

Pharmacology of AMR-MCH-14, an antagonist of the melanin-concentrating hormone receptor-1 for the treatment of obesity

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INTRODUCTION

Obesity is a growing concern for public health in industrialized nations across the globe. In the United States alone, over 60% of the population is overweight and over 30% of these people are obese.¹ Obesity is associated with a variety of comorbidities such as diabetes, dyslipidemia, coronary heart disease, stroke and certain cancers.² Current pharmaceutical treatments suffer from weak efficacy and significant side effects that limit their use. In fact, CB1 antagonist rimonabant recently was withdrawn from the European market due to CNS side-effects, in particular depression/suicide. Therefore, a major need exists for safer, more effective weight loss agents.

Melanin concentrating hormone (MCH) is a cyclic, 19 amino acid neuropeptide expressed in the zona incerta and lateral hypothalamus that regulates food intake and body weight.³ Antagonists of the MCH₁ receptor have been shown to be a promising new approach for the treatment of obesity.⁴

AMR-MCH-14 is representative of a novel structural class of selective, high affinity MCH₁ receptor antagonists identified by AMRI. The in vitro and in vivo properties of AMR-MCH-14 are presented.

METHODS and RESULTS

The affinity of AMR-MCH-14 for the MCH₁ receptor (Fig. 1) was determined using a binding assay with [³H]AMR-MCH-1 and cloned human MCH₁ receptors.⁵ The functional antagonism of AMR-MCH-14 was established with an aequorin-based Ca²⁺ mobilization assay (carried out by Euroscreen). A panel of more than 80 GPCRs, ion channels and cytochrome P450s was used to demonstrate the selectivity of AMR-MCH-14 for the MCH₁ receptor. Selectivity against the hERG potassium channel was established using a mini-patch clamp assay.

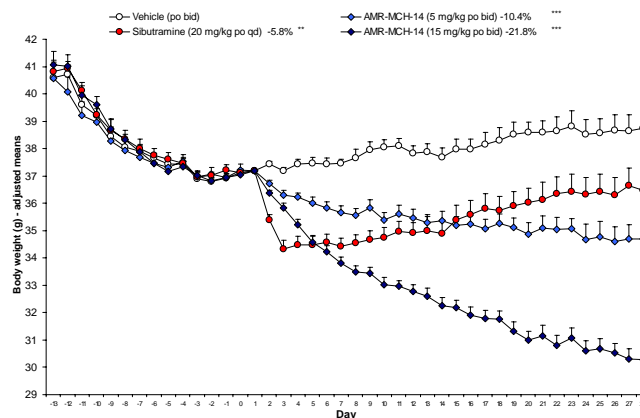
The in vivo efficacy of AMR-MCH-14 was demonstrated in a chronic, 28-day feeding study with male dietary-induced obese (DIO) C57BL/6J mice (Fig. 2). The mice were group housed and given free access to a high fat diet (D12451 45% of Kcal derived from fat; Research Diets, New Jersey, USA) and tap water for 14 weeks to induce obesity. At the end of the 14 week period the animals were singly housed for an additional two week period and placed on reverse phase lighting (lights off for 8 h from 09:30 – 17:30 h). After a 14-day baseline run in period with bi-daily oral vehicle dosing, animals were treated with AMR-MCH-14 twice daily (at 08:45 h and 14:45 h) by oral gavage at doses of 5 mg/kg and 15 mg/kg. Changes in body weight and food intake were compared to positive control sibutramine. Unlike sibutramine, which showed rapid onset of weight loss followed by mild weight gain, AMR-MCH-14 was characterized by gradual weight loss that continued throughout the course of the four week study. Measurement of food intake showed a sustained reduction in the groups treated with AMR-MCH-14 (Fig. 3). In contrast, sibutramine reduced food intake in the first week, and then increased food consumption in weeks two through four. At termination, the retroperitoneal and epididymal fat pads were dissected out and weighed, demonstrating that the weight loss caused by AMR-MCH-14 and sibutramine was associated with reductions in fat mass (Fig. 4). Analysis of leptin levels from terminal plasma samples revealed significant improvement in plasma leptin levels concomitant with fat loss (Fig. 5). Also following the DIO mouse study, coronal sections of the brain containing the caudate putamen were removed and used to determine the ex-vivo MCH₁ receptor occupancy (Fig. 6).

1. In Vitro Profile of AMR-MCH-14

Assay	AMR-MCH-14
MCH ₁ Binding ¹ (K _d , nM)	4.8 ± 1.4
MCH ₁ Funct. Antagonism ¹ (IC ₅₀ , nM)	14
CYP Isoform Inhibition ² (IC ₅₀ , μM)	>10 CYP 3A4; IC ₅₀ = 7.2 ± 4.3
t _{1/2} (HLM) ³ (min)	364 (n=2)
t _{1/2} (MLM) ⁴ (min)	382 (n=2)
Receptor Selectivity Panel ⁵	80 receptors evaluated 5-HT _{1B} , K _i = 0.35 μM 5-HT Transporter K _i = 0.96 μM
hERG ⁶ (IC ₅₀ , μM)	2.1 ± 0.6

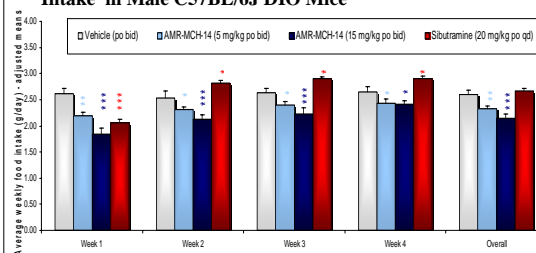
¹Human receptor; ²CYP isoforms tested, 1A2, 2B6, 2C9, 2C19, 2D6, 3A4; ³HLM = Human Liver Microsomes; ⁴MLM = Mouse Liver Microsomes; ⁵Panel of 80 receptors, including GPCRs, ion channels tested at 1 μM; ⁶Mini patch clamp

2. Effect of Chronic Administration of AMR-MCH-14 on Body Weight in Male C57BL/6J DIO Mice



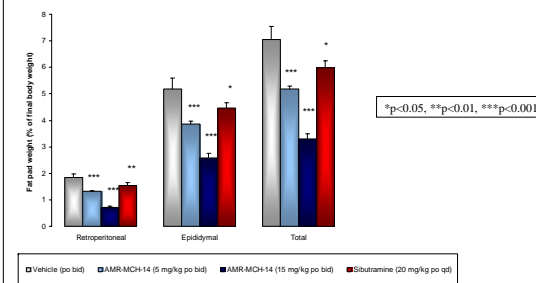
• AMR-MCH-14 caused sustained weight loss of 10.4% and 21.8% at 5 mg/kg bid and 15 mg/kg bid, respectively.

3. Effect of Chronic Administration of AMR-MCH-14 on Food Intake in Male C57BL/6J DIO Mice



• AMR-MCH-14 caused sustained reduction in weekly food intake (by 16% and 29% in week 1; 9% and 16% in week 2; 9% and 15% in week 3 and 9% in week 4 for 5 mg/kg and 15 mg/kg bid dose groups, respectively).

4. Effect of AMR-MCH-14 on Fat Pad Weight



• AMR-MCH-14 caused reductions in fat mass of 32.3% and 61% compared to vehicle for the 5 mg/kg bid and 15 mg/kg bid dose groups, respectively.

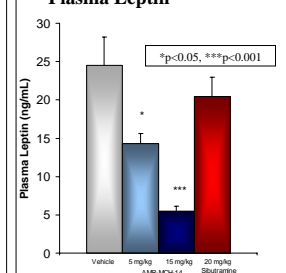
CONCLUSIONS

- AMR-MCH-14 is a selective, high affinity MCH₁ receptor antagonist.
- AMR-MCH-14 causes gradual, sustained weight loss in obese mice.
- Weight loss is accompanied by reduction in food intake.
- Weight loss is associated with reduction in fat mass and accompanied by reduction in circulating plasma leptin levels.

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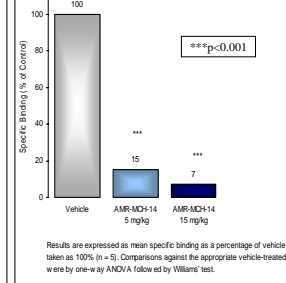
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5. Effect of AMR-MCH-14 on Plasma Leptin



• Significant reductions in circulating plasma leptin levels of 37.5% and 75.9% were observed following chronic administration of 5 mg/kg bid and 15 mg/kg bid AMR-MCH-14, respectively.

6. Ex Vivo Receptor Occupancy



• Ex-vivo analysis of MCH₁ receptor showed 85% and 93% occupancy, 6 hours following the final administration of 5 mg/kg bid and 15 mg/kg bid, respectively, in the 28-day study.