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Sibutramine, a serotonin–norepinephrine reuptake inhibitor, causes fibrosis in rats

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ABSTRACT

Sibutramine hydrochloride monohydrate is a weight loss agent indicated for the treatment of obesity. Although it has been banned from most markets, studies are still relevant as it is often a hidden ingredient in herbal and over the counter slimming products. Sibutramine induces liver fibrosis with steatosis in female Sprague-Dawley rats fed a high-energy diet without significant weight gain. In this study, using the same animal model, the effect of Sibutramine on lung morphology was investigated using histological evaluation of the terminal bronchiole and transmission electron microscopy evaluation of the respiratory tissue. From these results Sibutramine was found to induce lung fibrosis in Sprague-Dawley rats as increased collagen synthesis, mast cell accumulation and aggregates of Bronchus Associated Lymphoid Tissue (BALT) in the terminal bronchiole as well as increased collagen deposition in the respiratory tissue was seen.

1. Introduction

Sibutramine hydrochloride monohydrate is a monoamine reuptake inhibitor. Its therapeutic effects are produced mainly by the inhibition of norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) reuptake at neuronal synapse sites in the central nervous system (CNS). This inhibition causes an increase in the synaptic concentration of these substances and consequently an increase in the feeling of satiety and energy expenditure and a resultant decrease in food intake (Lechin et al., 2006; Eroglu et al., 2009; Sansbury and Hill, 2014). After absorption, Sibutramine is metabolised by the hepatic Cytochrome P450 enzymes, forming the pharmacologically active metabolites, M1 and M2, which are responsible for the resultant hypophagic effect and subsequent weight-loss (Hwang et al., 2014; Luque and Rey, 2002; Padwal and Majumdar, 2007). Despite Sibutramine's initial success as weight reducing agent, it was later banned from most markets following a series of reported adverse events including death. However, it is still available via the internet and many herbal products have been shown to contain far higher concentrations of this product than that which was prescribed.

Most of the human body's 5-HT is located in the enterochromaffin cells within the gastrointestinal tract, where it is responsible for regulating intestinal movements. It is also stored in platelets contributing to haemostasis and blood clotting. In certain cell types, 5-HT has been identified as a growth factor and has been shown to increase the proliferation of fibroblasts and consequent collagen synthesis that can lead to fibrosis (Fabre et al., 2008). These findings have further been substantiated by Dees et al. (2011) who linked elevated 5-HT levels to vascular disease and tissue fibrosis due to its ability to stimulate extracellular matrix production in both sclerotic and healthy fibroblasts. There are two 5-HT receptor subtypes that have been identified to play an important role in the lungs specifically. These are receptors 5-HT_{2A} and 5-HT_{2B} (Fabre et al., 2008). Following Sibutramine administration, the reuptake of 5-HT is blocked resulting in the activation of serotonergic receptors, and ultimately prolonged tissue exposure. It can therefore be hypothesised that prolonged exposure to raised 5-HT levels will cause increased risk for lung associated fibrosis as a result of 5-HT mediated activation of fibrogenesis.

Diet induced obese animals have become the standard for studies investigating obesity and its co-morbid complications. In such a model, using Sprague-Dawley rats, Jackson et al. (1997) described the hypophagic effects of Sibutramine and identified the key adrenoreceptors and serotonergic receptors involved in its pharmacological mechanism. In a study to determine the safety of Sibutramine it was observed that, following Sibutramine administration, ultrastructural changes in morphology associated with

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fibrosis in liver tissue in Sprague-Dawley rats fed a high energy diet that did not result in weight gain, could be observed by Oberholzer et al. (2013). In this study the effects Sibutramine on lung morphology was further investigated by using light- and transmission electron microscopy.

2. Materials and methods

2.1. Implementing the Spraque-Dawley rat model

Sprague-Dawley rats fed a high energy diet were implemented in this study. Ethical clearance was obtained from the University of Pretoria Animal Use and Care committee (ethical clearance number: h015-11). Thirty female, sexually mature Sprague-Dawley rats, each of average weight (200–250 g) were used in this study and were maintained at the University of Pretoria Biomedical Research Centre (UPBRC). The animals were housed conventionally in cages complying with the sizes laid down in the SANS 10386:2008 recommendations. A room temperature of 22 °C (±2); relative humidity of 50% (±20) and a 12 h light/dark cycle was maintained. Enrichment was provided according to standard procedures at the UPBRC. This experiment was conducted over a period of 88 days.

The animals were randomly divided into three groups [NC (Normal chow), HED (high energy diet) and HED-S (high energy diet plus Sibutramine administration)] with ten animals per group as indicated in Table 1.

All animals were fed on the normal diet [Epol mice cubes; a division of Rainbow farms (Pty) Ltd, Westville, Johannesburg, South Africa] for the first 4 days. This served as an acclimatisation period. For statistical purposes, day 0 was taken on the first day the animals in the HED groups received the high energy diet. Animals in the NC group remained on the normal chow diet for the duration of the study. Animals in the HED group were on the HED from day 5 until day of termination (days 0–88). Animals in the HED-S group were on the HED from day 5 until day of termination, but were treated with Sibutramine for the last 28 days before termination. This allowed time for the animals to gain weight on the HED before treatment with the weight loss agent commenced. Four weeks is also the standard time period for toxicology studies. During the last 28 days, animals in the NC and HED groups were not treated with the solvent of Sibutramine as it was dissolved in sterile water and water was provided ad libitum to all animals.

2.2. High energy diet (HED)

The HED diet consisted of 12% corn oil, 43% condensed milk and 45% Epol pellets. The nutritional value was estimated to be 4.4 Kcal/g with 13% protein, 18% fat and 69% carbohydrate content. The normal chow diet was replaced with the HE diet and was available to the animals ad libitum.

2.3. Sibutramine administration

Animals in the HED-S group were treated with 1.32 mg/kg b.wt. dose of Sibutramine hydrochloride monohydrate (BIOCOM biotech; Clubview, South Africa) dissolved in sterile water for 28 days. This dosage concentration was extrapolated from an equivalent human dose (i.e. 15 mg/day) using the formula for dose translation based on body surface area (BSA) as described by Reagan-Shaw et al. (2007). Sibutramine was administered to each animal in this group every morning (08h00) via oral gavage. This route of administration ensured that all animals within an experimental group received exact, equal concentrations of the test compound and was done by proper handling methods by trained individuals to avoid any unnecessary discomfort to the animals.

2.4. Tissue for light microscopy

On the final day of experimentation (day 28) rats were terminated via Isoflurane overdose. Tissue samples were collected via dissection and lung samples were fixed in 2% formaldehyde in phosphate buffered saline for 12–24 h and were then dehydrated in an ethanol series, cleared with xylene and embedded in paraffin wax. Sections of 4–6 μm were stained with picrosirius red and luna stain respectively and images viewed at 10× and 40× magnification with a Nikon Trans Optiphod transmitted light microscope (Nikon Instech Co., Kanagawa, Japan).

2.5. Picrosirius red staining

For the evaluation of the formation of different collagen fibres, the slides were stained with picrosirius red. The slides were de-waxed following standard protocols and then stained with Haematoxylin for 8 min after which they were washed for 10 min in running water. The slides were then counterstained with picrosirius red (1 mg/ml in saturated picric acid solution) for 1 h, after which they were washed in two changes of acidified water and dehydrated three times in 100% ethanol. The slides were then examined using both polarised and normal light.

2.6. Luna stain

The luna stain was used to indicate the presence of mast cells in the tissue. Slides were de-waxed and rehydrated with 95% ethanol. Afterwards they were stained with aldehyde fuchin for 30 min, rinsed 3 times in 95% ethanol and then stained with haematoxylin for 10 min. Following haematoxylin staining, the slides were rinsed with running tap water for 10 min followed by a single rinse in 95% ethanol. Methyl orange stain was applied for 10 min after which slides were then dehydrated and mounted.

2.7. Tissue for transmission electron microscopy

Lung samples were fixed in 2.5% glutaraldehyde/formaldehyde for 1 h, rinsed three times in 0.075 M sodium potassium phosphate buffer (pH 7.4) for 15 min before being placed in secondary fixative, 1% osmium tetraoxide solution, for 1 h. Following fixation, the samples were rinsed again as described above. This was followed by dehydration in 30%, 50%, 70%, 90% and three changes of 100% ethanol. Samples were then embedded in resin and ultra-thin sections (80–100 nm), cut with a diamond knife using an ultramicrotome, were contrasted with uranyl acetate for 5 min followed by 2 min of contrasting with lead citrate, after which samples were allowed to dry and examined with the JEOL transmission electron microscope (TEM) (JEM 2100F).

3. Results

The distribution of collagen in lung tissue of NC, HED and HED-S treated rats was evaluated. Fig. 1 shows the terminal bronchiole with the associated connective tissue stained with picrosirius red. Images taken with and without polarised light are shown indicating thicker collagen fibres (stained yellow) as well as thinner fibres (stained green). Fig. 1 A and B are representative of lung tissue of the control animals (NC group), with the well-defined terminal bronchial wall with its characteristically folded respiratory epithelium and regularly arranged oval bundles of smooth muscle clearly visible. The connective tissue surrounding the terminal bronchial