passes deeper into the respiratory tract, more soluble gases are adsorbed and particles are deposited on the airways and alveoli. With deposition of particles diffuse changes occur that affect the lining of the airways—the epithelium and the structure of the bronchioles. This can result in symptoms of chronic obstructive pulmonary disease (COPD) such as emphysema, chronic bronchitis, asthma, idiopathic pulmonary fibrosis.\(^{(2)}\)

The production of analogue radiographic images involves two main processes— the x-ray exposure of the film emulsion (silver halide) and the chemical processing of the films. The x-ray exposure results in the formation of invisible images (latent images) that are formed as small clusters of metallic silver atoms on the silver halide due to the reduction of interstitial silver ions by photoelectrons. These latent images are made visible and permanent on the film base by chemical processing resulting in the reduction of silver ions to metallic silver. The processing solutions commonly in use in radiodiagnostic departments are the developer and fixer solutions. The x-ray film developer is composed of many components with Metol hydroquinone (MQ) and Phenol hydroquinone (PQ) as the main agents.

The processing of radiographs is as old as the x-ray exposure of radiographic films and the production of radiographic images. However, possibly because radiation hazards showed quicker appearances, hazards from the chemical processing of films appeared unrecognized. Evidences however, showed that serious hazards could result from the chemicals used for the processing of radiographic films. Sherwood\(^{(3)}\) observed a range of severe effects in individuals exposed to these chemicals even at low concentrations. Formaldehyde and Glutaraldehyde, major components of x-ray film processing chemicals have predilection for the respiratory system\(^{(4)}\) and can produce such effects as haemorrhage, alveolar wall thickening, pulmonary vessel dilation and inflammatory cell invasion of the lungs.\(^{(5)}\) Sulphur dioxide and acetic acid are also other components of processing chemicals that pose risks.\(^{(6)}\) Other symptoms related to exposure to processing chemicals include severe headache, gastrointestinal tract upset, urinary urgency, chest tightness, peripheral artery spasm, sore throat, sore eyes, runny nose/catarrh, skin rashes, nausea, joint pains and unexpected fatigue.\(^{[7-10]}\) Asthma was also observed in radiology darkroom staff exposed to the processing chemicals for moderate length of time.\(^{(11)}\) These collection of symptoms seen in radiology darkroom workers was termed “darkroom disease.”\(^{(12)}\)

Possibly the observation of these medical problems was the reason for the quest for the shift from analogue (darkroom) to digital (filmless) radiography which is already gaining grounds in the advanced countries of the world. In developing countries including Nigeria, radiology procedures are still highly darkroom based making it necessary to draw attention to the existence of these problems so that there will be collective efforts by individuals, organizations and governments to look for solutions to these problems.

This study is therefore, aimed at investigating the effects of x-ray film developer solution on the lungs of wistar rats. Wistar rats are used because their genetic, biological, physiological and behavioural characteristics can be reasonably compared to those of human beings.\(^{(13)}\)

**MATERIALS AND METHODS**

Ethical approval for this experimental research was obtained from the Research and Ethical Approval Committee of the Faculty of Health.
Sciences and Technology of Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria. The research was carried out between 1st and 30th July, 2014.

Fifteen (15) apparently healthy wistar rats of 20-24 weeks and weighing between 209g and 210g were used. These rats were obtained from the animal farm of Nnamdi Azikiwe University, Nnewi Campus. These rats were randomly divided into three groups (A-C) of five rats each - groups A and B as the experimental groups and group C as the control group. Each group of rats was put in a labeled metal cage and observed for one week to acclimatize in their new environment before the start of the study.

Materials used for the study included 3 metal cages, bell jar, weighing balance, dissecting set, an electron microscope, 5ml syringes, super starter feeds, feeding and water troughs, 10% formalin, cotton gauze, surgical gloves, chloroform, plain sample bottles, 500g weight developer powder, 10litre water tank, 50ml measuring jug, 2 small (3litre) plastic bowels.

Experimental Procedure: A full concentration of the developer solution (0.05g per cm³) was prepared by dissolving the 500g packet of developer powder in 10litres (10,000cm³) of water. 5000cm³ of the prepared developer solution was then diluted by equal volume of water to obtain a developer solution with half the concentration of the original solution (0.025g per cm³).

Wistar rats in the experimental group A were exposed to the fumes from the full concentration developer solution contained in a 3 litre plastic bowel placed close to the cage harbouring them. By the same method, the rats in the experimental group B were exposed to the fumes from the developer solution of half concentration. The rats in the control group, C, were not exposed to any developer solution fumes. The cages harbouring the rats were kept in different rooms of a house. The rats were fed with super starter feeds and water as prescribed by the manager of the animal house from where they were obtained. Each of the rooms housing the rats was lit by a 15watt energy bulb as advised by the manager of the animal house.

On the 15th day of the experiment, a rat was randomly selected from each group for dissection. Before dissecting the rats, each was weighed and their weights noted as follows: Rat from group A, 209g; rat from group B, 210g and rat from group C, 210g. Each of the rats was then anesthetized by placing it in a bell jar with a wire mesh floor over cotton gauze moistened with chloroform and observed for signs of decreased motility and unsteady gait for about 20 seconds. Each rat was then brought out of the bell jar and painlessly sacrificed with the proper incision made on the midline of the ventral aspect from the thoracic region to the abdomen. The lungs of each rat was then harvested, preserved in 10% formalin in a plain sample bottle and sent to the Medical Laboratory Sciences Department of Nnamdi Azikiwe University, Nnewi Campus for analysis.

On that same 15th day, two rats were randomly selected from each of the experimental groups (A and B) for pulmonary aspiration with the respective developer solutions. Before aspirating the rats with the developer solutions, the rats were labeled A1, A2, B1, B2 and weighed. The weights were: A1-205g, A2-206g, B1-210g and B2-208g.

To aspirate the rats with the developer solutions, each rat was anesthetized using the same procedure described earlier for the sacrificed rats above. Each rat was then brought out of the bell jar. Using a 5ml syringe each rat was aspirated with 0.5ml of the solution: rats A1 and A2, with the full strength developer solution and rats B1 and B2, with the half strength developer solution.
To instill the solution, the mouth of the rat was opened. The tongue was pulled to a side using a swap stick and the solution was introduced to pass down through the pharynx. The aspirated rats were then kept on a slab and observed to recover from the effects of the anesthesia and were put back into their respective cages where they stayed and continued with the non-aspirated rats in the group.

On the 30\textsuperscript{th} day (end of the experiment) all the rats in each group were painlessly sacrificed after anesthetizing with chloroform as described earlier for the rats sacrificed on the 15\textsuperscript{th} day.

**Tissue collection:** With a surgical blade a ventral midline incision was made from the thoracic region to the abdomen of each rat. The lungs were harvested and preserved in a vial of 10\% formalin. The organs were labeled according to their groups and mode of exposure to the developer solution (i.e. inhalation only or inhalation plus aspiration). The specimens were then sent to the Medical Laboratory Sciences department for examination and analysis of the histological and morphological changes that might have occurred.

**Tissue Preparation:** The lung tissues were measured (length x breadth x height), weighed and cut into thin slices of 3-5mm thick and 1cm long and studied under an electron microscope. The microscopic appearance was first observed for irregularities in shape, consistency, colour, firmness and presence of lesions and ulcers. After microscopic examination, the small pieces of tissues (3-5mm thick and 1cm long) were cut and put into labeled tissue cassettes and preserved in 10\% formalin before processing.

**Tissue Processing:** The tissues were manually processed by immersing them into a 10\% formalin bath for 1hour after which they were passed through 70\%, 90\% and 95\% absolute alcohol I, II and III for 2hours each and absolute alcohol IV overnight to dehydrate the tissues completely. On removing the tissues from the absolute alcohol, the tissues were passed through three changes of xylene (I, II, III) for 1hour 30minutes each to remove the alcohol. The tissues were then impregnated with paraffin wax by passing them through two wax baths (I and II) for 2hours and 1hour respectively. After this, the tissues were embedded in molten paraffin wax in a mould. This was achieved by using a heated forceps to orient the tissue in the mould until it lay in the desired plane. The corresponding labels (on the tissue cassettes) were transferred from the paraffin wax bath and placed against the side of the mould adjacent to the tissue. The mould was then transferred to ice block to solidify. On solidification, the tissue block was removed from the mould and attached to a wooden block using a heated knife.

**Sectioning the tissue block:** The wooden block to which the tissue block was attached was placed in the block holder in the microtome parallel to the microtome knife. Before sectioning, the tissue block was trimmed to expose the surface of the tissue by adjusting the microtome knife to 10\µm. Sections were then cut with the rotary microtome and the ribbons were placed onto 20\% alcohol on a large (5cm x 7.5cm) slices to remove minor folds and creases from the sections. The ribbon was then gently placed on a water bath preheated to about 45\degree C so as to float out the tissue. Using a clean slide, the tissue was collected, allowed to dry and then labeled using diamond pencil. The slide was then placed on a hot plane at 5\degree C for the tissue to adhere to the slide.

**Staining the tissue sections:** The sectioned tissue were stained using Erhlich’s haematoxylin and Eosin staining technique according to Avwioro \cite{14} for demonstration of the general tissue structure. The slides were taken to water and stained for 20minutes in Erhlich’s haematoxylin. The stained slides were then washed in distilled water with agitation for...
10 minutes after which they were differentiated in 1% acid alcohol for few seconds and then blued in Scott’s tap water for 2 minutes. This was followed immediately by counter staining in 1% aqueous eosin for 2 minutes. The stained slides were rinsed in water for 30 seconds, then dehydrated in ascending grades of alcohol (70%, 90% and 95% absolute I and II) for 2 minutes each. The stained slides were then cleared in xylene and mounted in dibutylphthalate polystyrene xylene (DPX).

**Microscopy and photomicrography:** Microscopic examination of the cut sections was carried out using Swift binocular microscope with in-built lighting system. Sections with striking features were selected for photomicrography using Olympus photomicroscope with coloured films. Results were expressed in terms of observed physical/behavioural changes in the rats and histological changes in the alveolar cells, epithelial lining of the bronchioles, bronchi and other cellular components of the lungs.

**RESULTS**

Towards the end of the experiment, the rats in the experimental groups were observed to show decreased social activities and no longer respond well to their feeds and water. They also exhibited non-significant loss in weight.

Photomicrographs of the lungs of the rat from the experimental group B (exposed to full concentration of developer solution) sacrificed on the 15th day showed macrophage infiltration and thickening of basement membrane (Plate 1) while that of the sacrificed rat from experimental group B (half strength developer solution) showed distorted interstitial tissue architecture, mild infiltrate of macrophages with pleomorphic nuclei (Plate 2).

At the end of the 30 days of the experiment, the photomicrograph of the lungs of the rats from group A exposed to the developer solution by both inhalation and aspiration (rats A1 and A2) showed distorted interstitial tissue architecture and mild infiltrate of macrophages (Plate 3) while the photomicrograph of the rats in that same group but exposed to the developer solution by inhalation only showed mild distortion of interstitial tissue architecture and infiltrate of fibrous tissues within the stroma (Plate 4).
The lungs of the rats from experimental group B that were exposed to half concentration of the developer solution by both inhalation and aspiration (rats B1 and B2) and sacrificed on the 30th day showed distorted interstitial tissue architecture and mild infiltrate of macrophages (Plate 5) but the lung of the rats exposed to the same developer concentration by inhalation only revealed infiltrate of macrophages and signs of inflammatory oedema (Plate 6).
The photomicrographs of the lungs of the rats in the control group (Group C) showed normal tissue staining with Erhlich’s Haematoxylin and Eosin staining (Plate 7).

DISCUSSION

Respiratory exchange of gas is a very essential process of life undertaken by the respiratory system. Any impediment to this process is always life threatening and so requires urgent attention for a solution.

The observed sluggish activities and loss of weight in the experimental group of rats are possibly signs indicative of the presence of a problem. Such decreased activities and weight loss are common in individuals with respiratory problems. The confirmation of the problem was the observed distortion of interstitial tissue architecture, infiltrate of macrophages and thickening of basement membrane which are defense responses initiated by the lungs and respiratory tract tissues to respond to inflammatory processes/injury caused by the inhaled developer fumes. The macrophages are large, mobile, highly phagocytic cells that become mobile when stimulated by inflammation and migrate to the affected area.

The findings in this study relate to the findings by Kheradmand et al [15] and Geiser [16] in lungs exposed to formaldehyde inhalation. The process of acute inflammation is a protective measure initiated by certain tissue cells, mainly resident tissue macrophages, mastocytes, histiocytes etc aimed at removing injurious stimuli and initiate a healing process. Whenever there is infection or cell injury these defense tissue cells are activated and release inflammatory mediators that are responsible for the clinical signs and symptoms of inflammation. In humans the pulmonary inflammatory responses will manifest as acute respiratory infection with early symptoms as nasal congestion, runny nose, cough, sore throat, body and joint aches and fatigue which might advance to high fever and chills.

It was discovered from this study that the degree of pulmonary histological changes was related to the concentration of the developer solution, the period of exposure and the quantity of the developer solution entering the lungs. The distortion of interstitial tissue architecture and formation pleomorphic nuclei agreed with the mechanism of excavation and desquamation of surface epithelium and derangement leading to ulceration of alveoli reported by Njoya et al. [17] The absence of any of the histological changes in the control group implicated the developer solution as the cause of those histological changes. The findings from this research may provide explanations for the symptoms found among radiographer in New Zealand exposed to fumes from darkroom film processing chemicals reported by Spicer et al. [9] and also the respiratory abnormalities among photographic developers reported by Kipen et al. [8] This may also account for the guest for filmless and digital radiography which is now practiced in many advanced countries of the world. But for many developing counties including Nigeria, darkroom radiography is practiced in more than 80% of radiology centres. And empirical observations reveal more respiratory problems among radiology workers than their counterparts in other professions.

Tissue macrophages, we know, are derived from monocyte blood cells and initiate defense and immune response against invading organisms and particles. However, some disease-causing organisms like the mycobacterium that causes tuberculosis and the leishmania that causes leishmaniasis have the ability to evade the macrophages defenses and rather enter the vacuoles of the macrophage and replicate [18] leading to serious ill-health.
When the duration of the experiment (period of exposure of the rats to the developer solution) and the concentration of the developer that caused the histological changes were extrapolated to a 70kg human subject, it was found out that such effects would appear in about 25 years. The implication is that in countries like Nigeria where the working period is 35 years, a darkroom worker will complete the remaining 10 years with respiratory problems and will retire from service miserably.

**CONCLUSION**

Various histological changes were observed in Wistar rats exposed to varying concentrations of x-ray film developer solutions. The changes were shown by infiltrates of macrophages, distortion of interstitial tissue architecture, pulmonary oedema, alveolar tissue fibrosis and formation of pleomorphic nuclei. The severity of the histological changes was related to the concentration of the developer solution and the period of exposure to the solution. In humans, such changes could manifest within 25 years of exposure at work place.

In view of the likely consequences of exposure of radiology workers (radiographers and darkroom technicians) to the x-ray film developer solutions efforts should be made to hasten the move from darkroom radiography to filmless or digital radiography in developing countries. Alternatively, more radiographers and darkroom technicians per radiology department should be employed to reduce the period of exposure to film processing chemicals and hence the probability of respiratory problems. Also the use of automatic film processors should be encouraged because exposure to developer solutions via automatic processors is less than for manual processors.

**REFERENCES**


