BU10038 as a safe opioid analgesic with fewer side-effects after systemic and intrathecal administration in primates

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Abstract

Background: The marked increase in mis-use of prescription opioids has greatly affected our society. One potential solution is to develop improved analgesics which have agonist action at both mu opioid peptide (MOP) and nociceptin/orphanin FQ peptide (NOP) receptors. BU10038 is a recently identified bifunctional MOP/NOP partial agonist. The aim of this study was to determine the functional profile of systemic or spinal delivery of BU10038 in primates after acute and chronic administration.

Methods: A series of behavioural and physiological assays have been established specifically to reflect the therapeutic (analgesia) and side-effects (abuse potential, respiratory depression, itch, physical dependence, and tolerance) of opioid analgesics in rhesus monkeys.

Results: After systemic administration, BU10038 (0.001–0.01 mg kg⁻¹) dose-dependently produced long-lasting antinociceptive and anti-hypersensitive effects. Unlike the MOP agonist oxycodone, BU10038 lacked reinforcing effects (i.e. little or no abuse liability), and BU10038 did not compromise the physiological functions of primates including respiration, cardiovascular activities, and body temperature at antinociceptive doses and a 10–30-fold higher dose (0.01–0.1 mg kg⁻¹). After intrathecal administration, BU10038 (3 μg) exerted morphine-comparable antinociception and anti-hypersensitivity without itch scratching responses. Unlike morphine, BU10038 did not cause the development of physical dependence and tolerance after repeated and chronic administration.
The opioid epidemic has greatly affected a large population worldwide. Although mu opioid peptide (MOP) receptor agonists remain the most widely used analgesics, the abuse liability and respiratory arrest associated with MOP agonists have contributed to escalating medical and economic burdens in the global community. Through decades of research, numerous scientific strategies have tried to develop safe, non-addictive analgesics, but none has been demonstrated in humans.

The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is the fourth opioid receptor subtype, which generally inhibits neuronal transmission. Unlike a partial MOP agonist, buprenorphine, which can produce respiratory depression, NOP agonists do not inhibit respiratory function. More importantly, NOP agonists interact with buprenorphine in a synergistic manner to produce antinociceptive effects without respiratory depression. Given the inhibitory regulation of dopamine neurotransmission by the NOP receptor, coactivation of both MOP and NOP receptors may produce analgesia with fewer side-effects, that is, a wider therapeutic window.

Editor’s key points

- Opioids can be effective analgesics, but have unwanted side-effects including addiction, dependence, tolerance, and respiratory depression.
- Analgesics with activity at both mu and nociceptin opioid (MOP, NOP) receptors may have analgesic potential with reduced unwanted effects.
- The analgesic, cardiovascular, and respiratory effects of a bifunctional MOP/NOP agonist were assessed in primates.
- Analgesia without significant adverse effects was demonstrated. Further studies are needed to explore the clinical potential of such approaches.

Conclusions: These in vivo findings demonstrate the translational potential of bifunctional MOP/NOP receptor agonists such as BU10038 as a safe, non-addictive analgesic with fewer side-effects in primates. This study strongly supports that bifunctional MOP/NOP agonists may provide improved analgesics and an alternative solution for the ongoing prescription opioid crisis.

Keywords: analgesics; opioid; opiate addiction; opioid-related disorders; respiration; rhesus macaque

Safe, non-addictive opioid analgesic in primates

Methods

Subjects

All animal care and experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee of Wake Forest University (Winston-Salem, NC, USA). This study is reported in accordance with the animal research: reporting of in vivo experiments (ARRIVE) guidelines for reporting experiments involving animals. Sixteen adult male and female rhesus monkeys (Macaca mulatta), 10–19 yr of age, weighing 6.6–12.3 kg, were purchased from U.S. National Primate Centers for biomedical research, and they were kept at an indoor facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (Frederick, MD, USA). The animals were individually housed in cages with 6–12 ft² (floor area) and 2.7–5.4 ft (height) in species-specific rooms with environmental controls set to maintain 21–25°C, 40–60% relative humidity, and a 12-h light–dark cycle. Their daily diet consisted of approximately 20–28 biscuits (Purina Monkey Chow;Ralston Purina Co., St. Louis, MO, USA), fresh fruit, and water ad libitum. Small amounts of primate treats and various cage-enrichment devices were supplied as forms of environmental enrichment. Animals were not exposed to any opioid compounds for 1 month before experiments.

In vitro characterisation

Receptor binding

Affinities for the individual opioid receptors were determined in displacement binding assays in recombinant human opioid
receptors transfected into Chinese hamster ovary (CHO) cells as previously described. The displaced selective radioligands were \[^{3}H\]N/OFQ (NOP), \[^{3}H\]DAMGO (MOP), \[^{3}H\]Cl-DPDE (DOP, delta opioid peptide receptor), and \[^{3}H\]U69593 (KOP, kappa opioid peptide receptor).

\[^{35}S\]GTP\(\gamma\)S binding

The \[^{35}S\]GTP\(\gamma\)S binding stimulation assay, like the receptor binding assay, was performed in human opioid receptors transfected CHO cells as described previously. Agonist efficacy at these opioid receptors was determined in comparison with the standard selective agonists, that is, N/OFQ (NOP), DAMGO (MOP), DPDE (DOP), and U69593 (KOP).

Sensory assays

Acute nociception

The warm water tail-withdrawal assay was used to evaluate the thermal antinoceptive effects of BU10038 and morphine. Through the positive reinforcement techniques, monkeys were trained to cooperate for the pole-and-collar transfer to a primate restraint chair. They were seated in primate restraint chairs, and the lower parts of their shaved tails (~15 cm) were immersed in a thermal flask containing water maintained at 42, 46, or 50°C, which was randomly presented. Through numerous training sessions, monkeys have become adapted to this experimental setting. Water at 42 and 46°C was used as non-noxious stimuli (i.e. no tail-withdrawal movement), and water at 50°C was used as an acute noxious stimulus (i.e. 2–3 s tail-withdrawal latency). All tail-withdrawal latencies were measured at each temperature using a computerised timer by individuals who were blinded to the experimental conditions. If a monkey did not remove its tail within 20 s (cut-off), the flask was removed and a maximum time of 20 s was recorded. Test sessions began with baseline measurements at each temperature. Subsequent tail-withdrawal latencies were measured at multiple time points after subcutaneous or intrathecal administration of the test compound. For dose–response curves, the test compound was administered by a cumulative dosing procedure with a 30 min interinjection interval. Tail-withdrawal latencies were measured at 20 min after each injection. A single dose of MOP receptor-selective antagonist naltrexone (0.03 mg kg\(^{-1}\), s.c.) or selective NOP receptor antagonist J-113397 (0.1 mg kg\(^{-1}\), s.c.) was administered 15 min before determination of dose–response curves to determine the MOP and NOP receptor components mediating BU10038-induced antinociception. The doses and pretreatment time for naltrexone and J-113397 were chosen based on previous studies.

Capsaicin-induced thermal alldynia

The antiallodynic effects of BU10038 were evaluated by using a 1 h pretreatment regimen (i.e. 1 h before capsaicin administration). Capsaicin (1.2 mg ml\(^{-1}\) × 0.3 ml) was administered topically via a bandage attached on the terminal 3–5 cm of the tail for 15 min. The alldynic response was manifested as reduced tail-withdrawal latency from a maximum value of 20 s to ~2–3 s in 46°C water. This alldynic effect peaks at 15 min after removal of the capsaicin bandage, and this is the time point to measure the tail-withdrawal latency in 46°C water (i.e. to determine the antiallodynic effects of the test compound).

Itch scratching responses

Scratching activity as a behavioural response to itch sensation was recorded on videotapes when monkeys were in their home cages. Each 15 min recording session was conducted at multiple time points after intrathecal administration of BU10038 or morphine. A scratch was defined as one brief (<1 s) episode of scraping contact of the forepaw or hind paw on the skin surface of other body parts. Total scratches were counted and summed for each 15 min time block by individuals who were unaware of the experimental conditions.

Drug self-administration

Monkeys with indwelling intravenous catheters and subcutaneous vascular access ports were used to evaluate the reinforcing effects of the test compound under a progressive-ratio schedule as described previously. Briefly, the monkeys’ operant responding was evaluated using injections of 3 mg kg\(^{-1}\) oxycodone or saline until responding was stable (mean ± standard error of the mean, SEM = 3 injections for three consecutive sessions with no trend). Dose–response curves were determined in each monkey by substituting a range of doses of BU10038 (0.1–3 mg kg\(^{-1}\) per injection, i.v.) in a randomised order. Doses were available for at least five consecutive sessions and until responding was deemed stable.

Physiological responses

Freely moving monkeys implanted with the D70-PCTR telemetry transmitter were used to evaluate the effects of BU10038 on physiological functions as described previously. Respiratory rate, heart rate, blood pressure, and temperature were measured and analysed with Ponemah software version 5.2 (Data Sciences International, St. Paul, MN, USA). For acute drug effects, data from the 30 min interval before drug administration were collected as baseline and then at each time point (i.e. 1, 6, 24, and 48 h) after administration of BU10038 (0.01, 0.1 mg kg\(^{-1}\), i.m.). For detecting precipitated withdrawal signs after 3 days (i.e. one injection per day at 09:00 AM) of BU10038 administration (0.01 mg kg\(^{-1}\), i.m.), data from the 30 min interval before antagonist were collected and then continuously for 2 h after administration of antagonist J-113397 (0.03 mg kg\(^{-1}\), i.m.) and naltrexone (0.01 mg kg\(^{-1}\), i.m.) on Day 5. The mean value of each 15 min time block was generated from each subject to represent the measure outcome for each single data point.

Surgical implantation

The surgical procedures, intrathecal catheterisation and implantation of telemetry device, have been successfully conducted and the surgical details can be found in previous studies. Before surgery, animals were given atropine (0.04 mg kg\(^{-1}\), i.m.), buprenorphine (0.01–0.03 mg kg\(^{-1}\), i.m.), and cefotaxime (500 mg, i.v.) for pain relief and prevention of infection. Then animals were anaesthetised with ketamine (10 mg kg\(^{-1}\), i.m.) and intubated and maintained under anaesthesia with inhaled isoflurane (1–2% in 1 L min\(^{-1}\) O\(_2\)). A catheter was placed in a saphenous vein for administration of lactated Ringer’s solution during the surgery. Intraoperative monitoring was conducted to determine the depth of anaesthesia and physiological status. Vital signs, such as heart rate, respiration rate, indirect blood pressure, and body temperature, were recorded at the initiation of the surgery, periodically...
throughout the procedure, and in the immediate post-operative recovery period. Animals received buprenorphine (0.003–0.02 mg kg$^{-1}$, i.m.) and meloxicam (0.15 mg kg$^{-1}$, s.c.) after operation to manage pain and inflammation, and ceftriaxone (2.2 mg kg$^{-1}$, i.m.) to manage post-surgical infections. Postoperative care and incision site observations were performed daily until healing was complete, which was evaluated by on-site veterinarians. In addition, attending veterinarians provided medical care on a round-the-clock basis including weekends and holidays. All animals were monitored daily by veterinarian and laboratory staff and maintained in good health throughout the entire study period.

**Data analysis**

Mean (SEM) values were calculated from individual data for all study end points. Comparisons were made for the same monkeys across all test sessions in the same experiment. Data were analysed using either two-way analysis of variance (ANOVA) with repeated measures (data of telemetry and itch) or one-way ANOVA with repeated measures (data of drug self-administration), followed by Bonferroni’s multiple comparisons test. The criterion for significance for all tests was set at $P<0.05$. To analyse nociceptive responses, individual tail-withdrawal latencies were converted to the percentage of maximum possible effect (MPE) using the formula defined as:

$$\text{MPE} = \left(\frac{\text{test latency} - \text{control latency}}{\text{cut-off latency, 20 s} - \text{control latency}}\right) \times 100. \quad (1)$$

Because MPE data are not normally distributed as 100% MPE cannot be exceeded and also our sample size is limited, at each time point, we used the Kruskal–Wallis test to compare the MPE across treatment groups and to compare each treatment group with the vehicle group. MPE data are displayed as...
median values with inter-quartile ranges in the Supplementary Tables. Kruskal–Wallis test is a one-way ANOVA on ranks and does now assume a normal distribution. To compare the time effect in each treatment group, we used the repeated-measures one-way ANOVA on ranks for analysis. This approach is similar to repeated-measures one-way ANOVA but uses ranks instead of original values for analysis. To calculate both treatment effect and time effect, we used the repeated-measures two-way ANOVA on ranks.

**Drugs**

BU10038 HCl (provided by Dr. Stephen M. Husbands, University of Bath, Bath, UK) was dissolved in a solution of dimethyl sulphoxide/10% (mass/vol) (2-hydroxypropyl)-β-cyclodextrin in a ratio of 3:97. Morphine sulphate, oxycodone HCl, and naltrexone HCl (National Institute on Drug Abuse [NIDA], Bethesda, MD, USA) were dissolved in sterile water. J-113397 (Tocris Bioscience, Minneapolis, MN, USA) was dissolved in a solution of dimethyl sulphoxide/Tween 80/sterile water in a ratio of 1:1:8. Capsaicin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 70% (vol/vol) ethanol. For systemic administration, drugs were administered at a volume of 0.1 ml kg⁻¹. The systemic delivery route determines on the setting of primate subjects and the safety of laboratory personnel performing the injection procedure. When monkeys were sitting in the primate chair (e.g. for measurement of tail-withdrawal responses), the test compound was delivered subcutaneously in the back (i.e. around the scapular region). When monkeys were in their home cages (e.g. for measurement of physiological responses by the telemetry device), the test compound was delivered intramuscularly into the thigh. For intrathecal administration, monkeys with intrathecal catheters and subcutaneous access ports were used. A total volume of 1 ml was administered through the access port followed by 0.35 ml of sterile saline to flush out the dead volume of the port and catheter. For acute administration, there was a minimum of 1 week interval between drug administrations. Based on our prior experience across different ligands and study end points and systemic and intrathecal delivery routes, a 1 week inter-injection interval is sufficient to avoid potential confounding factors, that is, baseline responses and the potency and magnitude of drug effects can be repeatedly observed in the same subjects. For chronic administration, morphine was administered intrathecal twice daily (first injection at 09:00 AM and second injection at 04:00 PM) and BU10038 was administered intramuscularly or intrathecally once every 2 days (injection at 09:00 AM) for 4 weeks. This chronic dosing strategy was selected based on the duration of analgesic action between BU10038 (24 h) and morphine (6 h), that is, an approximate four-fold difference. As the analgesic is re-administered to patients after its analgesia is subsiding in the clinical setting, we used this repeated dosing regimen to compare and determine if BU10038 and morphine retain their analgesic effects after animals were repeatedly exposed to and maintained under a similar duration of analgesic action.

**Results**

**Receptor and [³⁵S]GTPyS binding profile of BU10038**

BU10038 is a C14-O-naltrexone derivative (Fig. 1a). Table 1 shows that BU10038 has binding Kᵢ values between 1 and 15 nM at all opioid receptor subtypes. Distinct from naltrexone, BU10038 has a relatively good binding affinity at the NOP receptor, that is, 14.8 vs >10 000 nM. Table 2 shows the in vitro functional activity of BU10038 as measured by the [³⁵S]GTPyS binding assay. BU10038 does not have detectable efficacy at DOP and KOP receptors. At the MOP receptor, BU10038 has approximately 18% stimulation relative to DAMGO, which is similar to that of buprenorphine. At the NOP receptor, BU10038 has approximately 34% stimulation relative to N/OFQ. Overall, these findings indicate that BU10038 is a bifunctional MOP/NOP partial agonist.

**Systemic BU10038 produces potent and long-lasting antinociceptive and antiallodynic effects**

MOP agonists are known to change nociceptive threshold and produce antinociception in primates and humans. Therefore, the warm water tail-withdrawal assay was used to determine the functional efficacy of BU10038 for changing the nociceptive threshold. BU10038 (0.001–0.01 mg kg⁻¹, s.c.) produced antinociceptive effects against an acute noxious stimulus, 50°C water, in a dose- (F₃, 9 = 25.5; P < 0.05) and time-dependent (F₉, 2₇ = 13.8; P < 0.05) manner (Fig. 1b). The minimum effective dose of BU10038 to produce full antinociception was 0.01 mg kg⁻¹. The duration of action produced by this dose was 30 h and subsided by 48 h. To determine the anti-hypersensitive efficacy of BU10038, we used a clinically relevant model, capsaicin-induced allodynia, which has been widely applied to evaluate analgesics in humans. BU10038 attenuated capsaicin-induced thermal allodynia in 46°C water dose- (F₃, 9 = 5.1; P < 0.05) and time-dependently (F₉, 2₇ = 30.2; P < 0.05) (Fig. 1c). Next, we conducted antagonist studies by using the MOP-selective dose of the opioid receptor antagonist naltrexone and the NOP antagonist J-113397. Pretreatment with naltrexone (0.03 mg kg⁻¹) or J-113397 (0.1 mg kg⁻¹) produced similar degrees (i.e. approximately three-fold dose ratio) of the rightward shift of the dose–response curve for BU10038-induced antinociception (Fig. 1d). These findings indicate that both MOP and NOP receptors contributed to the anti-nociceptive effects of BU10038. The antinociceptive duration of BU10038 (0.01 mg kg⁻¹, s.c.) was much longer than that of morphine (1.8 mg kg⁻¹, s.c.) (i.e. >24 vs 6 h) (Fig. 1e). Based on the dose–response curves, BU10038 was more potent than morphine (ED₅₀ = 0.003 vs 1 mg kg⁻¹) (Fig. 1f). Overall, systemic BU10038 displays a favourable analgesic profile in primates.

**BU10038 does not produce reinforcing effects**

To examine and compare the reinforcing strengths of compounds, we used a progressive-ratio schedule of

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<th>Compound</th>
<th>NOP</th>
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reinforcement which has been commonly used for evaluating abuse potential. Monkeys were given the opportunities to intravenously self-administer oxycodone and various doses of BU10038 (0.1–3 µg kg⁻¹ per injection). Substitution of saline between test compounds resulted in a low number of reinforcers (i.e. three or fewer injections). Oxycodone (3 µg kg⁻¹ per injection) produced strong reinforcing effects (Fig. 2a–e). In contrast, there was no significant difference between the reinforcing strengths of saline and BU10038 (F = 1.6; P > 0.1) (Fig. 2a–e). Collectively, BU10038 may have much less abuse liability than the MOP analgesic oxycodone.

Higher doses of BU10038 do not compromise physiological functions

In order to characterise the safety window of BU10038, we measured a variety of physiological parameters in monkeys implanted with radio-telemetric transmitters. A systemic dose (0.01 mg kg⁻¹, i.m.) of BU10038 that produced full antinociception did not affect the respiratory function (respiration rate and minute volume), cardiovascular activity (heart rate, QRS interval, and blood pressure), and body temperature of monkeys (Fig. 3a–f). At a dose (0.1 mg kg⁻¹, i.m.) approximately 10–30 times higher than its antinociceptive doses, BU10038 also did not significantly change any physiological parameters (all F values: 0.5–4, P > 0.1) during the 48 h period (Fig. 3a–f). These findings indicate that BU10038 is a safe analgesic without respiratory and cardiovascular concerns in primates.

Intrathecal BU10038 produces potent antinociceptive and antiallodynic effects

After intrathecal administration, BU10038 (0.3–3 µg) produced antinociceptive effects against an acute noxious stimulus, 50°C water, in a dose- (F₃,₉ = 17.5; P < 0.05) and time-dependent (F₄,₁₂ = 12.6; P < 0.05) manner (Fig. 4a). The minimum effective dose of BU10038 to produce full antinociception was 3 µg. The duration of action produced by this dose was 30 h and subsided by 48 h. Intrathecal BU10038 also attenuated capsaicin-induced thermal allodynia in 46°C water dose- (F₃,₉ = 18.9; P < 0.05) and time-dependently (F₃,₉ = 19.9; P < 0.05) (Fig. 4b). The antinociceptive duration of intrathecal BU10038 3 µg was much longer than that of morphine 30 µg (Fig. 4c). To examine whether intrathecal BU10038 elicits itch sensation, we compared its effects with morphine, which elicits scratching responses in monkeys. Although BU10038 (3 µg) produced potent antinociception and antihypersensitivity, it did not significantly increase scratching responses (F₁,₃ = 0.6; P = 0.5). In contrast, morphine (30 µg) elicited scratching responses in the

Table 2 Opioid agonist stimulation of [³⁵S]GTPγS binding in CHO cells expressing recombinant human opioid receptors or NOP receptors. Data are the average from two experiments, each carried out in triplicate. *Too little stimulation to determine EC₅₀. CHO, Chinese hamster ovary; DOP, delta opioid peptide receptor; MOP, mu opioid peptide; NOP, nociceptin/orphanin FQ peptide; KOP, kappa opioid peptide receptor

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Fig 2. Comparison of reinforcing effects of oxycodone and BU10038 as measured by drug self-administration in monkeys. (a–e) Number of injections received as a function of dose in monkeys responding to oxycodone (O, 3 µg kg⁻¹ per injection), saline (S, ~0.14 ml kg⁻¹ per injection), or BU10038 (0.1–3 µg kg⁻¹ per injection) under a progressive-ratio schedule of reinforcement. (a–d) Data of individual monkey (M1–M4). Each data point represents mean (SEM) (n = 3–5 sessions). (e) Data of grouped monkeys. Each data point represents mean (SEM) (n = 4). *P < 0.05, a significant difference from saline. SEM, standard error of the mean.
same subjects ($F_{1, 3} = 12.1; P < 0.05$) (Fig. 4d). Taken together, BU10038 displays a promising spinal analgesic profile in primates.

Repeated exposure to BU10038 is devoid of physical dependence

After repeated exposure to opioid analgesics, primates and humans quickly develop physical dependence.\(^{16,38,39}\) In particular, antagonist-precipitated withdrawal signs are manifested as changes in respiratory and cardiovascular activities in primates.\(^{14,16}\) After repeated administration of BU10038 (0.01 mg kg\(^{-1}\), i.m., daily for 3 days), a combination of naltrexone (0.01 mg kg\(^{-1}\), i.m.) and J-113397 (0.03 mg kg\(^{-1}\), i.m.) did not precipitate withdrawal signs, that is, no changes in all physiological parameters measured herein (all $F$ values <3, $P > 0.1$) (Fig. 5a–e). Therefore, BU10038 does not produce physical dependence after 3 days of repeated administration.

Chronic exposure to BU10038 does not cause tolerance

After repeated exposure to opioid analgesics, animals and humans may develop tolerance.\(^{40,41}\) After a long-term exposure to systemic morphine (i.e. two injections of 1.8 mg kg\(^{-1}\) daily for 4 weeks), morphine-treated monkeys developed tolerance to antinociception.\(^{32}\) In the same group of animals, after the same duration of chronic administration, BU10038 (0.01 mg kg\(^{-1}\), i.m.)-treated monkeys did not show tolerance to antinociception produced by 0.003 and 0.01 mg kg\(^{-1}\) (Fig. 6a). Similarly, chronic exposure to intrathecal morphine (i.e. two injections of 30 mg daily for 4 weeks) led to a significant decrease in the antinociceptive effects of morphine ($F_{1, 3} = 32.5; P < 0.05$) (Fig. 6b). There was no change in the antinociceptive effects of BU10038 after chronic exposure to intrathecal BU10038 (3 mg) for 4 weeks (Fig. 6c). These results demonstrate that unlike morphine, chronic administration of systemic or intrathecal BU10038 does not develop tolerance.

Discussion

This study provides four significant findings indicating the therapeutic potential of BU10038, a novel bifunctional MOP/NOP agonist, as a safe, non-addictive analgesic with reduced side-effects. First, BU10038 produces potent and long-lasting antinociception and antihypersensitivity by activating MOP and NOP receptors. Second, BU10038 lacks reinforcing effects (i.e. little or no abuse potential), and it is safe and does not compromise respiratory and cardiovascular functions at, or 10
times above, analgesic doses. Third, BU10038 exerts spinal analgesic action without itch. Fourth, unlike morphine, BU10038 may not produce physical dependence or tolerance after repeated and chronic administration.

We have identified derivatives of the opioid receptor antagonist naltrexone with additional NOP receptor affinity and efficacy with low efficacy at the MOP receptor. BU10038 is one of these compounds, specifically the 14-O-phenylpropanoyl ester of naltrexone. We believe the phenylpropanoyl side chain of BU10038 extends into the region occupied by the t-butyl group of buprenorphine, which may explain the similar, but non-identical pharmacological profile.43,44 Buprenorphine is a partial MOP agonist, but it is commonly used in both human and veterinary medicine to effectively treat various pain conditions.45,46 Because MOP agonists increase nociceptive threshold and inhibit capsaicin-induced allodynia in humans,32,35 full antinociceptive and antiallodynic effects of BU10038 suggest that its functional efficacy as an analgesic may be similar to MOP agonists. It is worth noting that NOP antagonists enhanced the antinociceptive effects of bifunctional MOP/NOP agonists in rodents.47 However, NOP antagonists attenuated those of

![Fig 4. Effects of intrathecal administration of BU10038 on modulating sensory processing in monkeys. (a) Antinociception against acute noxious stimulus, 50°C water. (b) Antihypersensitivity against capsaicin-induced allodynia in 46°C water. (c) Comparison of antinociceptive duration of BU10038 (3 μg) and morphine (30 μg). (d) Comparison of itch scratching responses elicited by BU10038 (3 μg) and morphine (30 μg). Each data point represents mean (SEM) (n=4). All compounds were delivered intrathecally. *P<0.05, significantly different from vehicle condition from the first time point to the corresponding time point. SEM, standard error of the mean.](image)

![Fig 5. Lack of physical dependence on BU10038 in monkeys after short-term repeated administration. BU10038 (0.01 mg kg⁻¹) was administered once daily for 3 days. On Day 5, the antagonists naltrexone (0.01 mg kg⁻¹) and J-113397 (0.03 mg kg⁻¹) were used to precipitate withdrawal signs that were measured in monkeys implanted with telemetric probes before and after antagonist treatment. (a) Respiration rate. (b) Minute volume. (c) Heart rate. (d) Mean arterial pressure. (e) Body temperature. Data are shown as changes from baseline values (i.e. before antagonist treatment). Each data point represents mean (SEM) (n=4) from each individual data averaged from a 15 min time block. All compounds were delivered intramuscularly. SEM, standard error of the mean.](image)
bifunctional MOP/NOP agonists in primates. As drugs that work in rodents often fail when tried in humans, the non-human primates could serve as a surrogate species for humans to further investigate and develop bifunctional MOP/NOP agonists as analgesics.

Compared with highly abused drugs such as MOP agonists and psychostimulants, BU10038 does not produce reinforcing effects. In our intravenous drug self-administration procedure in primates, considered a gold standard to evaluate the abuse liability of drugs, BU10038 shows little to no abuse potential. In addition, BU10038 at antinociceptive doses and a 10–30-fold higher dose did not cause respiratory depression or affect cardiovascular function. Given the respiratory depression or arrest caused by MOP agonists, BU10038 demonstrates a wider safety window in primates. Overall, the functional profile of systemic BU10038 is similar to that of BU08028. These in vivo findings in primates support the scientific strategy that bifunctional MOP/NOP agonists are alternative analgesics which may have a direct impact on the worsening opioid crisis.

Neuraxial/spinal drug administration is the procedure that delivers drugs in close proximity to the spinal cord. To date, intrathecal delivery of opioids has become one of the standard procedures for perioperative analgesia and is used successfully in different clinical contexts. However, itch is one of the side-effects associated with the spinal use of MOP agonists and compromises the use of opioid analgesics in pain management. Lack of itch scratching responses by intrathecal BU10038 reinforces the hypothesis that coactivation of MOP and NOP receptors synergistically exerts analgesia with fewer side-effects. The spinal dorsal horn is the major locus not only for the integration of peripheral sensory input and descending supraspinal modulation, but also for regulating peripherally and centrally elicited pain. Given that intrathecal drug delivery can provide effective pain intervention as an alternative delivery route, bifunctional MOP/NOP agonists can be used as spinal analgesics to substantially advance human medicine.

After repeated administration, opioid analgesics often cause adverse events, such as physical dependence and tolerance. After short-term exposure (i.e. 3 days), morphine-treated primates displayed precipitated withdrawal signs. In contrast, BU10038-treated primates did not develop physical dependence. After long-term exposure (i.e. 4 weeks), morphine-treated primates developed tolerance to antinociceptive effects of morphine. In contrast, BU10038-treated primates did not show tolerance by either systemic or intrathecal route, even after 4 weeks of chronic administration. Although more frequent dosing and longer durations of treatment could result in tolerance, these findings may indicate that bifunctional MOP/NOP agonists such as BU10038 have advantages over morphine in repeated or chronic dosing regimens. Given the neuroplasticity of NOP receptors under chronic pain states, future studies are warranted to investigate whether bifunctional NOP/MOP agonists cause tolerance to develop more slowly compared with MOP agonists in patients with chronic pain.

Collectively, the therapeutic potential of BU10038 extends from that of a recently reported BU08028 with partial agonist activity at MOP and NOP receptors. Systemic or spinal delivery of BU10038 is devoid of several adverse effects associated with clinically used MOP agonists after acute and chronic administration. It is pivotal to further investigate the functional profiles of bifunctional MOP/NOP ligands by using a variety of pharmacological tools with different efficacy at MOP vs NOP receptors. For example, cebranopadol is a newly developed analgesic with mixed MOP and NOP full agonist activity and has been in several clinical trials for its analgesic efficacy. However, cebranopadol generalises to a morphine discriminative stimulus. It will be important to know the similarities and differences between bifunctional partial and full MOP/NOP agonists in terms of their abuse potential, safety window, and tolerability profile. Primate models will continue to be a translational bridge to facilitate the research and development of bifunctional MOP/NOP agonists as safe, non-addictive analgesics.
Author’s contributions
Study design/planning: NK, HD, MCK.
Study conduct: All authors.
Data analysis: All authors.
Writing paper: NK, HD, SMH, MCK.

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Declaration of interest
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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2018.10.065.

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