THE MORPHOLOGY OF THE RAT FOETAL LUNG FROM CHLOROQUINE TREATED MOTHERS

by

A. O. OKANLAWON
Anatomy Department
College of Medicine
University of Lagos
P.M.B. 12003, Lagos, Nigeria

INTRODUCTION

Chloroquine is used in man for the treatment of malaria, rheumatoid arthritis and collagen diseases(1). Malaria alone affects more than 200 million people in the world (2) and is a major problem in many tropical and subtropical areas. For most of these people, chloroquine (a 4-aminoquine active only against asexual erythrocytic stage) is still the main and often the only drug used for malaria control, especially in rural areas where it is used as self medication by the population since the drug may be bought on the open market (2). However prolonged treatment with chloroquine has been reported to result in retinopathy, and Cochleovastubular apparatus paresis (1).

Chloroquine has also been shown to cause increased phospholipid storage in liver, spleen, lung and other body tissues (3) when administered chronically to man and animals. With regard to tissue phospholipid storage, it is clear that the lysosome is the principal site to be considered when seeking the biochemical basis for this condition (4,5). A variety of intralysosomal catabolic pathways are inhibited by chloroquine partly because of its ability to raise intralysosomal pH (4).

Electron microscopic study of tissue from human and rats (3) treated with chloroquine indicates that a prominent feature of the Lipidosis is the presence in the Cytoplasm of numerous autophagic vacuoles and multilamellar bodies (also called Myelin figures). These multilamellar bodies are enriched in acid phosphatase indicating their lysosomal origin and in addition contain large amount of phospholipid (5). Multilamellar bodies are structurally similar to the lamellar bodies of type II alveolar cells which are considered the storage sites of intracellular surfactant prior to secretion (6). The importance of surfactant as a surface active agent in the prevention of pulmonary collapse in the Newborn at the end expiratory volume has been well established (7). Thus any defect in the synthesis and or release of surfactant results in the Respiratory distress Syndrome (RDS) of the newborn and is largely found in preterm babies whose lungs are still too immature to synthesize surfactant.

This work represent an attempt to determine, by an indirect approach using structural observation, the effect of chloroquine on surfactat release during foetal lung maturation.

MATERIALS AND METHODS

All experiments were conducted on albino Sprague-Dawley rats which had unrestricted access to rats' feed (PFIZER NIG. LTD.) and Water, in a room with a temperature range of 24–26°C, and day light exposure from 06.00 to 19.00 hours daily. The day of mating was considered day zero.

Chloroquine phosphate (25mg/kg) was injected intraperitoneally on day 20 and 21 of gestation and the animals were killed 24 hours later. The control group received saline (0.9% Nacl). Another group of animal treated with chloroquine on day 20 and 21 also received adrenaline (50mg/kg) on day 21. The drugs were given within one hour of each other. All animals were sacrificed on day 22 (Term) and the foetuses were immediately removed, and weighed.

The foetal lungs were also immediately removed, bottled dry, weighed and fixed in 10% formaline. Only the left lung was used for morphological studies, while the right lung was reserved for DNA estimation. Paraffin sections, 5U-thick were prepared from 4 to 5 foetuses from each of the control and experimental groups. The sections were stained with haematoxylin and eosin. Three slides, at least, were examined from each foetus.

The stages of foetal lung development were identified according to the criteria described by Boyden (8) and Hislop and Reid (9). The distinguishing feature of the pseudoglandular period is the presence in the lung tissue of tubules lined by a light-microscopically distinct epithelium composed of columnar cells and surrounded by mesenchyme while the canalicular period is characterised by the presence of cuboidal cells. The specific feature of the terminal-sac period is the presence of structures (called saccules) which have a relatively large lumen and rather smooth walls and form clusters.

STATISTICS

Statistical comparison were performed with the one-way analysis of variance (ANOVA) with subsequent Fisher's

least significant different test (10). Where necessary, values of $P < 0.05$ were considered significant. Significance was tested at the 0.05 and 0.01 levels.

RESULTS

Body weights and lung weight increase rapidly over the latter days at gestation (Table 1). The lung weight was neither affected by chloroquine nor by the chloroquine and adrenaline injection (Table 2). In constrast chloroquine and adrenaline injected foetuses had larger body weight ($P < 0.01$) than control or chloroquine-injected foetuses. The ratio of lung weight to body weight was not affected by either chloroquine or chloroquine and adrenaline injection.

TABLE 1: BODY AND LUNG WEIGHT IN FOETAL RATS AT VARIOUS GESTATION AGES (G.A.) (MEAN + S.E)

G.A. G.A.	NO. OF FOETUS*	BODY WT.(G)	LUNG WT. (G)	LUNG WT. (MG) BODY WT. (MG)
19	16 (2)	0.87 ± 0.02	28.50 ± 1.96	0.0326 ± 0.0024
20	10 (2)	1.66 ± 0.16	49.66 ± 4.38	0.0299 ± 0.0016
21	15 (2)	3.47 ± 0.08	97.29 ± 4.02	0.0280 ± 0.0013
22	13 (2)	4.05 ± 0.11	135.00 ± 6.12	0.0330 ± 0.009

* = Number of litters is shown in Parentheses.

TABLE 2: EFFECT OF CHLOROQUINE, CHLOROQUINE AND ADRENALINE ON BODY AND LUNG WEIGHT AT DAY 22 GESTATION (MEAN + S.E.)

GROUP	NO. OF FOETUSES*	BODY WT. (G)	LUNG WT. (MG)	LUNG WT. (MG) BODY WT. (MG)
CONTROL	13 (2)	4.05 ± 0.11	135.00 ± 6.12	0.0330 ± 0.0009
CHLOROQUINE (CH)	16 (3)	4.26 ± 0.11	119.8 ± 18.3	0.0279 ± 0.0026
CH + ADRENALINE	18 (3)	4.76 ± 0.14**	134.0 ± 5.4	0.0282 ± 0.0035

* Number of litters is shown in Parentheses, ** $P < 0.01$

Figure 1 showed that the air spaces were smaller and the interstitium appeared more cellular in the chloroquine-treated than in controls. The air spaces in the chloroquine-treated group have smooth walls and are lined by columnar epithelium. The lung thus appear to be in the camalicular phase of lung development. Figure 2 shows the effect of adrenaline on chloroquine treated animals. The lungs of these animals appeared more mature compared with the chloroquine-treated group. The interstitium less compact and the air spaces have relatively large lumen and thin walled. The lung thus appeared to be in the late terminal-sac phase of development.

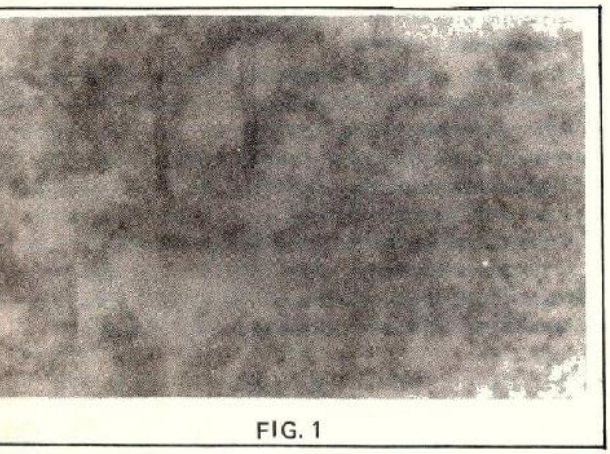

FIG. 1

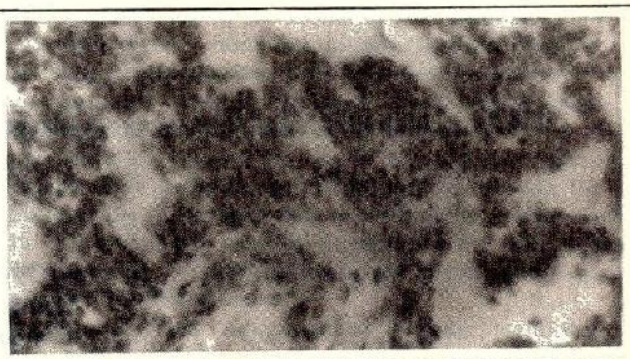

FIG 2: FOETAL LUNG FROM MOTHERS INJECTED WITH CHLOROQUINE AND ADRENALINE DAY 22 = TERM x 400 H/E STAINING TECHNIQUE

DISCUSSION

The present result suggest that there is a retardation of foetal lung maturation secondarily to chloroquine treatment which was prevented by adrenaline. Chloroquine is an antimalarial drug with amphiphilic properties (3). It has been established that the amphiphilic nature of the drug plays a role in the degree of phospholipidosis seen in animal experiments (3, 11). In chloroquine-treated rat foetuses, the phospholipid content of lavaged lung tissue has been shown to be actually higher than in control (12). Therefore it was suggested that chloroquine may be a potential therapeutic agent for diseases caused by surfactant deficiency, like the respiratory distress syndrome. In contrast morphological data from this experiment suggest that the lungs are immature as a result of chloroquine treatment. The evidence is therefore that the whole lung level of lavaged phospholipid is not an accurate reflection of pulmonary maturity.

Administration of AY-9944, a cholesterol inhibitor, directly into rabbit foetuses resulted in a similar condition: a retardation of lung development despite elevated surfactant (13). Maturation of the foetal lung appeared to stop completely during AY-9944 treatment even though an increased accumulation of lamellar bodies occurred in type II cells. In another study, Thomas-Laurie et al (14) reported that prepartum maternal administration of chlorphentermine (an anoretic) resulted in the affected neonates gasping for breath, indicating an impairment in pulmonary function. In the neonates, their lungs wee judged to be mature; their plycogen levels were similar to those of the controls and the content of disaturated phosphatidycholine, the major surface-active lipid in the lungs, was elevated over that present in control foetuses. They however reported that the level of this surface-active material was lower in Amniotic fluid of drug-treated mothers when measured one day prior to term. These data suggest that there may be an impairment in the secretion of surfactant into the alveolar air space.

Additional support for this suggestion was obtained in the adrenaline experiment. Adrenaline injected in chloroquine-treated animals improved the maturation of foetal lung. The

simplest explanation of this observation is that adrenaline increased the secretion of lung surfactant into the alveolus (6, 15). The precise mechanism is not well understood. However it has been suggested that increased secretion of surfactant caused by adrenaline might be due to increased calcium mobilisation, as calcium is required for coupling membrane fusion and surfactant release (6).

Surfactant secretion is also an active process which requires calcium (6). It is significant to note that chloroquine inhibits oxidation in the mitochondrion (16) and also inhibits calcium binding (17). Also chloroquine has an inhibitory effect on prostaglandin formation (5, 18) an agent which is also needed for surfactant secretion in addition to calcium. These result suggest that chloroquine may have been interfering with calcium mobilisation (17) for the coupling of membrane fusion and surfactant release.

It is of interest to observe that the foetal body weight was larger in the chloroquine plus adrenaline experiment. Although it is known that adrenaline inhibits insuline secretion and stimulates hepatic glucose formation from glycogen (glycogenolysis) leading to hyperglycaemia, glucose intolerance, glucosuria and weight loss (19) we do not have any explanations for the increased weight in chloroquine plus adrenaline experiment. A generalised hyperglycaemia in the foetus is not an adequate explanation because hypermetabolism induced by adrenaline should lead to weight loss.

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