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The novel triple monoamine reuptake inhibitor tesofensine induces sustained weight loss and improves glycemic control in the diet-induced obese rat: Comparison to sibutramine and rimonabant

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ABSTRACT

Tesofensine, a novel triple monoamine reuptake inhibitor, produces a significant weight loss in humans. The present study aimed at characterizing the weight-reducing effects of tesofensine in a rat model of diet-induced obesity. Sibutramine and rimonabant were used as reference comparators. Compared to baseline, long-term treatment with tesofensine (28 days, 1.0 or 2.5 mg/kg, p.o.) resulted in a significant, dose-dependent and sustained weight loss of 5.7 and 9.9%, respectively. Sibutramine (7.5 mg/kg, p.o.) treatment caused a sustained weight loss of 7.6%, whereas the employed dose of rimonabant (10 mg/kg, p.o.) only produced a transient weight reduction. While all compounds exhibited a significant inhibitory effect on food intake which gradually wore off, the hypophagic effect of tesofensine was longer lasting than sibutramine and rimonabant. In contrast to tesofensine, the body weight of pair-fed rats returned to baseline at the end of the study, which may indicate that tesofensine stimulated energy expenditure. The differential efficacy on weight reduction was also reflected in lowered body fat depots, as tesofensine and sibutramine most efficiently reduced abdominal and subcutaneous fat mass which was paralleled by reduced plasma lipid levels. In an oral glucose tolerance test, only tesofensine significantly suppressed the plasma insulin response below the level that could be obtained by paired feeding, indicating that tesofensine further improved glycemic control. In conclusion, the robust weight loss with long-term tesofensine treatment is likely due to a combined synergistic effect of appetite suppression and increased energy expenditure.

1. Introduction

The consequences of the global rise in obesity prevalence have become increasingly apparent and unless current trends are reversed both individual and societal burdens will be enormous. In particular, obese individuals have a higher risk of developing type 2 diabetes, dyslipidemia, and cardiovascular diseases (Haslam and James, 2005). Conversely, a weight loss of 5–10% of initial body weight is associated with reduced incidence of type 2 diabetes and improvements in cardiovascular risk profiles, thus reducing obesity co-morbidity (Goldstein, 1992). However, current pharmacotherapy used in population-based obesity management insufficiently reduces body weight and co-morbidity risk parameters and tolerance often develops to the appetite suppressing effect of anti-obesity pharmacotherapy (Bray and Ryan, 2007; Fernstrom and Choi, 2008), which warrants research for more efficacious drugs.

It has become increasingly clear that there is a close association between obesity and altered homeostasis of monoaminergic neurotransmission as indicated by the weight-reducing effect of pharmacologically stimulated monoaminergic function in obese individuals. Recent clinical trials indicate that tesofensine, a novel triple monoamine reuptake inhibitor (Lehr et al., 2008), is very effective in producing weight loss in obese individuals, an effect which is accompanied by a significant reduction in body fat mass, plasma triglycerides and insulin resistance (Astrup et al., 2008a,b). When corrected for placebo and diet effects, long-term tesofensine treatment produces a weight loss of 10.6% in obese patients (Astrup et al., 2008a), which is twice that achieved by other anti-obesity agents, including the noradrenaline and serotonin reuptake blocker, sibutramine, and the cannabinoid CB_1 receptor antagonist, rimonabant (Bray and Ryan, 2007).

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The effectiveness of tesofensine in obese patients suggests that optimization of intrinsic triple monoamine reuptake inhibitor activity can further improve obesity treatment. Preclinical studies indicate that synergistic effects on body weight loss are obtained by stimulating the activity of more than one monoaminergic neurotransmitter system (Nelson and Gehlert, 2006). Consequently, combined blockade of noradrenaline and serotonin reuptake by co-administration of pharmacological agents which selectively inhibit the transport of noradrenaline (NAT) or serotonin (SERT) results in reduced food intake and body weight that neither monoamine reuptake inhibitor could achieve on its own. This anti-obesity effect of dual serotonin and noradrenaline reuptake inhibition (SNRI) is particularly relevant for sibutramine. The pharmacological effects of sibutramine is closely associated with its tertiary amine metabolites which are much more potent NAT and SERT inhibitors than the parent compound (Connoley et al., 1999; Jackson et al., 1997b), suggesting that both mechanisms are important for the magnitude of weight loss obtained by sibutramine. Interestingly, the dual NAT and dopamine transporter (DAT) inhibitors, bupropion and mazindol, cause weight loss by combined induction of hypophagia and thermogenesis (Billes and Cowley, 2007). In combination, positive modulation of noradrenaline and serotonin in combination with stimulated dopaminergic activity may thus be advantageous for sustaining appetite and weight control in long-term obesity pharmacotherapy.

The aim of the current study was to further characterize the effects underlying the sustained weight loss produced by long-term tesofensine treatment in obese individuals. The major objective was to establish to what extent appetite suppression contributed to the anti-obesity effect of tesofensine, using a rat model of diet-induced obesity (DIO). To allow for evaluation of the anti-obesity efficacy of tesofensine, sibutramine and rimonabant were included as reference comparators.

2. Materials and methods

2.1. Compounds

Tesofensine (molecular weight = 520.4 g/mol) was synthesized at NeuroSearch A/S. Sibutramine (molecular weight = 316.3 g/mol) and rimonabant (molecular weight = 500.3 g/mol) were obtained from Chemos GmbH (Regenstauf, Germany).

2.2. Animals

Selectively bred male rats, 22–24 weeks of age, displaying enhanced likelihood of developing diet-induced obesity (DIO) bred on a Sprague–Dawley background were used (Paulsen et al., 2010). All experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals and were approved by the Danish Committee for Animal Research. The animals were housed and fed in AAALAC accredited facilities maintained on a standard 12-h light/dark cycle (lights on, 6:00 AM; lights off, 6:00 PM) at a room temperature of 19.5–24.5 °C and relative humidity of 45–65%. All animals had free access to water. At 4 weeks of age, the rats were made obese by switching to a moderately high-fat diet (#12266B; 31.8% kcal from fat, energy density 4.41 kcal/g, Research Diets Inc., New Jersey) in pellet form. The rats were housed in a group environment (2 rats per cage) until 1 week before the study (body weight approximately 550–625 g) when they were singly housed. The rats had ad libitum access to high-fat diet and water unless otherwise stated.

2.3. Drug treatment

Four days prior to initiation of the experiment, 17 week old rats were stratified according to their body weight in order to obtain body weight matched groups, and subsequently allocated to the following treatment regimens (at least $n = 8$ per group): Vehicle (0.5% hydroxypropyl methylcellulose, Sigma, St. Louis, MI); tesofensine (1.0 or 2.5 mg/kg, 1.9 or 4.8 μmol/kg, used as citrate salt); sibutramine (7.5 mg/kg, 23.7 μmol/kg), or rimonabant (10 mg/kg, 20.0 μmol/kg). All compounds were prepared freshly and administered once daily for 28 days via oral gavage 2 h before lights out (10 h into the light phase). The first oral dose was given on day 0. The DIO rats treated with tesofensine (2.5 mg/kg) were matched with pair-fed controls of similar body weight. Each pair-fed DIO control was dosed with vehicle and given a daily food allotment equal to that consumed by its drug-treated counterpart over the previous 24 h period. A daily check was made to ensure that the previous food ration had been consumed. Feeding efficiency was calculated as total calorie consumption per gram body weight change over the entire 28-day dosing period. Following an oral glucose tolerance test (see below), the rats were sacrificed by CO_2 anaesthesia. Body white adipose tissue compartments were removed and weighed. Fat depot weight analysis included mesenterial, retroperitoneal, epididymal and subcutaneous inguinal fat, and total fat depots were calculated as sum of fat mass in these four compartments relative to total body weight.

2.4. Oral glucose tolerance test and blood chemistry analysis

Animals were mildly fasted as they had access to only 50% of their daily energy requirements in the preceding 20 h. Food intake was monitored daily prior to the oral glucose tolerance test and the food administered on the day prior to the oral glucose tolerance test was calculated for each individual group as a mean of the two prior days of food intake. The last drug injection was given the day prior to the oral glucose tolerance test. The oral glucose load was given as gavage via a gastric tube connected to a syringe ensuring accurate dosing. The oral glucose tolerance test was carried out at 8.00 AM. Blood samples for plasma glucose and insulin analysis were taken from a tail vein in heparinized tubes after oral administration of 2.0 g glucose/kg. Plasma insulin was measured in duplicates for each data point using an ultra-sensitive ELISA based assay (Mercodia AB, Sweden). Glucose and insulin AUC calculations were calculated as total AUC based on data from 0 to 180 min. The baseline tail vein blood sample was also analysed for plasma total triglycerides and total cholesterol, using standard enzyme assay kits on a fully automated analyser (Vitros DTII, Ortho Diagnostics, Rochester, NY).

2.5. Statistical evaluation

All data were imported into Excel 5.0 spread sheets and subsequently subjected to relevant statistical analyses (GraphPad Prism 4.03 software). Results are presented as mean ± S.E.M. (standard error of the mean) unless otherwise stated. Statistical evaluation of the data was carried out using a one-way or a repeated measure two-way analysis of variance (ANOVA) with appropriate post-hoc analysis between control and treatment groups in cases where statistical significance was established ($P<0.05$; Tukey or Bonferroni).

3. Results

3.1. Body weight analysis

Chronic oral administration of tesofensine to DIO rats caused a significant reduction in total body weight gain ($F_{5,77} = 79.4$, $P<0.0001$), see Fig. 1A. The reduction in body weight gain during tesofensine treatment was stabilized at approximately two weeks after initiation of drug treatment, irrespective of the dose used (Fig. 1A). However, the highest dose (2.5 mg/kg, maximal weight loss on day 18 = 10.0 ± 0.7%, weight loss on day 28 = 9.9% ± 0.7%) induced