Selected pulmonary effects induced by fenfluramine in the rat – a comparison with chlorphentermine

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Abstract: We have investigated the potential capacity of fenfluramine to induce pulmonary hypertension in the rat and the extent to which it induces pulmonary phospholipidosis, in comparison to chlorphentermine. Right ventricular hypertrophy and medial hypertrophy of the pulmonary trunk and artery may indicate pulmonary hypertension. Foam cells in the alveolar sacs indicate pulmonary phospholipidosis. Rats were dosed chronically with either fenfluramine (35 mg/kg), chlorphentermine (35mg/kg) or saline daily for 12 weeks. The right ventricular was assessed for hypertrophy. Sections of the pulmonary trunk and artery were examined qualitatively for medial hypertrophy and lung sections for foam cells in alveolar sacs. Fenfluramine, unlike chlorphentermine, induced mild right ventricular hypertrophy ($P < 0.05$). Neither of the drugs induced medial hyptertrophy of the pulmonary trunk or artery. Both drugs induced foam cells in the alveoli i.e. pulmonary phospholipidosis, but fenfluramine did so to a lesser extent than chlorphentermine.

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Introduction: Pulmonary hypertension [1–5] and pulmonary phospholipidosis [6] have been associated with fenfluramine administration to humans and animals respectively. There is uncertainty regarding the potential capacity of fenfluramine to induce pulmonary hypertension [1]. This condition is suspected to arise with appetite suppressants, in particular chlorphentermine [7] and aminorex [8].

The objective of this study was to assess the potential of fenfluramine to induce pulmonary hypertension and the extent to which it induces pulmonary phospholipidosis, in comparison to chlorphentermine in the rat.

An elevated pulmonary arterial blood pressure, right ventricular hypertrophy [9] and medial hypertrophy of the pulmonary trunk and artery [9] indicate pulmonary hypertension, while foam cells in the alveolar sacs indicate pulmonary phospholipidosis [10]. Because chlorphentermine has been shown to induce pulmonary hypertension [7] and pulmonary phospholipidosis [10] in rats, we used it as a control.

Methods: 15 female BD9 rats were obtained from the Provincial Animal Centre, Kuilsriver, South Africa, and were housed in a well-ventilated animal room kept at 20 ± 3°C and a 12 h light:dark cycle. The rats had free access to food and tap water. Each rat was weighed before the commencement of drug administration and at 2 week intervals.

The rats were divided into three groups of five. Each group was injected intraperitoneally with either fenfluramine (donated by Potchefstroom University, obtained from Servier Laboratories) (35 mg/kg), chlorphentermine (35 mg/kg) or saline once daily 6 days per week for 12 consecutive weeks. Chlorphentermine hydrochloride was synthesized according to the procedure for clortermine hydrochloride given in the *Pharmaceutical Manufacturing Encyclopedia* [11].

The only difference between chlorphentermine and clortermine is that for the former compound the chlorine atom is in the para- instead of the ortho- position on the benzene ring. Thus for the synthesis of chlorphentermine, p-α-dichlorotoluene was used as the starting material instead of o-α-dichlorotoluene as for clortermine.

At the end of the treatment period, each rat was anaesthetized with ether (Holpro, Cape Town, South Africa). Heparin (1,000 U/kg) (Intramed, Johannesburg, South Africa) was injected into the external jugular vein, the chest opened surgically and as much blood as possible removed by direct cardiac puncture. The heart and lungs were removed carefully and stored in buffered formalin for pathological analysis.

After the heart was fixed completely in buffered formalin, the atria were dissected away and the wall of the right ventricle was dissected carefully from the remainder of the heart. The free wall of the right ventricle and the left ventricle with the attached intraventricular septum were blotted dry, placed in an oven at 60°C for 48 h to allow the formalin to evaporate, and then weighed accurately using an electronic microbalance.

Right ventricular hypertrophy was ascertained by assessing the mass of the free wall of the right ventricle as a ratio to the left ventricle plus the intraventricular septum [8]. The larger the ratio of right to left ventricular mass, the greater the degree of right ventricular hypertrophy.

The formalin-fixed lungs were cut at the hilum to expose the pulmonary artery at this specific point. Sections of this site, the rest of the lungs and the pulmonary trunk were obtained for the histological study. The section of the pulmonary trunk was taken at the midpoint of its length. Verhoeff's stain for elastic fibres was used to determine qualitatively whether thickening of the muscular layer of the pulmonary artery or trunk occurred; and haemotoxylin and eosin stain to identify foam cells in the alveolar sacs [10].

Data are expressed as means ± SD. Student's *t*-test was

used to make statistical comparisons and a P value < 0.05 was considered significant.

Results and discussion: Although the rat is widely used in cardiopulmonary studies, it is not routinely used for pulmonary artery catheterization because of its small blood vessels. This makes successful and reproducible pulmonary artery catheterization difficult. Consequently, we used indirect methods to assess the pulmonary hypertensive potential of fenfluramine.

Table 1 shows the ratios of the mass of the free wall of the right ventricle to that of the left ventricle plus the intraventricular septum for the rats treated with fenfluramine, chlorphentermine or saline. The mean ratio obtained for the fenfluramine-treated rats was significantly different ($P < 0.05$) from the control ratio obtained for the saline-treated rats. That obtained for the chlorphentermine-treated rats was not significantly different ($P < 0.4$) from the control.

The results show that fenfluramine, unlike chlorphentermine, induced right ventricular hypertrophy. The observation for chlorphentermine is consistent with those of others [7,10]. The significance of the results obtained in our investigation is best evaluated by comparing them with those obtained in rats by other investigators.

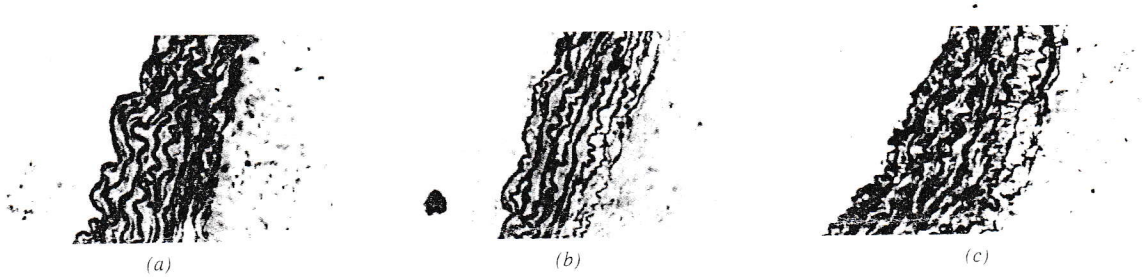
Table 1. Ratios of the ventricular mass after chronic administration of fenfluramine, chlorphentermine or saline to rats for 12 weeks (means ± SD; n = 5)

$$\left[\frac{RV}{LV+S} \pm SD\right] \times 10^3$$

Fenfluramine	Chlorphentermine	Saline
264 ± 16.1*	252 ± 12.5	234 ± 14.0

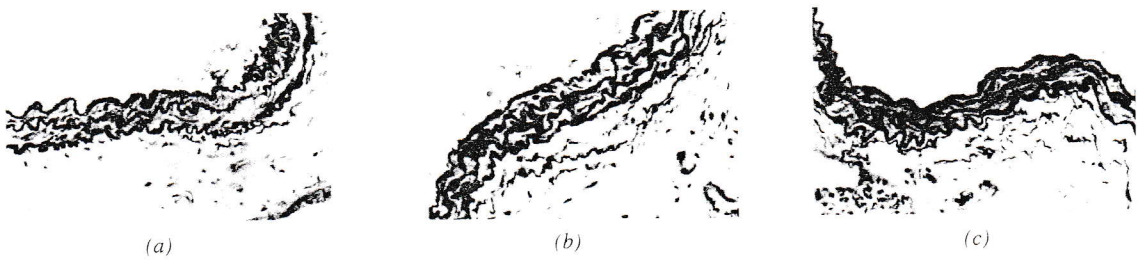
**P < 0.05 as compared with saline-treated rats. RV - right ventricle, LV - left ventricle, S - intraventricular septum. Saline-treated rats serve as control.*

The mean ratio of the ventricular mass of 234 obtained for control rats in our study correlated well with that of 238 obtained by Kay *et al.* [12]. In the latter study, monocrotaline induced right ventricular hypertrophy and a value of 454 was obtained. For comparison, the reciprocal of the values obtained in the study of Kay *et al.* [12] was calculated. This value is much higher than 264 obtained for our fenfluramine-treated rats.



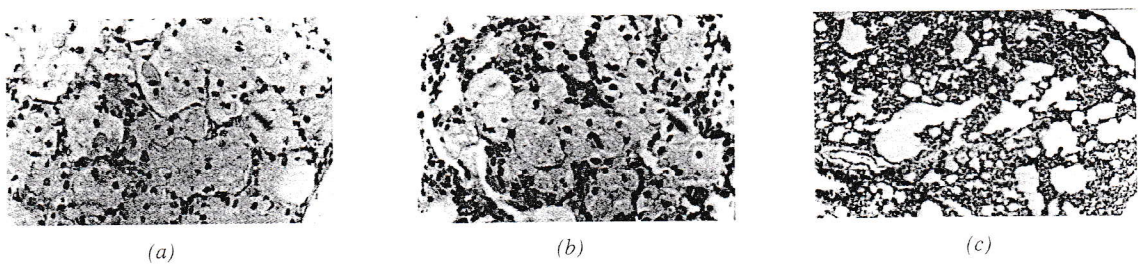
(a) *(b)* *(c)*

Figure 1. Transverse section of the pulmonary trunk. Rats were treated with either (a) fenfluramine (35 mg/kg), (b) chlorphentermine (35 mg/kg) or (c) saline for 12 weeks. Verhoeff's stain × 40.



(a) *(b)* *(c)*

Figure 2. Transverse section of the pulmonary artery. Rats were treated with either (a) fenfluramine (35 mg/kg), (b) chlorphentermine (35 mg/kg) or (c) saline for 12 weeks. Verhoeff's stain × 40.



(a) *(b)* *(c)*

Figure 3. Foam cells in the alveolar sacs of rats treated with either (a) fenfluramine (35 mg/kg), (b) chlorphentermine (35 mg/kg) or (c) saline for 12 weeks. Haemotoxylin and eosin stain × 100.

Although fenfluramine induced right ventricular hypertrophy, it did not induce the condition to the same magnitude as monocrotaline. The degree of right ventricular hypertrophy induced by fenfluramine is mild in extent, is not significantly different from that obtained with chlorphentermine and is within the normal range for the rat.

The pulmonary trunks (Figure 1) and arteries (Figure 2) were of normal structure and no evidence of medial thickening was found in the rats dosed with either fenfluramine, chlorphentermine or saline. In all sections the media were thin and retained between internal and external elastic laminae. Elastic fibres were arranged normally and no fragmentation was observed in any of the sections studied.

Our results are consistent with those of Heath *et al.* [10], who showed that chronic administration of chlorphentermine (50 mg/kg) for 50 days to rats did not induce medial hypertrophy of the pulmonary trunk or artery. Similarly, Smith *et al.* [13] did not find medial hypertrophy of the pulmonary artery after chronic administration of chlorphentermine to rats in drinking water (400 mg/l) for 1 year. In our investigation, we performed a qualitative analysis of the medial layer of the pulmonary vessels.

Microscopic examination revealed that after chronic administration of fenfluramine and chlorphentermine, foam cells were present in the alveolar sacs of the lungs. Only two of five fenfluramine-treated rats showed foam cells (Figure 3a) whereas all chlorphentermine-treated rats ($n = 5$) showed foam cells (Figure 3b). No foam cells were found for the saline-treated rats (Figure 3c). This indicated that the drugs induced pulmonary phospholipidosis in the rat.

The foam cells were oval, large and contained a bluish-purple stained nucleus. They were not evenly distributed throughout the lung tissue and in some sections they filled the entire alveolar sac, whereas in others, few foam cells were present or occurred singly. The foam cells in the chlorphentermine-treated rats were more abundant and generally larger than those in the fenfluramine-treated rats. The lungs were examined for any other structural changes but no such changes were observed.

The study indicated that fenfluramine, unlike chlorphentermine, induced mild right ventricular hypertrophy. It induces phospholipidosis to a lesser extent than chlorphentermine.

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