ADS012, a single strain live biotherapeutic product, attenuates tolerance to repeated morphine dosing in mice

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Background

Depletion of gastrointestinal bacteria by broad-spectrum antibiotics, such as vancomycin, results in the loss of the development of antinociceptive tolerance to chronic morphine, suggesting the gut microbiome may play a significant role. To refine this understanding, we sought to determine if the treatment of a single strain of bacteria with a very narrow bactericidal activity could have an impact on the development of morphine tolerance induced in mice by repeated subcutaneous (s.c.) morphine administration. The bacteria chosen was ADS012, a *Bacillus velezensis* strain with *C. difficile* bactericidal activity.

Aim

To determine if ADS012, an oral single strain live biotherapeutic product (SS-LBP) of *Bacillius velezensis*, can reduce the development of tolerance in mice induced by repeated morphine administration

Methods

Morphine tolerance was induced in 32 mice by repeated morphine-HCl dosing (10 mg/kg, s.c., bid, days 1-10) (Table 1). 10 control mice were not treated with morphine. Mice treated with ADS012 were dosed orally, twice daily with 5 x 10^8 colony forming units/dose. All animals were monitored for body weight loss and stool production.

Response to pain was assessed by a tail flick test using Ugo Basile Tail Flick Instrument. Mice were placed and held on the Ugo Basile Tail Flick instrument surface with the tail straight back and across an infrared light source. The heat source and timer were turned on by pressing the start button, and automatically switched off when the animal flicks its tail off the emitter. Latency time was recorded and analyzed as an analgesic effect. Tail flick test was performed 4 times: at baseline, on day 1 after the first dose, on day 6 after the morning morphine dose and similarly at the end of the study (day 10).

For electrophysiology, the mice were put under sedation, and stimulating electrodes were connected to the tibial nerve and recording electrodes to the C3 and C4 somatosensory positions of the brain. The tibial nerve was stimulated, and pain signals were measured as the Peak-to-Peak (P-2-P) amplitude of the P25 wave.

Electrophysiology was performed at baseline before morphine dosing on 15 animals, and on all animals on day 1 after the first dose, on day 6 after morphine morning dosing, and at the end of the study on day 10 after morphine morning dosing.

Morphine and morphine-3-glucuronide in plasma 1.5 hrs post-morphine injection were quantitated by HPLC.

Results

Repeated morphine-HCl led to morphine tolerance as determined by a gradual decrease in tail flick latency from Day 1 to Day 10 (12.3 to 4.8 sec; $p<0.0001$) (Fig 1). Treatment with ADS012 (5 x 10^8 CFU, PO; bid days 1-10) resulted in an attenuation of the development of morphine tolerance as demonstrated by higher tail flick latency relative to saline controls at Day 10 (9.3 sec vs 4.8 sec; $p<0.0001$).

For measurement of pain independent of animal behavior, tibial-nerve induced sensory evoked potential (SEP) was chosen (Fig 2). Repeated morphine-HCl dosing led to an increase in sensory evoked potential (SEP) p25 amplitude measured using posterior tibial nerve stimulation and C3/C4 scalp recording (14.9 uV to 61.8 uV; $p<0.01$) (Fig 3). Treatment with ADS012 resulted in attenuation of the development of morphine tolerance over time as demonstrated by lower amplitude in SEP p25 electrophysiology measurements relative to untreated mice (17.3 uV vs. 61.8 uV; $p<0.0001$).

ADS012 did not significantly impact morphine or morphine-3b-glucuronide plasma levels indicating that the mechanism of tolerance attenuation was not due to an impact on drug metabolism (Fig 4).

In addition, ADS012 did not significantly affect constipation which was severe and independent of the level of antinociceptive tolerance, though there was a trend towards increased fecal production (Fig 5).

Conclusions

These results suggest that the single strain LBP ADS012 attenuates the development of tolerance to repeated morphine dosing and highlights the gut-brain axis in morphine tolerance. LBP ADS012 can be an important adjunct to limit levels of morphine exposure needed over time to maintain analgesia.

Table 1. Study design of morphine tolerance due to repeated subcutaneous morphine dosing

Group	Opioid		Agent Treatment		Dosing Schedule	Latency time to Tail flick	Sensory evoked potential	Terminal Collection
Morphine (N=12)	Morphine	10 mg/kg bid s.c.	saline	-	Days 1-10	Days 0, 1, 6, 10	Days 0, 1, 6, 10	Day 10 Serum
Morphine + ADS012 (N=20)	Morphine	10 mg/kg bid s.c.	ADS012	$5x10^8$ CFU bid PO				
ADS012 (N=10)	-	-						

Fig 1. ADS012 significantly attenuated morphine tolerance as evaluated by increased latency to tail flick

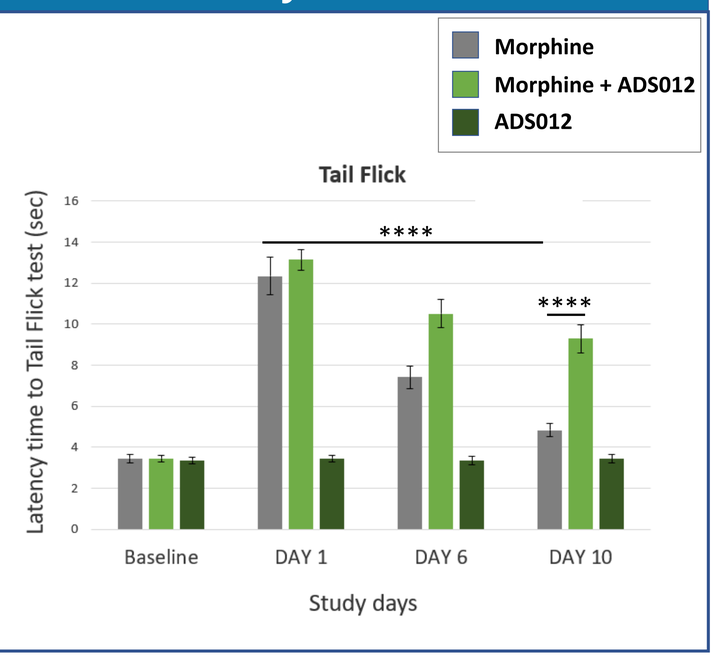


Fig 2. P25 wave recording from a naïve mouse before and after acute morphine showing analgesia

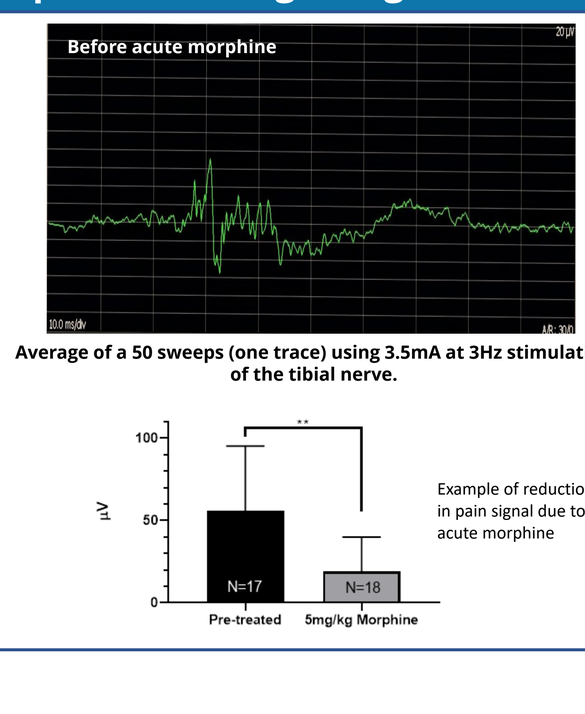


Fig 3. ADS012 attenuated development of morphine tolerance as determined by reduced sensory evoked potential (pain signal in brain)

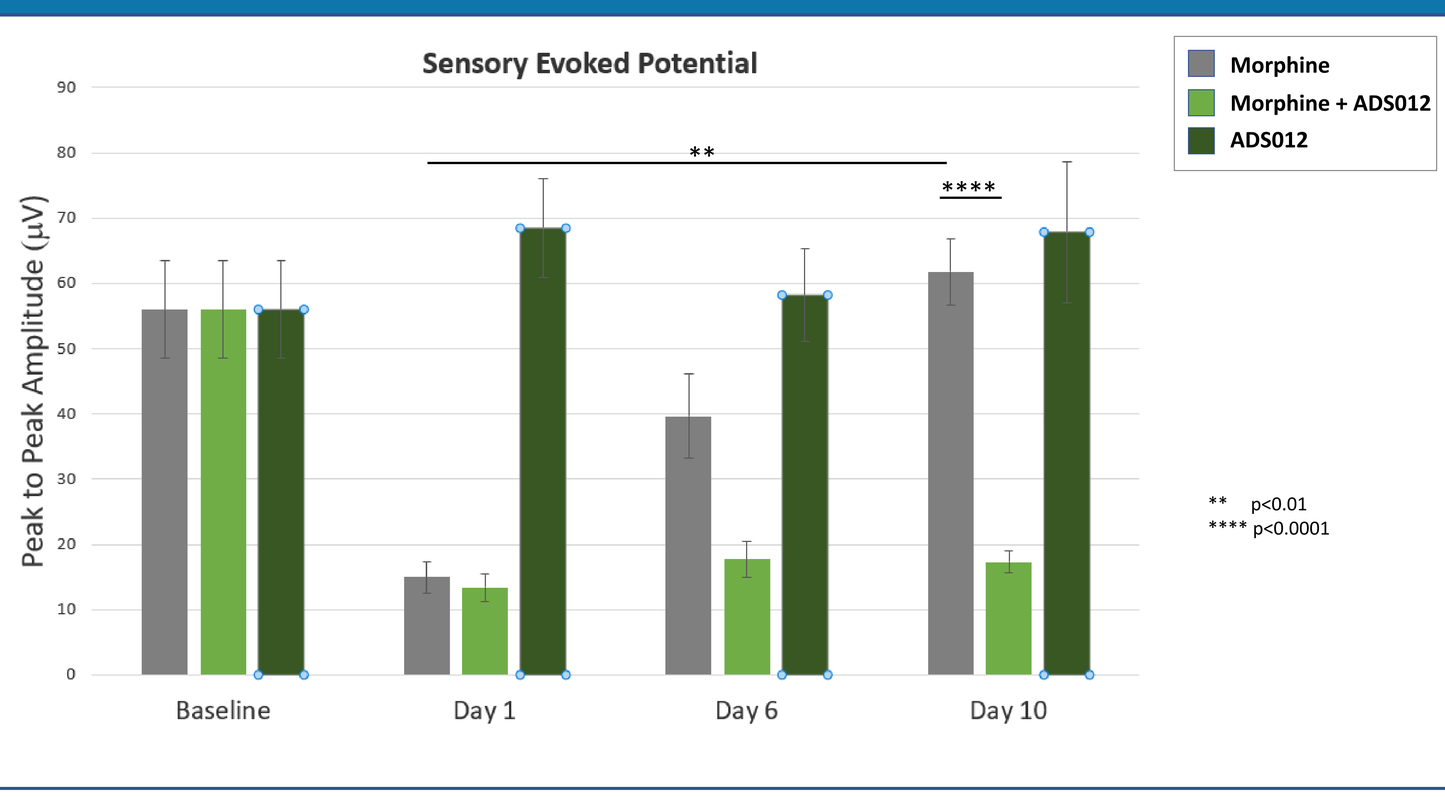


Fig 4. ADS012 did not significantly impact morphine metabolism

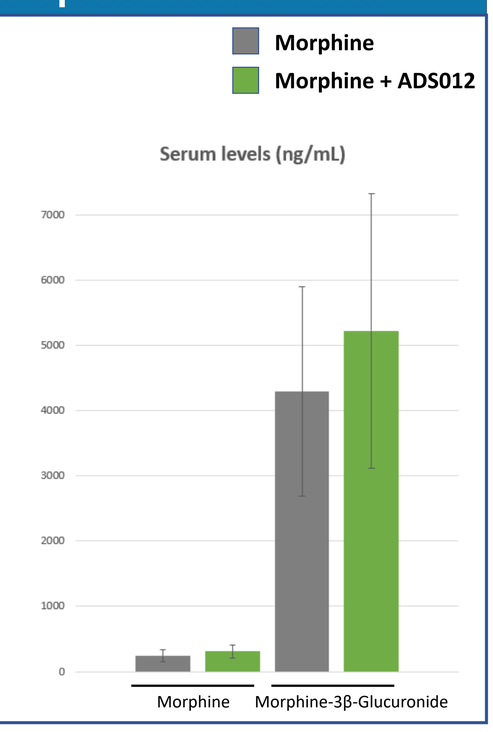
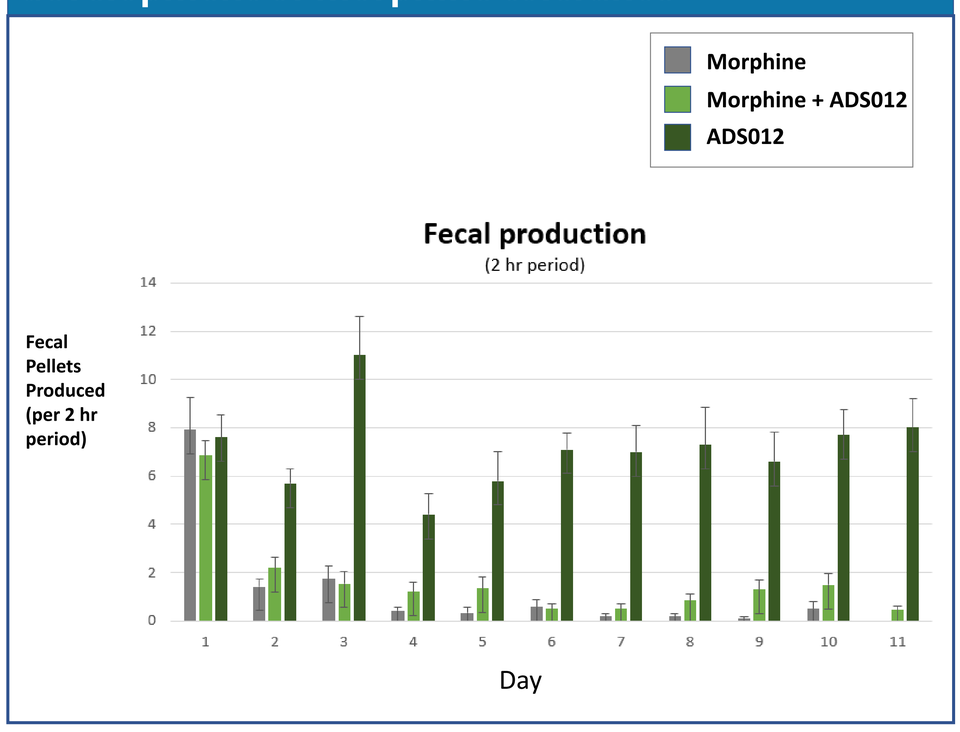


Fig 5. ADS012 did not significantly affect constipation which was independent of the development of morphine tolerance



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