

# Comparative pharmacology of AMR-MCH-1 and AMR-MCH-2, MCH<sub>1</sub> receptor antagonists 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.<sup>1</sup> Obesity is associated with a variety of comorbidities such as diabetes, dyslipidemia, coronary heart disease, stroke and certain cancers.<sup>2</sup> Current pharmaceutical treatments suffer from weak efficacy and significant side effects that limit their use. In fact, the CB1 antagonist rimonabant was recently withdrawn from the European market due to CNS side-effects, in particular depression/suicide.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. In mammals, MCH is highly expressed in the zona incerta and lateral hypothalamus and regulates food intake and energy homeostasis through interaction with the MCH<sub>1</sub> receptor.<sup>3</sup> Antagonists of the MCH<sub>1</sub> receptor have been shown to be a promising new approach for the treatment of obesity.<sup>4</sup>

AMR-MCH-1<sup>5</sup> and AMR-MCH-2 are representative of novel structural classes of selective, high affinity MCH<sub>1</sub> receptor antagonists identified by AMRI. The in vitro and in vivo properties of AMR-MCH-1 and AMR-MCH-2 are presented.

## METHODS

The affinity of AMR-MCH-1 and AMR-MCH-2 for the MCH<sub>1</sub> receptor was determined using a binding assay with [<sup>3</sup>H]AMR-MCH-1 and cloned human MCH<sub>1</sub> receptors.<sup>6</sup> The functional antagonism was established with an aequorin-based Ca<sup>2+</sup> mobilization assay. In vitro metabolic stability assessment was carried out in the presence of human and mouse liver microsomes.

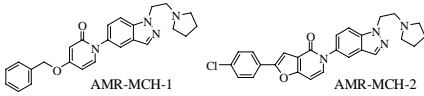
The in vivo efficacy of AMR-MCH-1 was demonstrated in a chronic, 28-day feeding study with male dietary-induced obese (DIO) C57BL/6J mice. The mice (4-6 weeks of age; Harlan UK Ltd.) were group housed and given free access to a high fat diet (D12451 45% of Kcal derived from fat; Research Diets, New Jersey, USA) and tap water for 14 weeks to induce obesity. At the end of the 14 week period, the animals were singly housed for a further 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-1 twice daily (at 08:45 and 14:45) by oral gavage at doses of 30 mg/kg and 60 mg/kg or with sibutramine once daily (20 mg/kg po) at 8:45 and vehicle at 14:45. Food intake, water intake and body weight were recorded daily. On the morning of day 28, the animals were dosed one final time. Blood samples for bioanalysis were taken at 0.5, 1, 3 and 6 hours following the final dose. At termination, the retroperitoneal and epididymal fat pads were dissected out and weighed. Brains were removed and divided in two. Coronal sections containing the caudate putamen were cut from one half of each brain. Ex vivo occupancy of striatal MCH<sub>1</sub> receptors was determined from these sections. The remaining half brains were used for quantification of AMR-MCH-1.

Sub-chronic DIO mouse feeding studies on AMR-MCH-1 and AMR-MCH-2 were carried as described above with the exception of duration (5 days vs. 27 days), dosing (qd vs. bid) and termination (non terminal studies).

## RESULTS

AMR-MCH-1 was found to bind to the human MCH<sub>1</sub> receptor with a K<sub>d</sub> value of 2.6 nM and determined to be a functional antagonist at the MCH<sub>1</sub> receptor with an IC<sub>50</sub> value of 14 nM (Table 1). AMR-MCH-1 demonstrated significant and sustained reductions in food intake and body weight in a chronic feeding study in male DIO mice. At twice daily oral doses of 30 mg/kg and 60 mg/kg, AMR-MCH-1 produced weight losses of 11.1% and 13.9%, respectively, compared with 5.8% for positive control sibutramine (20 mg/kg po qd) (Figure 1). AMR-MCH-1 also caused sustained reduction in weekly food intake (by 24% and 22% in week 1; 11% and 12% in week 2; and 12% and 7% in week 3 for 30 mg/kg and 60 mg/kg, respectively; non-significant reductions were observed in week 4) (Figure 2). Fat pad analysis indicated that the weight loss caused by AMR-MCH-1 was associated with reductions in total fat pad mass of 27.5% and 44.6% compared to vehicle for the 30 mg/kg bid and 60 mg/kg bid dose groups, respectively (Figure 3). Ex-vivo MCH<sub>1</sub> receptor occupancy showed 74% receptor occupancy at 30 mg/kg and 85% receptor occupancy at 60 mg/kg, 6 hours following the final dose (Figure 4). The brain levels of AMR-MCH-1 at the 6 hour time point were found to be 1,377 ng/g and 3,226 ng/g for the 30 and 60 mg/kg bid doses, corresponding to brain to plasma ratios of 0.72 and 0.86, respectively (Table 2). Improved brain penetration was achieved through a modified structural class, represented by AMR-MCH-2. AMR-MCH-2 maintained high affinity for the MCH<sub>1</sub> receptor, with a K<sub>d</sub> value of 5.2 nM and a functional antagonist IC<sub>50</sub> value of 23 nM (Table 1). AMR-MCH-2 demonstrated improved efficacy versus AMR-MCH-1 in 5-day feeding studies in DIO mice with once a day oral dosing (qd). At 30 mg/kg qd, AMR-MCH-2 showed 4.5% weight loss compared to the 2.8% weight loss provided by AMR-MCH-1 at twice the dose (60 mg/kg qd) (Table 3). The improved efficacy was correlated with increased brain exposure. At 6 hours following a single 10 mg/kg oral dose of AMR-MCH-2, DIO mice were found to have brain concentrations of 2,253 ng/g with a brain to plasma ratio of 16. These data indicate that AMR-MCH-1 is a high affinity MCH<sub>1</sub> receptor antagonist that causes sustained weight loss in obese mice and that structural modification to AMR-MCH-2 yields improved brain penetration and improved efficacy.

**Table 1. In Vitro Profile of AMR-MCH-1 and AMR-MCH-2**

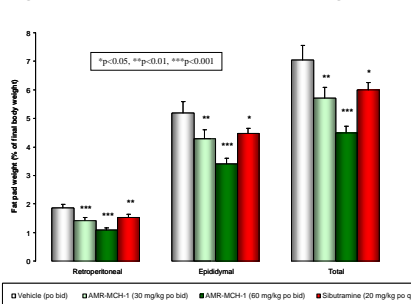


Assay	AMR-MCH-1	AMR-MCH-2
MCH <sub>1</sub> Binding <sup>1</sup> (K <sub>d</sub> , nM)	2.6 ± 0.2	5.2 ± 1.5
MCH <sub>1</sub> Funct. Antagonism <sup>1</sup> (IC <sub>50</sub> , nM)	14 (n=2)	23
t <sub>1/2</sub> (HLM) <sup>3</sup> (min)	303 ± 50	>1000
t <sub>1/2</sub> (MLM) <sup>4</sup> (min)	695 ± 122	595

<sup>1</sup>Human receptor; <sup>3</sup>HLM = Human Liver Microsomes; <sup>4</sup>MLM = Mouse Liver Microsomes

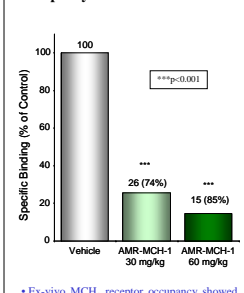
\* AMR-MCH-1 and AMR-MCH-2 are high affinity MCH<sub>1</sub> receptor antagonists with favorable metabolic stability in the presence of human and mouse liver microsomes.

**Figure 3. Effect of AMR-MCH-1 on Fat Pad Weight**



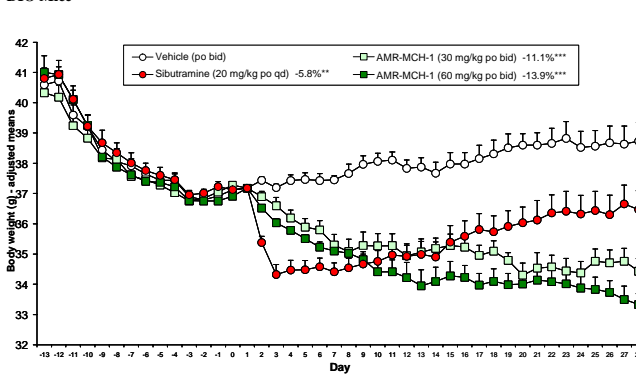
\* AMR-MCH-1 caused reductions in total fat pad mass of 27.5% and 44.6% compared to vehicle for the 30 mg/kg bid and 60 mg/kg bid dose groups, respectively.

**Figure 4. Ex Vivo MCH<sub>1</sub> Receptor Occupancy**



\* Ex-vivo MCH<sub>1</sub> receptor occupancy showed 74% receptor occupancy at the 30 mg/kg dose and 85% receptor occupancy at the 60 mg/kg dose, 6 hours following the final dose in the 28-day DIO mouse study.

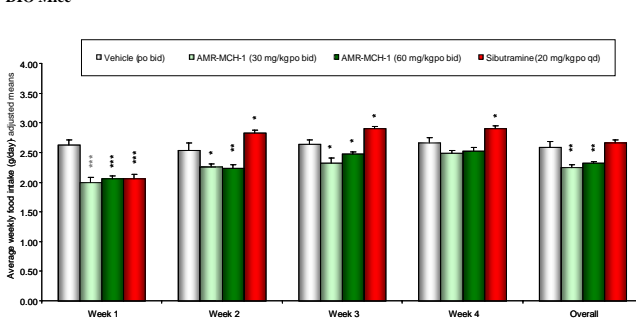
**Figure 1. Effect of Chronic Administration of AMR-MCH-1 on Body Weight in Male C57BL/6J DIO Mice**



Data are adjusted means (n = 8-9); SEMs are calculated from the residuals of the statistical model. Data analyzed by ANCOVA with body weight on Day 1 as covariate. For clarity, no significant differences from vehicle are illustrated on this figure. Figures in legend are percentage difference from control on Day 28 (vs after 27 days dosing). \*\*\*p<0.01, \*\*\*\*p<0.001.

\* AMR-MCH-1 caused sustained weight loss of 11.1% and 13.9% at 30 mg/kg bid and 60 mg/kg bid, respectively.

**Figure 2. Effect of Chronic Administration of AMR-MCH-1 on Food Intake in Male C57BL/6J DIO Mice**



Data are adjusted food intake means (n = 9); SEMs are calculated from the residuals of the statistical model. Data analyzed by ANCOVA with baseline (average of days 6-9) as covariate followed by Wilcoxon test for AMR compounds and multiple t test for sibutramine. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

\* AMR-MCH-1 caused sustained reduction in weekly food intake (by 24% and 22% in week 1; 11% and 12% in week 2; and 12% and 7% in week 3 for 30 mg/kg and 60 mg/kg, respectively; non-significant reductions were observed in week 4).  
 \* AMR-MCH-1 caused a mild and transient reduction in water intake (p<0.05) during days 1-4 at 60 mg/kg bid (data not shown).

**Table 2. PK Parameters Following Oral Dosing of AMR-MCH-1 to DIO Mice**

PK Parameter	1 Dose (po)		27-Days (po, bid)	
	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg
[Brain] 6 h (ng/g)	1484 ± 242	1377 ± 388	3226 ± 804	
[Plasma] 6 h (ng/g)	2224 ± 744	1950 ± 520	3774 ± 784	
T <sub>1/2</sub> 6 h	0.7 ± 0.2	0.72 ± 0.12	0.86 ± 0.18	
B <sub>max</sub> (h)	0.83 ± 0.29	0.83 ± 0.29	0.67 ± 0.29	
C <sub>max</sub> (ng/mL)	4977 ± 1230	4083 ± 339	7420 ± 1232	
AUC <sub>0-6h</sub> (h*ng/mL)	ND	14760 ± 1423	27862 ± 2653	
Weight Loss	ND	11.1%	13.9%	

\* AMR-MCH-1 showed dose proportional PK with no evidence of plasma or brain accumulation.

**Table 3. Brain and Plasma Exposure and Weight Loss Following Oral Dosing of AMR-MCH-1 and AMR-MCH-2 to DIO Mice**

AMR-MCH-1		AMR-MCH-2	
DIO Mouse Oral Exposure (6 h post single dose)		DIO Mouse Oral Exposure (6 h post single dose)	
Dose (po)	30 mg/kg	Dose (po)	10 mg/kg
[Brain] (ng/g)	1484 ± 242	[Brain] (ng/g)	2253 ± 266
[Plasma] (ng/mL)	2224 ± 744	[Plasma] (ng/mL)	141 ± 34
B/P Ratio	0.7 ± 0.2	B/P Ratio	16.3 ± 2.4
5-Day DIO Mouse Feeding Study (Once Daily Dosing)		5-Day DIO Mouse Feeding Study (Once Daily Dosing)	
Dose (po)	60 mg/kg	Dose (po)	30 mg/kg
Weight Loss	2.8% (p<0.05)	Weight Loss	4.5% (p<0.001)

\* AMR-MCH-2 showed increased brain exposure compared with AMR-MCH-1 in single dose PK studies

\* AMR-MCH-2 showed improved weight loss versus AMR-MCH-1 in 5-day DIO mouse feeding studies with once daily dosing

## CONCLUSIONS

- AMR-MCH-1 and AMR-MCH-2 were shown to be high affinity MCH<sub>1</sub> receptor antagonists.
- AMR-MCH-1 caused gradual, sustained weight loss in obese mice in a chronic feeding study.
- Weight loss was accompanied by reductions in food intake and fat mass.
- Structural modification of AMR-MCH-1 to give AMR-MCH-2 provided increased brain penetration and improved efficacy.

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