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Pulmonary Heart Disease Induced by Disenecioyl Dehydroretronecine: A Semisynthetic Pyrrolizidine Alkaloid Pyrrole[1] (39603)

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Ingestion of the pyrrolizidine alkaloids (PAs), phytotoxins found in plants of the genera *Senecio, Crotalaria,* and *Heliotropium,* causes severe hepatic and cardiopulmonary lesions in man and animals (1). Pyrrolizidine alkaloids themselves appear to be relatively nontoxic; however, as a result of metabolism to PA pyrroles the toxicity is markedly enhanced (2). Pyrrolic metabolites cause pulmonary fibrosis, pulmonary hypertension, and cardiac hypertrophy (3, 4).

In the past it has been difficult to evaluate the mechanisms of action of the PAs and their pyrrole metabolites primarily because of the difficulty in preparing them in a radioactive form. Recently this difficulty has been partially circumvented by the synthesis of the radioactive semisynthetic pyrrolizidine alkaloid, [^{3}H]disenecioyl retronecine (5). In the present report, data are presented which show that disenecioyl dehydroretronecine, the pyrrole of disenecioyl retronecine, produces lung lesions similar to those of naturally occurring PA pyrroles.

Materials and methods. Disenecioyl dehydroretronecine (DSDR) was prepared as previously described (6). Briefly, retronecine (7β-hydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine) was acylated with senecioyl chloride (3,3-dimethyl-acryloyl chloride) to yield the semisynthetic PA disenecioyl retronecine (di-3,3-dimethyl acrylic acid ester of retronecine). This was then oxidized with $KMnO_4$ to disenecioyl dehydroretronecine (di-3,3-dimethyl acrylic acid ester of dehydroretronecine).

Twenty-five male ARS-Sprague-Dawley rats, initially weighing 45-55 g, were randomly divided into a control group of 10 rats and an experimental group. Control rats received 0.05 ml of dimethylformamide (DMF) iv into the left jugular vein while the experimentals were injected via the same route with 15 mg of DSDR/kg body weight (21 mg DSDR/ml DMF). Rats were sacrificed 14, 21, 28, and 35 days postinjection by cannulating the trachea and infusing the lungs with 4% formalin in saline at 3 cm of water pressure (8). The lungs and heart were removed *en block* and placed in jars containing fresh Carstair's fixative (8). Other tissues were fixed in 10% buffered formalin. After fixation, the hearts were cut and the right ventricle (RV), left ventricle (LV), and interventricular septum (IVS) were individually weighed. The right ventricular ratio (RVR) was calculated by the formula: RVR = RV g/(LV + IVS g). A RVR greater than 0.50 was considered as indicative of right ventricular hypertrophy (RVH) (9). Sections from the lung and other major organs were stained with hematoxylin and eosin. Additional lung sections were stained with Carstair's stain for fibrin (8).

Results. Three experimental rats which died spontaneously on Days 6 and 9 postinjection and three experimental animals sacrificed 14 days postinjection did not develop RVH and showed only a diffuse, mild septal hyperplasia and fibrosis in the lungs. The nine experimental animals sacrificed 21 to 35 days after injection had an $\bar{X}$ RVR of 0.58, of these, eight had RVH. The eight control rats sacrificed at the same time period had an $\bar{X}$ RVR of 0.26. Table I summarizes the pulmonary alterations found in the animals sacrificed 21 to 35 days postinjection. In contrast to controls, extensive arteriolar intimal hypertrophy and hyperplasia

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were commonly seen (Fig. 1). These lesions, while focal, were seen distributed throughout the lungs of animals with RVH. Other pulmonary alterations included increased numbers of alveolar macrophages, septal edema, interstitial fibrosis, and accumulation of fibrin in the walls of the arterioles. Typically, the right myocardium of animals with RVH showed myocyte hypertrophy, interstitial edema and hypercellularity, and focal necrosis (Fig. 2). Structural alterations were not seen in the left ventricle or interventricular septal myocardium.

Discussion. This and a previous report from our laboratory (6) demonstrate that DSDR causes hepatic and cardiopulmonary lesions similar to those produced by the naturally occurring PA pyrroles dehydromonocrotaline (4) and dehydroretronecine (7). Like the latter two, DSDR would appear to exert its vasotoxic effects on the endothelium of the first microvascular system it enters. Here it initiates a sequence of changes leading to pulmonary microvascular occlusion, resulting in increased resistance to pulmonary blood flow and the subsequent development of right heart hypertrophy. Associated with these changes are septal thickening due to edema and hypercellularity, increased numbers of alveolar macrophages, and, in the right heart, myocyte hypertrophy and focal myocytolysis. Since only minimal isolated alterations were seen in the veins of the lung, it may be assumed the semisynthetic PA pyrrole failed to escape the lung. We feel that the cardiac lesions seen in this study are therefore secondary to the pulmonary lesions.

The lung lesion produced by the PAs differs slightly from those caused by the PA pyrroles. As with the pyrroles, there is thickening of the alveolar septae, increased alveolar macrophages, and occasionally a periarteritis (3). Unlike the pyrroles, the PAs cause medial hypertrophy of the small muscular arteries of the lung. This hypertrophy has been attributed to the development of pulmonary hypertension (10). However,

TABLE I. LESIONS DEVELOPING IN RATS AFTER A SINGLE IV INJECTION OF DISENECIOYL DEHYDRORETRONECINE.[a]

Lesion	Treatment: DSDR[b] 15 mg/kg	Treatment: Control 0.05 ml DMF[c]
Arteriolar intimal hypertrophy and hyperplasia	8[d] (9)[e]	0 (8)
Interstitial cellular hyperplasia	8 (9)	0 (8)
Interstitial fibrosis	7 (9)	0 (8)
Septal edema	6 (9)	0 (8)
Fibrin in wall of arterioles	6 (9)	0 (8)
Periarteritis	3 (9)	0 (8)

[a] Animals sacrificed 21 to 35 days following injection.
[b] DSDR = Disenecioyl dehydroretronecine.
[c] DMF = Dimethylformamide.
[d] Number of animals with lesion.
[e] Number of animals injected.

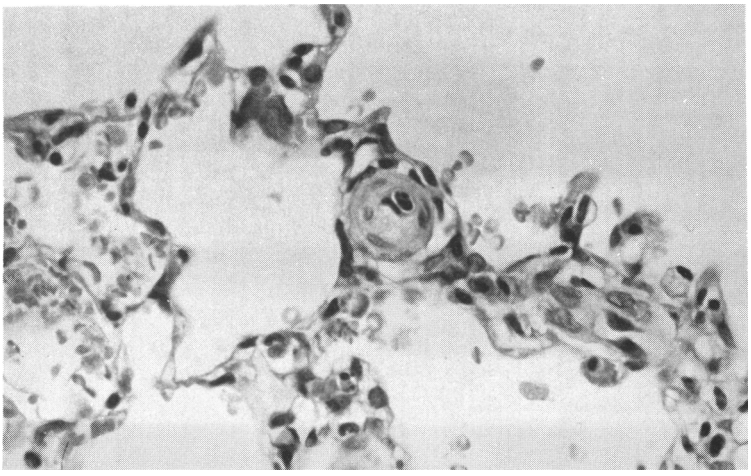

FIG. 1. Effect of disenecioyl dehydroretronecine on the lung in a rat sacrificed 28 days postinjection. The rat had a right heart ratio of 0.63. Note the occluded arteriole and the septal hypercellularity. H & E stain. × 270.

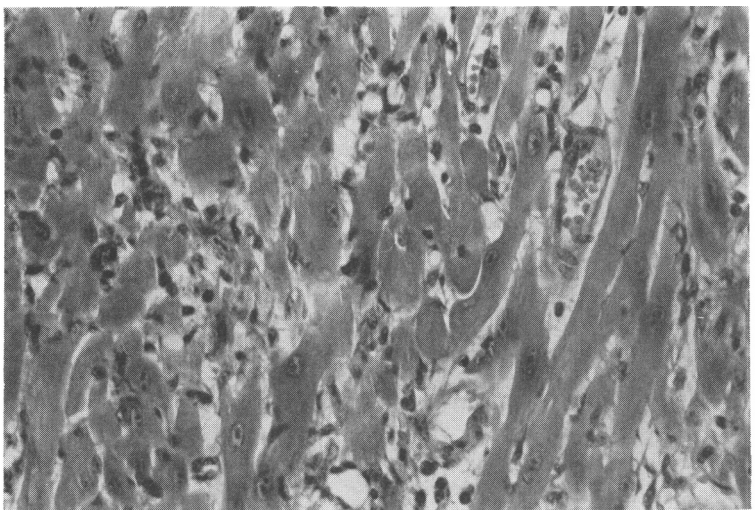

FIG. 2. Right ventricle from a rat sacrificed 21 days after being injected with disenecioyl dehydroretronecine. The animal had a right ventricular ratio of 0.66. This photograph demonstrates the hypertrophy of myocytes, interstitial hypercellularity, and parenchymal collapse seen in the rats which developed right ventricular hypertrophy. H & E stain. × 290.

in the present reported study, the development of cor pulmonale in the experimental rats occurred without medial hypertrophy. Thus, the medial hypertrophy of the pulmonary arteries seen after PA intoxication may be due to a different mechanism than simply the development of pulmonary hypertension. Possibly the parent alkaloid or some other PA metabolite, like the dehydropyrrolizidine alcohols, e.g., dehydroretronecine, either alone or synergistically, initiates the medial changes. Certainly the reaction of the PA metabolites with muscle cells is an established fact since rhabdomyosarcomas were produced in a large percentage of rats given dehydroretronecine (11).

Summary. Rats injected with disenecioyl dehydroretronecine, a recently synthesized semisynthetic pyrrolizidine alkaloid pyrrole, developed cardiopulmonary lesions similar to those caused by naturally occurring PA pyrroles. These alterations included pulmonary edema, alveolar edema and septal fibrosis, occlusion of the pulmonary arterioles, increase in alveolar macrophages, and right heart hypertrophy.

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