Pulmonary Alveolar Proteinosis
Developing from Desquamative Interstitial Pneumonia
in Long Term Toxicity Studies of Iprindole* in the Rat

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Summary. Rats fed on a diet containing 0.1% iprindole for up to 12 months were observed to develop pulmonary alveolar proteinosis, which evolved through a stage of desquamative interstitial pneumonia. The changes were studied sequentially by light and electron microscopy and histochemistry.

Degenerative changes in the alveolar capillary endothelium led to interstitial oedema, whilst there was marked lipidic degeneration of the type I alveolar epithelial cells. Myelin figures derived from the epithelial cells were initially taken up by alveolar macrophages which came to fill the air spaces. When these cells later broke down, compaction of the released lamellar lipid, originally derived from epithelial cells, resulted in the appearances of alveolar proteinosis.

In conjunction with previous reports of alveolar proteinosis developing in man and experimental animals following exposure to a wide variety of dusts, the present findings suggest that alveolar proteinosis represents a non-specific response to lung injury.

Introduction

We have previously shown that the anti-depressive drug iprindole administered orally in large doses to rats results in pulmonary histiocytosis which resembles human desquamative interstitial pneumonia (Vijeyaratnam and Corrin, 1972a). We have also described the primary changes in the alveolar wall which provoke this outpouring of alveolar macrophages: soon after iprindole administration is started degenerative changes appear in the alveolar epithelium and capillary endothelium, and interstitial oedema develops (Vijeyaratnam and Corrin, 1972b). It was anticipated that with continued iprindole administration these changes might progress to interstitial fibrosis, and long-term experiments were therefore undertaken. In these however, the mild alveolar wall thickening remained static and no fibrosis developed, both the exudative changes gradually altered and from a desquamative interstitial pneumonia-like picture evolved the appearances of pulmonary alveolar proteinosis. The pathogenesis of both these conditions in man is obscure and their relationship poorly understood. A description of the experimental changes, which have been studied sequentially at both the light and electron microscopical level, should therefore be of value.


1 Virchows Arch. Abt. A Path. Anat., Bd. 358
Materials and Methods

The animals used were 3-month-old specific pathogen-free Charles River strain male rats weighing 170 g. They were divided into two groups, 26 test animals and 14 controls, and each animal was weighed at the beginning and end of the experiment. All animals were fed a powdered diet ad libitum, to which iprindole (John Wyeth and Brother Ltd., Maidenhead, Berks.) was added in the case of the test rats in a concentration of 0.1% by weight. Four test rats and 2 controls were killed by exposure to pure nitrogen at the end of 3, 4, 6, 9, and 12 months. Iprindole was withdrawn from the diet of the surviving test rats at 12 months and all the remaining animals were killed 5 months later.

At death the thorax was opened and 2 ml of cold (4 °C) 0.1 M cacodylate-buffered 4 per cent paraformaldehyde (pH 7.4) was gently instilled through the trachea into the lungs. The trachea was then tied off and the lungs immersed in the fixative for 10 mins. Tissue blocks, of sizes suitable for light and electron microscopy, were then cut and fixation continued at 4 °C for 1–5 days. For light microscopy the tissues were processed to paraffin and 7 μm sections were stained with haematoxylin and eosin, periodic acid—Schiff reagents with and without diastase treatment and for iron. Frozen sections were stained for neutral fats with oil red 0 and Sudan Black and for phospholipids by the silver hydroxylamate method of Adams, Bayliss and Ibrahim (1963). The Schultz reaction and the digitonin method were employed for the detection of cholesterol and its esters. Oxidative and hydrolytic enzymes were localised by techniques used previously (Vijeyaratnam and Corrin, 1972a). For electron microscopy the tissues were post-fixed at 4 °C for 1 hour in 1 per cent osmium tetroxide containing 0.12 per cent sucrose buffered to pH 7.4 with veronal acetate. Suitable thin Epon-embedded sections stained with uranyl acetate and lead citrate were examined in a Siemens Elmiskop I.

Results

The rats appeared to be in good health and showed no signs of respiratory distress. None died spontaneously during the experimental period, but the test rats did not gain weight as well as the controls. (Av. body weights after 12 months: test rats 510 g, control 600 g.) Post mortem, pale irregular areas were found on the lung surfaces of test rats, gradually increasing in number and size to measure 6–8 mm in diameter by 12 months.

Light Microscopy

The most striking lesion seen in the lungs after 3 months of medication is the presence of numerous cells within many alveolar spaces. The intra-alveolar cells are similar to those observed in the desquamative interstitial pneumonia-like condition present at 6 weeks (Vijeyaratnam and Corrin, 1972a), but show marked vacuolation and have an abundant foamy cytoplasm (Fig. 1). Multinucleated forms are numerous. Nuclei are pyknotic in some cells and are not seen in many others. The free intra-alveolar cells progressively increase in number and size but later appear to break down, filling the alveoli with pale eosinophilic granular material (Fig. 2). Thus by 6 to 9 months, several alveolar spaces are filled with this granular material, whilst others still contain numerous intact cells. Some alveoli contain both granular material and cells, many of which are in various stages of degeneration and disintegration. The pale granular eosinophilic material is weakly periodic acid-Schiff positive. After 1 year of drug treatment, the cellular breakdown products in some regions condense to form a more deeply eosinophilic mass (Fig. 3) which is strongly periodic acid-Schiff positive and diastase resistant. In these areas the appearances mimic those of human