Evaluation of two novel compounds compared to methadone on morphine withdrawal in a rat model of physical dependence

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SYGNATURE DISCOVERY

Opioid use disorder (OUD) and the resultant opioid toxicity crisis is a significant public health concern. Opioid maintenance therapy (OMT) and in particular methadone maintenance therapy (MMT) are highly effective at preventing withdrawal syndrome, reducing cravings and increasing quality of life by reducing harms to people who use opiates. However, MMT has economical drawbacks such as witnessed daily administration and safety risks such as higher risk of overdose[1]. In this study, we first determined the maximum tolerated dose of two novel compounds and then evaluated their efficacy, compared to methadone, at alleviating morphine withdrawal syndrome in rats..

1 Methodology

Following baseline, male Sprague Dawley rats were orally administered morphine (30 mg/kg) twice daily for 14 days to induce morphine dependence. Abrupt cessation of morphine administration initiated withdrawal. Body weight, food and water intake and 44 behaviours were assessed daily. Methadone (15 mg/kg), Levo-1-ME and Levo-2S-ME were administered orally during morphine withdrawal.

Study Phase	Baseline			Induction of Dependence													Morphine Withdrawal						
Study Day	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Observations	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	Δ	Δ	Δ	Δ	Δ	Δ	Δ

Food, Water, Body Weight

↑ = Measurement of bodyweight and food and water intake, with behavioural observations am only.
Δ = Measurement of bodyweight and food and water intake, with behavioural observations am and pm.

2 Determining Maximum Tolerated Dose

Rats were orally administered Levo-1-ME or Levo-2S-ME once daily starting on the first day of morphine withdrawal (D15). Treatment started at 60 mg/kg and was increased in the next cohort if morphine withdrawal resembled vehicle treated rats, and there were no adverse effects of Levo-1-ME or Levo-2S-ME treatment. If adverse effects were present the dose was reduced.

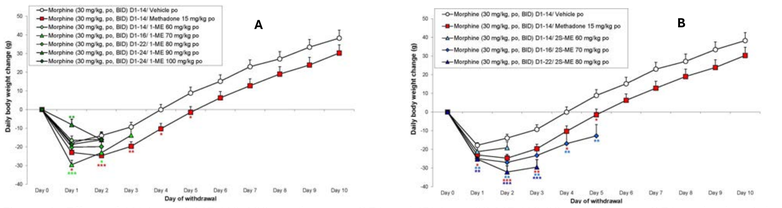

Figure 1: Effect of (A) Levo-1-ME (60-100 mg/kg, p.o., QD) and (B) Levo-2S-ME (60-80 mg/kg, p.o., QD) on bodyweight during morphine withdrawal, compared to vehicle and methadone (15 mg/kg, p.o., QD) treatment.

There were no adverse behavioural observations and the effects on body weight and food and water intake resembled methadone treatment with Levo-1-ME (100 mg/kg) or Levo-2S-ME (80 mg/kg). Four treatments were selected for the efficacy study; Levo-1-ME and Levo-2S-ME (80 mg/kg, QD or 60 mg/kg, BID).

3 Effect of Levo-1-ME, Levo-2S-ME and methadone on bodyweight during morphine withdrawal

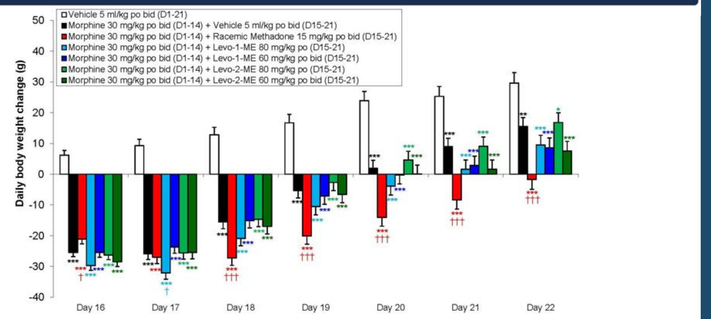

*Figure 4: Daily change in body weight with vehicle, methadone (15 mg/kg, p.o., BID) and Levo-1-ME and Levo-2S-ME (80 mg/kg, p.o., QD and 60 mg/kg, p.o., BID) treatment. Compared to vehicle by Dunnet's test *p<0.05, **p<0.01, ***p<0.001. Compared to morphine by Dunnet's †p<0.05, ††p<0.01, †††p<0.001.*

4 Effect of Levo-1-ME, Levo-2S-ME and methadone on food intake during morphine withdrawal

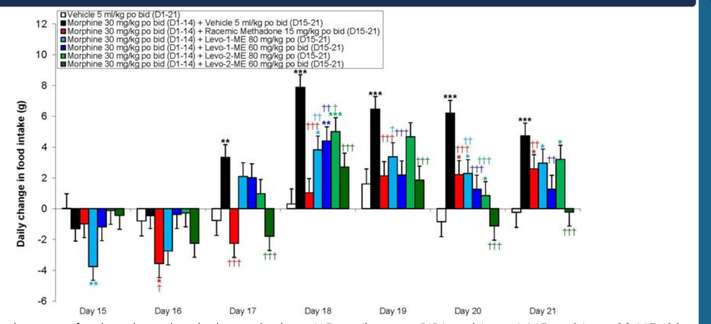

*Figure 4: Daily change in food intake with vehicle, methadone (15 mg/kg, p.o., BID) and Levo-1-ME and Levo-2S-ME (80 mg/kg, p.o., QD and 60 mg/kg, p.o., BID) treatment. Compared to vehicle by Dunnet's test *p<0.05, **p<0.01, ***P<0.001. Compared to morphine by William's † p<0.05, ††p<0.01, †††p<0.001.*

5 Effect of Levo-1-ME, Levo-2S-ME and methadone on water intake during morphine withdrawal

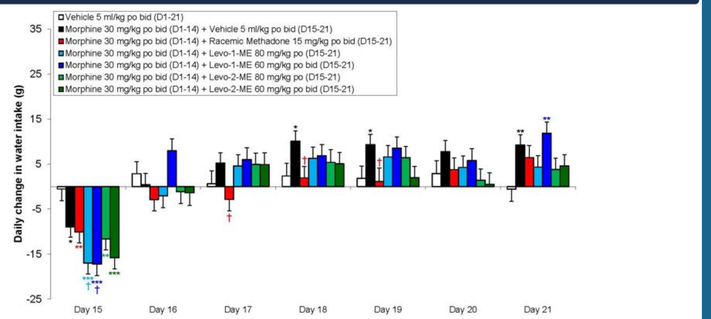

*Figure 4: Daily change in water intake with vehicle, methadone (15 mg/kg, p.o., BID) and Levo-1-ME and Levo-2S-ME (80 mg/kg, p.o., QD and 60 mg/kg, p.o., BID) treatment. Compared to vehicle by Dunnet's test *p<0.05, **p<0.01. Compared to morphine by Dunnet's †p<0.05.*

6 Behavioural observations during morphine dependence and morphine withdrawal

	Behavioural Observations	Morphine 30 mg/kg BID	Morphine Withdrawal	Methadone 15 mg/kg BID	Levo-1-ME 80 mg/kg QD	Levo-1-ME 60 mg/kg BID	Levo-2S-ME 80 mg/kg QD	Levo-2S-ME 60 mg/kg BID
Morphine Dependency Behaviours	Ataxia	+		+				
	Head Weaving	+						
	Straub Tail	+++		++++				
	Incr. Body Tone	+++		++++				
	Incr. LMA	++		+++	+			
	Incr. Reaction to Sound	++		++				
	Explosive Movements	+		++				
	Exophthalmos	+++		+++				
	Stained Fur	++						
Mixed Behav.	Hunched Posture	+	++	+	++	++	++	++
	Incr. Irritability	++	+++	+++	+	++	+	+
	Piloerection	+	+++	+	+++	++	+++	+++
Morphine Withdrawal Behaviours	Drooping Abdomen		+		+	+	+	+
	Arched Back		+		+			
	High Stepping		+	+	+	+	+	+
	Wet Dog Shakes		+				+	+
	Decr. Body Tone		++	+	+	+	++	+
	Tail Rattle		+	+		+		
	Incr. Respiration		++			+	+	+
	Diarrhoea		+					+
Methadone Complications	Abnormal Posture			+				+
	Jumping			+				
	Decr. Irritability			++				
	Decr. Respiration			+				
	Biting/Licking Cage			+				
	Eating Bedding			+				

Figure 5: Behavioural observations during morphine dependence phase (morphine 30 mg/kg BID) or morphine withdrawal phase (all other groups). Comparisons against vehicle by Wilcoxon Rank Sum test (all p<0.05-0.001). Mean score: +<0.025, ++0.25-0.49, +++0.5-0.74, ++++>0.75.

7 Discussion

- Morphine withdrawal syndrome is characterised by hyperphagia and hyperdipsia leading to a sudden increase in bodyweight. This effect is seen in patients due to compulsivity and craving of rewarding stimuli[2].
- Methadone alleviates morphine withdrawal induced hyperphagia and hyperdipsia, whilst Lev-1-ME and Levo-2S-ME alleviate hyperphagia only.
- Morphine induces a spectrum of behaviours which can be categorised during morphine dependence or morphine withdrawal.
- Methadone ameliorates most morphine withdrawal behaviours but also induces a pronounced opioid effect and additional potentially harmful behaviours.
- Levo-1-ME and Levo-2S-ME do not induce behaviours associated with morphine dependency or methadone treatment, whilst ameliorating some behaviours associated with morphine withdrawal.
- Levo-1-ME and Levo-2S-ME may be useful new tools for OMT with decreased side effect profile versus MMT.

[1] Stotts AL, Dodrill CL, Kosten TR. Opioid dependence treatment: options in pharmacotherapy. Expert Opin Pharmacother. 2009 Aug;10(11):1727-40.

[2] Maron JL. Addicted to Addictions: Why Recovering from One Deleterious Habit May Lead to the Next. Clin Ther. 2023 Dec;45(12):1161.