The aim of this study was to determine whether 28 days administration of CR845 induced physical dependence on withdrawal by recording the behavioural, physical and physiological signs that occurred upon abrupt cessation of dosing. The µ-opioid receptor agonist, morphine, which produces rapid tolerance and physical dependence on withdrawal, was used as the positive control in this experiment.

METHODS

The experiment was conducted in 46 male, Sprague-Dawley rats (150-175 g on arrival; Charles River, UK). They were individually-housed on a 12hr/12hr cycle (lights on 07:00h). Food and water were available ad libitum. After acclimatisation for 1 week, they were divided into 4 groups (N values):

1. CR845 5 mg/kg/day (15);
2. Saline 1 ml/kg/day (11);
3. Morphine 60 mg/kg/day 5 mg/kg, bid (10);
4. Deionised water 5 ml/kg, bid (10).

CR845 and its vehicle control were given intravenously (i.v.) and morphine and its vehicle control were given orally (i.p.). After a 7-day baseline period, rats were administered drugs or vehicle for 28 days (on-dose phase), after which treatment was terminated and the rats were monitored for a further 7 days (withdrawal).

Physiological measurements of body weight, food and water intake and rectal body temperature were taken once-daily during the baseline, drug administration and withdrawal phases of the study. For the first 4 days of withdrawal, temperatures were measured twice-daily. A battery of behavioural signs (e.g. locomotor changes, stereotypy, tooth-chattering, aggression, vocalisation) and physical signs (e.g. loss of condition) were recorded daily during the 7-day baseline period and twice-daily in the on-dose and withdrawal phases.

RESULTS

Rats were initially dosed with CR845 at 25 mg/kg iv which resulted in a rapid loss of ~12% of their bodyweight in the first 24hr (likely a consequence of the known aquaretic effect of CR845 agonists) (Figure 1C). It was therefore decided to reduce the dose. Following a 7-day washout period, rats were tested with 5 mg/kg/day dose of CR845 in the on-dose phase, rats gradually regained weight back to control levels, food and water intake was unchanged relative to controls (Figure 1A-C). Body temperature was increased in Weeks 3 and 4 by a mean of 0.3°C (p<0.05) (Figure 1D).

Behavioural changes observed on-dose in a significant proportion of CR845-treated rats included ataxia, hunched body posture, Straub tail, decreased locomotor activity, subdued behaviour, ptosis and exophthalmos (Table 1). On withdrawal from CR845 dosing, there was no effect on bodyweight, food and water intake or body temperature compared with the vehicle control group (Figure 1A-D). Behavioural signs observed on-dose with CR845 gradually reduced during the 7-day withdrawal period with no robust symptoms of physical dependence (Table 1). Relative to its vehicle control, morphine (30 mg/kg/day, bid) decreased mean bodyweight by 26 g (p<0.001) at the end of Week 1 attenuating to 62 g (N.S.) by the end of Week 4 (Figure 2C). Food and water intake and temperature were reduced throughout the on-dose phase by an average of 15.2% (p<0.001), 21.8% (p<0.001) and 0.8°C (p<0.001), respectively (Figure 1A-B,D). Morphine produced unequivocal signs of treatment, including ptosis, altered locomotor activity (initially decreased, then increased), initial subdued behaviour, increased body tone, Straub tail, increased reactivity to sound and ptosis, and decreased food intake. Ataxia, Straub tail, increased reactivity to sound and ptosis (Table 2). About 20% of rats were irritable, possibly due to dependence/withdrawal emerging within the first 24hr of the daily morphine doses due to its short half-life. Cessation of morphine treatment produced characteristic physiological withdrawal signs of weight loss, hypophagia, hypothermia and hypothermia (Figure 2A-D). As the rats recovered, hypophagia, hypothermia, and weight regain occurred and hypothermia disappeared (Figure 2A-D). Behavioural signs of withdrawal included irritability, tail rearing and aggression. Other signs that emerged during this period were wincing, altered body tone, ataxia and respiratory abnormalities (Table 2). The most severe withdrawal effects after cessation of morphine treatment were observed in the first 3 days (Table 2). Reduced effects of morphine dependence gradually reduced in frequency over the following withdrawal days (Table 2). Increased locomotor activity on withdrawal was sporadically observed in the vehicle control group (Table 2).

DISCUSSION

CR845 5 mg/kg iv is predicted to produce plasma exposures that are 10-fold (Cmax) and 50-fold (AUC) greater than the targeted human clinical exposures. CR845 produced clear signs of drug effect in the on-dose phase, but no robust behavioural, physiological or physical signs of dependence on withdrawal. In contrast, repeated morphine administration produced rapid tolerance and unequivocal physical and behavioural signs of dependence on withdrawal. If these preclinical findings translate to man, they predict that CR845 will not produce physical dependence with repeated administration.

CONFLICT OF INTEREST

D.J. Heath is an employee and shareholder of Renasci Ltd. J. Goddard, J. Gosden, S. Dykes, R. Brammer, R. Spencer and F. Menzagh are employees and shareholders of Care Therapeutics, Inc.