

ORIGINAL ARTICLE

Histological Study of Lungs in Rats Exposed to Chloroquine During Intrauterine life”

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ABSTRACT

Aims and Objectives: A study was carried out to see the effects of chloroquine on the lungs of rats exposed to chloroquine during their intrauterine life.

Type of Study : Descriptive cross sectional study

Materials and Methods: In this study, 12 pregnant female albino rats were used and divided in 4 groups, A (control) and B,C and D (experimental). Total gestational period in rats ranges from 20-22 days which in this study was divided into three trimesters of seven days each. Oral dose of chloroquine 700mg/kg body weight was given to group B in first trimester (day1 to day 7), group C in 2nd trimester (day 8 to day 14) and group D in third trimester (day 15 to term). After the control and experimental groups had delivered, their offsprings were selected at random (about 5/adult rat). On day 5 after birth, lungs were then dissected out, processed, blocks were made, cut and mounted. Sections were stained with haemotoxylin and eosin stains

Results: Histological sections of lungs of offsprings of group A showed typical normal histological structure of lungs. Microscopic sections of lungs of offsprings of group B showed no considerable change as compared to normal. Saccules and alveoli were mostly lined by type I pneumocytes. At some places type II pneumocytes and inflammatory cells were found. Inter-alveolar septum was thicker as compared to controls at few places. Histological sections of lungs of offsprings of C and D group showed that chloroquine reduced the expansion of saccules which normally occurred immediately preterm hence retarding foetal lung maturity. Saccular septa were much thicker and there was increased number of inflammatory cells in parenchyma. In offsprings of group D , saccular septa were the thickest and nearly all the saccules were lined by type II pneumocytes.

Conclusion: In conclusion, chloroquine caused retardation of maturation of lungs in rats. So its use should be discouraged in pregnancy.

Key Words: Chloroquine, lung development, albino rats, pregnancy

INTRODUCTION

Malaria remains the world's most devastating human infection and globally 125 million women are at risk of malaria every year¹. Prevention of malarial infection during pregnancy is an important concern as maternal malaria is associated with poor maternal and perinatal outcome. Pregnancy increases the susceptibility to malarial infection. The severity of malaria in pregnancy is thought to be due to general impaired immunity plus a diminution of acquired immunity to malaria in endemic areas. Malaria in pregnancy is different to the disease in the non-pregnant state².

Chloroquine has been used extensively for the prevention and treatment of malaria, is considered safe for use during pregnancy³. Chloroquine is a widely prescribed anti-malarial agent and is also used for treatment of autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis⁴. No harmful effects on the

fetus have been observed when chloroquine or hydroxychloroquine are used in the recommended doses for malaria prophylaxis⁵. The use of chloroquine for the prevention of malaria is considered safe at various stages of pregnancy but the resistance is common⁶. WHO currently recommends chloroquine for the effective treatment of plasmodium vivax malaria⁷.

Chloroquine crosses the placenta to the fetus with foetal concentrations approximately same as in mother . It is excreted in human breast milk⁸⁻¹⁰. Being a cationic amphiphilic drug, chloroquine strongly accumulates in the tissues of different organs. It causes the phospholipid storage disorder in the lungs. The alveolar macrophages show a pronounced response to drug treatment. There is a striking increase in the accumulation of alveolar macrophages in the alveolar spaces. The accumulated cells are larger in size and become engorged with lamellar inclusions¹¹⁻¹². In a study, it

was proved that uptake of chloroquine differed widely between slices of different organs with the sequence : lungs > kidneys = brain = liver > diaphragm = heart = skeletal muscles>adipose tissue¹³.Chloroquine treatment resulted in dilation of endosomes, lysosomes and lamellar bodies in type 11 pneumocytes. It also resulted in disappearance of lamellar organization of lamellar bodies¹⁴.

In a study, pregnant Sprague Dawley rats were injected with chloroquine phosphate (40mg/kg b.wt i.p) in the late canalicular (day 20) and early terminal sac (day 21) and sacrificed in the late terminal sac (day 22=term) stage of foetal lung development. Light microscopic examination of lungs showed reduction in volume density of parenchyma and saccular space, reduction in the volume of an average saccule and increase in the number of saccules per unit volume. These observations suggest that chloroquine retards foetal lung maturation by reducing the saccular expansion which takes place immediately preterm in preparation for post-natal gaseous exchange¹⁵⁻¹⁶. In another study, chloroquine 50 mg/kg b.w. was given to rats on day 20,21,and 22. It was found that chloroquine caused a premature development of the pneumocytes type II. Increased phospholipids (PL) content as well as disaturated phosphatidylcholine (DSPC) and phosphatidylglycerol (PG) were also found after drug treatment¹⁷.

Chloroquine (75 mg/kg b.w/day) given to rats resulted in increase in number and phospholipids content of alveolar macrophages. Excessive accumulation of phospholipids impaired the clearance function of alveolar macrophages¹⁸. In a study, histomorphometric techniques were used to study the effects of chloroquine on foetal lung maturation in rats. Results showed decrease in lung weight and increase surfactant associated phospholipids in lungs of offsprings. It was proved that chloroquine retards foetal lung maturation¹⁹.

In another study, pregnant rats were treated throughout the second half of pregnancy with chloroquine. Their offsprings (sacrificed immediately after birth) showed generalized lipidosis in lungs , liver, pituitary gland , adrenals , spinal cord and hypothalamus²⁰.

The present study is planned to see impact of chloroquine on lungs of newborn rats who were exposed to drug during intrauterine life and use evidence as recommendation for its use humans.

MATERIALS AND METHODS

In this study , 12 adult female rats (250-300 gms) and 4 adult male rats(300 to 350 gms) of Albino Wistar strain were used. Animals were kept in the Animal House of Post Graduate Medical Institute, Lahore for 15 days. They were provided with normal feed and tap water ad libitum. Male and female rats were kept in separate cages. Care was taken regarding optimal light and temperature. For conception three female rats and one male rat were kept together in a cage for a week and then male rat was removed from the cage. Female rats were observed daily for signs of pregnancy which was confirmed by presence of vaginal plug. Presence of vaginal plug was taken as day one of pregnancy. After conception male rats were separated and 12 female rats were divided into 4 groups A, B, C and D containing 3 animals each. Total gestational period in rats ranges from 20-22 days, which in this study was divided into three trimesters of 7 day each. The rats were weighed and marked. They were placed in their respective cages which were labelled by tags.

Group A:

This was a control group containing 3 animals, which were fed on normal diet throughout pregnancy.They were allowed to complete their gestational periods without drug intake.

Group B:

Containing 3 animals , were given oral dose of chloroquine 700mg/kg body weight during 1st trimester of pregnancy(day 1 to day 7).

Group C:

Containing 3 animals , were given oral dose of chloroquine 700mg/kg body weight during 2nd trimester of pregnancy(day 8 to day 14).

Group D:

Containing 3 animals, were given oral dose of chloroquine 700mg/kg body weight during 3rd trimester of pregnancy (day 15 to day term).

After the control and experimental groups had delivered, their offsprings were selected at random (about 5/adult rat). On day 5 after birth, they were anaesthetized by cotton pledget soaked with chloroform. Both lungs were then dissected out and placed on blotting paper to make them free of surrounding fluid . The lungs were processed in an autoprocessor. Blocks were made, cut, mounted and sections were stained with haemotoxylin and eosin stains .

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RESULTS

Control Group (A):

The offsprings showed typical histological structure of the lung. The epithelium was composed primarily of type 1 pneumocytes lining alveoli. Inter-alveolar septa were thin. Few fibroblasts were seen located in the corners of the alveoli and in the base of the secondary septa. Capillaries were seen adjacent to alveoli .

Experimental Groups

Group B:

The epithelium was composed primarily of type 1 pneumocytes lining alveoli. Type II pneumocytes were also found. Inter-alveolar septa were thick at few places. Septum between saccules was thick as compared to control at some places. Capillaries were seen adjacent to alveoli . Few inflammatory cells were also present. Saccular pattern was evident and at few places alveoli were formed by formation of secondary crests in saccules. (Fig 1).

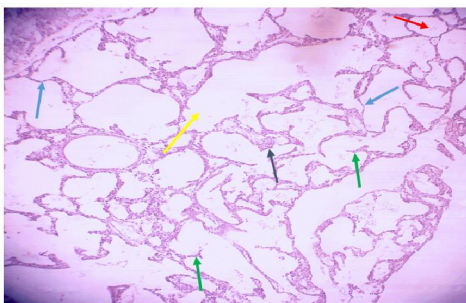


Fig 1: Histological section of lungs of offsprings of Group B

Yellow arrow __ Saccule
 Black arrow __ Type II Pneumocytes
 Blue arrow __ Type I Pneumocytes
 Red arrow __ Alveolus
 Green arrow __ Secondary crest in saccular wall

Group C :

The epithelium consisted mostly of type II pneumocytes, found in groups of 3-5 cells. At few places type I pneumocytes were also found . Capillaries were found in the walls of the sacculi and alveoli, close to the saccular and alveolar lumina. Secondary septa were seen to protrude from the saccular walls. Saccular septa were thicker as compared to group B. There was increased number of inflammatory cells in parenchyma. Lung showed the saccular pattern of lung development instead of alveolar pattern at this age (Fig 2).

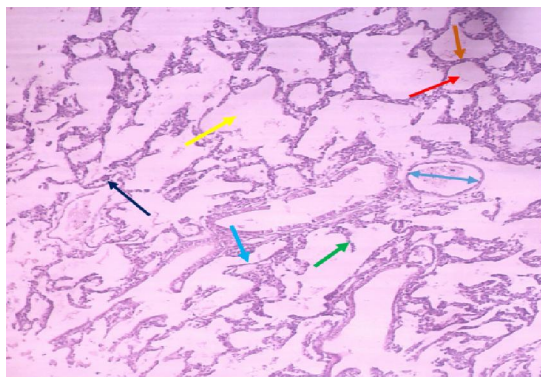


Fig 2: Histological section of lungs of offsprings of Group C

Yellow arrow __ Saccule
 Black arrow __ Type II Pneumocytes
 Blue arrow __ Type I Pneumocytes
 Red arrow __ Alveolus
 Green arrow __ Secondary crest in saccular wall
 Orange arrow __ Thick interalveolar septum
 Double arrow __ Blood vessel

Group D:

The epithelium consisted mostly of type II pneumocytes, found in groups. Septa were seen to protrude from the saccular walls. Saccular septa were very much thicker as compared to group C and B. There was increased number of inflammatory cells in parenchyma. Lung showed the saccular pattern of lung development instead of alveolar pattern at this age (Fig 3).

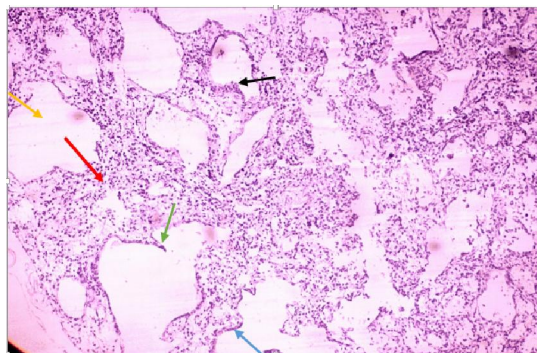


Fig 3: Histological section of lungs of offsprings of Group D

Yellow arrow __ Saccule
 Black arrow __ Type II Pneumocytes
 Blue arrow __ Thick septum between saccules
 Red arrow __ Inflammatory cells
 Green arrow __ Secondary crest in saccular wall

DISCUSSION

The results of present study revealed that chloroquine retarded the maturity of foetal lung in rats. There are very few published studies assessing the safety of medications during human pregnancies so data from animal teratogenicity studies are extremely valuable²¹. The beginning of lung development in rats takes place around the 10th day of intrauterine life. The lung buds are formed at the ventral surface of the foregut²². There are five main stages of lung development in rats. They include pseudoglandular, tubular, canalicular, saccular and alveolar stages. In pseudoglandular (fetal day 15 and 16) epithelial tubes lined by columnar epithelium was not associated with blood capillaries. In tubular stage (fetal day 17 and 18), blood capillaries begin to oppose epithelial tubes lined by cuboidal epithelium (type II cells). In canalicular stage (fetal days 19 and 20) sac like end segments showed progressive thinning of epithelial linings and cells differentiated to squamous type I cells. In terminal sac period (neonate through the 3rd day of life) the wall of terminal sacs showed a thin epithelial lining. Blood capillaries protruded close to airway. In alveolar stage (day 3 onwards), secondary crests develop in the saccular wall and result in formation of alveoli. Alveolar multiplication may occur throughout life in the rat²³⁻²⁶. Chloroquine being an amphiphilic cationic compound caused decrease in volume density of parenchyma, saccular space and average saccular volume in lung hence retarding foetal lung maturity¹⁴.

Present study revealed that chloroquine when given in pregnancy caused retardation of maturation of lungs in offsprings of rats which was evident by scarcity of type I pneumocytes and presence of type II pneumocytes in abundance especially in those animals exposed to chloroquine in 2nd and 3rd trimesters in their intrauterine life. In offsprings of Group C and D, saccular pattern of lung development was present. In a study, chloroquine when given to pregnant rats in late terminal stage, reduced saccular expansion which takes place immediately preterm in preparation for post-natal gaseous exchange and thereby retards foetal lung maturation¹⁵, a finding similar to the present study.

CONCLUSION

Chloroquine caused retardation of maturation of lungs in rats those were exposed to drug during their intrauterine life especially in last trimester of

pregnancy. Thus its use during pregnancy should be discouraged.

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