A NOVEL BIFUNCTIONAL OPIOID THAT LACKS TRADITIONAL OPIOID SIDE EFFECTS

By

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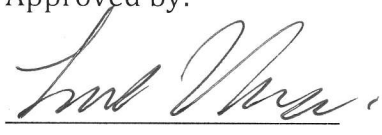

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Abstract

Prescription opioids, such as morphine, remain an important part in the management of pain. However, their clinical utility can be limited by side effects such as constipation, nausea, and high risk of addiction. Additionally, abuse of prescription opioids has been on the rise in recent years. Therefore, it is necessary to develop effective analgesics that lack the rewarding properties of currently used opioids. The neurokinin-1 receptor (NK-1) and its endogenous ligand, Substance P (SP), have been implicated in the control of nausea and vomiting, as well as mediating the rewarding effects of opioids. Here we have characterized the side effects of a novel efficacious opioid agonist/NK-1 antagonist, TY027. TY027 fails to elicit conditioned place preference, retching or vomiting, and does not inhibit gastric motility. These findings suggest that TY027 has a superior side effect profile when compared to currently used opioids, and most importantly, it does not produce rewarding effects that may lead to addiction.

Introduction

Opioid medications are widely used to control pain, and are effective for managing both acute and chronic pain states (1-4). However, opioid administration often results in unpleasant side effects. Some of the more common side effects include constipation, nausea, and vomiting. Opioids also possess rewarding effects, which can lead to abuse or addiction. Prescription opioid medications represent one of the most abused drugs in the US, with nearly 1 in 12 high school seniors reporting nonmedical use of Vicodin (hydrocodone/acetaminophen), and 1 in 20 reporting nonmedical use of OxyContin (oxycodone sustained-release) (5,6). The rewarding effects of opioids are mainly due to μ-opioid receptor agonism, as μ-opioid receptor knockout mice are

insensitive to the rewarding effects of opioids, and μ-opioid receptor antagonists block the rewarding effects of opioids (7-9). In order to help limit the potential for abuse, it is necessary to develop opioids that lack rewarding effects while still providing pain relief.

Opioid-induced constipation is a common side effect in patients taking opioids, with prevalence rates ranging from 15 - 90% of patients affected (10-12). This variability reflects the fact that age, gender, pathology, choice of opioid, route of administration, dose, and duration of treatment all affect the incidence of constipation. Constipation and related gastric disturbances can cause significant distress, and result in decreased quality of life (13,14). Additionally, tolerance rarely develops to the constipating properties of opioids (10). Non-pharmacological treatments for opioid-induced constipation include increased water and fiber intake, but drug interventions are often necessary. Laxatives are widely used, but are frequently ineffective, and may cause electrolyte imbalances. Stool softeners are well tolerated, but are rarely effective on their own (10, 13). Therefore, it is necessary to develop effective analgesics that lack the constipating effects of traditional opioids.

Nausea and vomiting (emesis) are other common side effects of treatment with opioids. The prevalence rates of nausea range from 10 - 50%, and can also affect quality of life (11, 15, 16). Evidence suggests that this may be a dose-related phenomenon, with dose escalation provoking more intense effects (17-19). Using a COX-2 inhibitor can have an opioid sparing effect, with lower opioid doses used, resulting in a reduced incidence of nausea and vomiting (18). However, there is evidence to suggest that pain itself can induce nausea and vomiting (20). When high doses of opioid are warranted, there are several therapies available. Anti-histamines, anti-cholinergics, and dopamine

receptor antagonists have been useful in treating nausea, but sometimes switching anti-emetics or combinations of anti-emetics are necessary to control the vomiting. Unfortunately, the anti-emetics themselves often posses side effects, including sedation and confusion (15, 16), which in turn limit their utility.

Substance P (SP) is a neuropeptide that binds preferentially to the neurokinin-1 receptor (NK-1), and may be involved in the rewarding properties and side effects of opioids. Previously, anatomical and functional evidence has linked the opioid and SP-NK-1 systems (21-24). The rewarding properties of morphine were absent in NK-1 knockout (KO) mice, suggesting a role for SP in morphine reward. This effect was also morphine specific; the NK-1 KO mice responded normally to other rewarding stimuli, such as cocaine or food (25-27). Furthermore, NK-1 antagonists have been found to have clinical utility as anti-emetics, currently approved to treat both chemotherapy-induced nausea and vomiting and postoperative nausea and vomiting (28).

Previous research in my lab had identified a lead compound, TY027 **(**Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-Bn$(CF_3)_2$**),** with μ/σ opioid agonist activity and NK-1 antagonist activity. It has a half-life of 4.83 hours in rat plasma, and was found to cross the blood-brain barrier. In nerve-injured rats, TY027 relieved thermal hyperalgesia and tactile allodynia in a dose-dependent manner. TY027 was active after both central and systemic administration. The purpose of this study was to evaluate the abuse potential of intravenously administered TY027, as well as determining its effects on both emesis and gastric transit.

Materials and Methods

Animals

Male Sprague-Dawley rats (175-300g; Harlan, Indianapolis, Indiana), and male ferrets (1.1-1.5 kg, Marshall BioResources) were kept in a temperature-controlled environment with a light/ dark cycle of 12 hours starting at 07:00.Food and water were available *ad libitum*. All animal procedures were performed in accordance with the policies and recommendations of the International Association for the Study of Pain, the National Institutes of Health and with approval from the Animal Care and Use Committee of the University of Arizona for the handling and use of laboratory animals.

Drug Preparation

Morphine sulfate (MS) was obtained from the National Institutes of Drug Abuse. TY027 (H-Tyr-D-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-3,5Bn(CF_3)$_2$) was synthesized as described by Yamamoto et al. (29, 30). Unless otherwise stated, MS and TY027 were dissolved in a vehicle of a 10% DMSO, 10% Tween 80, and 80% saline solution (DTS).

Jugular vein catheterization

Rats were anesthetized with a mixture of ketamine/xylazine, 100 mg/kg, 1mL/kg i.p. (80% ketamine/20% xylazine) and had their right jugular vein exposed. A piece of PE50 tubing (Cole-Parmer) was inserted 2 cm into the right jugular vein and secured in place with sutures, and then externalized on the back between the shoulder blades. After surgery animals were housed individually. Catheters were flushed with a 10% heparin solution in saline every 3 days.

Conditioned Place Preference

A three-chamber box was set up with different visual and tactile cues in the end chambers. To ensure no baseline differences, animals were given 20 minutes free access to explore all chambers. The score from both baselines were averaged for the

final baseline value. On conditioning days the animals were confined to a chamber for 20 minutes after receiving vehicle or drug intravenously via the jugular vein catheter. On test days the animals were given 20 minutes free access to all chambers again, and chamber scores were recorded.

Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
			Baseline 1	Baseline 2
Conditioning 1	Test day 1	Conditioning 2	Test day 2	Conditioning 3
Conditioning 4	Test day 3	Conditioning 5	Test day 4	

Table 1: CPP protocol

Emesis

Adult male ferrets were tested for ability of TY027 to induce emesis. Animals were left in the testing room in individual cages and observed for 30 minutes prior to any testing procedures. Ferrets were injected with the drug i.p., and then they were placed immediately in their cages. The number of retches and vomits were counted for the next hour, although most activity occurred in the first 30 minutes after administration. For emesis experiments, MS was dissolved in 0.9% saline.

Gastric transit

Male Sprague-Dawley rats were fasted overnight with water available *ad libitum*. The animals were briefly anesthetized with isoflurane, and received the drug intravenously via a lateral tail vein injection. They then received an oral gavage of a nutritive meal containing ^{51}chromium (^{51}Cr). After 15 minutes, the animals were again

briefly anesthetized with isoflurane before they were sacrificed by cervical dislocation. The abdomen was opened, and the stomach and small intestine were isolated. The small intestine was cut into 10 equal pieces, placed in glass vials, and the stomach was set aside by itself. The samples were analyzed by a scintillation counter for counts-per-minute to measure the passage of the ^{51}Cr.

Statistical Analysis

Data were analyzed by non-parametric two-way analysis of variance (ANOVA; post-hoc: Neuman-Kuels), one-way ANOVA, and Students T-test when appropriate in FlashCalc (Dr. Michael H. Ossipov, University of Arizona, Tucson). Data were plotted in GraphPad Prism4 and represent mean value ± SEM where significance was reached if $p \leq 0.05$ unless otherwise stated.

Results

Addiction Potential of Intravenous TY027

Vehicle (DTS) did not affect the time spent in the conditioned chamber after either 2 or 5 exposures (2D, 5D, respectively), while MS (3 mg/kg, dissolved in DTS) significantly increased the amount of time spent in the drug-paired chamber after both 2 ($p = 0.05$) and 5 ($p = 0.006$) drug exposures. TY027 did not significantly affect the time spent in the drug-paired chamber after 2 or 5 exposures.

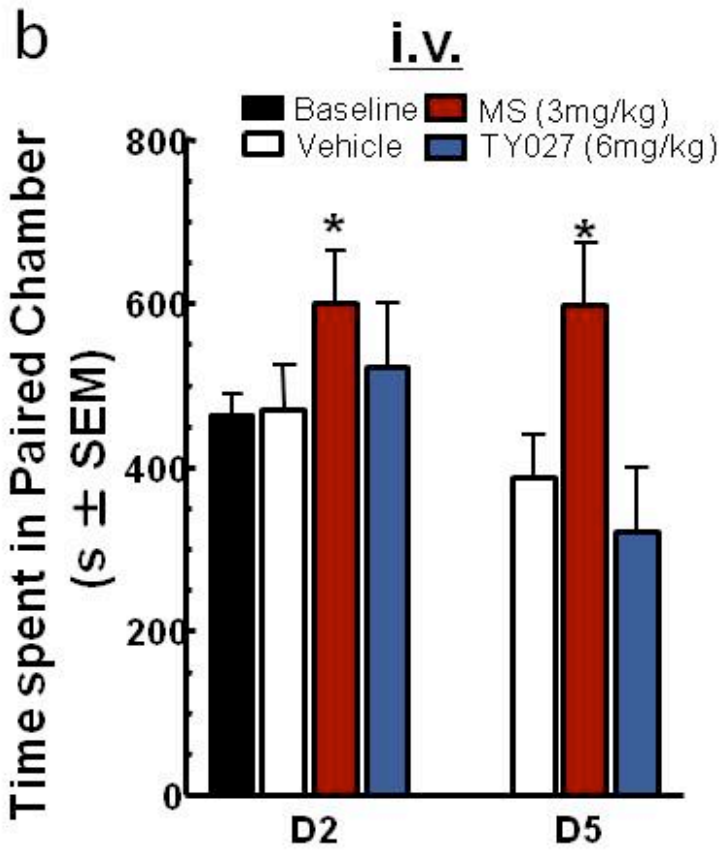

Figure 1: Intravenous injection of morphine produces CPP after both 2 and 5 exposures (600.8 ± 62.5 s, p = 0.05 and 597.4 ± 75.7, p = 0.006, respectively), while administration of TY027 does not result in significant preference (p = 0.19, and 322.2 ± 77.0 s, p = 0.06, respectively). D2 corresponds to test day 2, and D5 to test day 4

Opioid Induced Emesis

Saline and DTS both failed to produce any retching or vomiting in 12 of 12 ferrets. A single administration of MS (0.6 mg/kg) induced retching in 12 of 12 ferrets ($p < 0.001$) and vomiting in 7 of 12 animals ($p < 0.05$). A single dose of TY027 (3 mg/kg) induced 13 retches and 1 vomit in one animal, with no retches or vomits from the other 11 animals. TY027 was not significantly different from vehicle with respect to both number

of animals retching/vomiting and the number of retches/vomits per animal ($p = 0.34$). Both were significantly different from MS ($p < 0.001$).

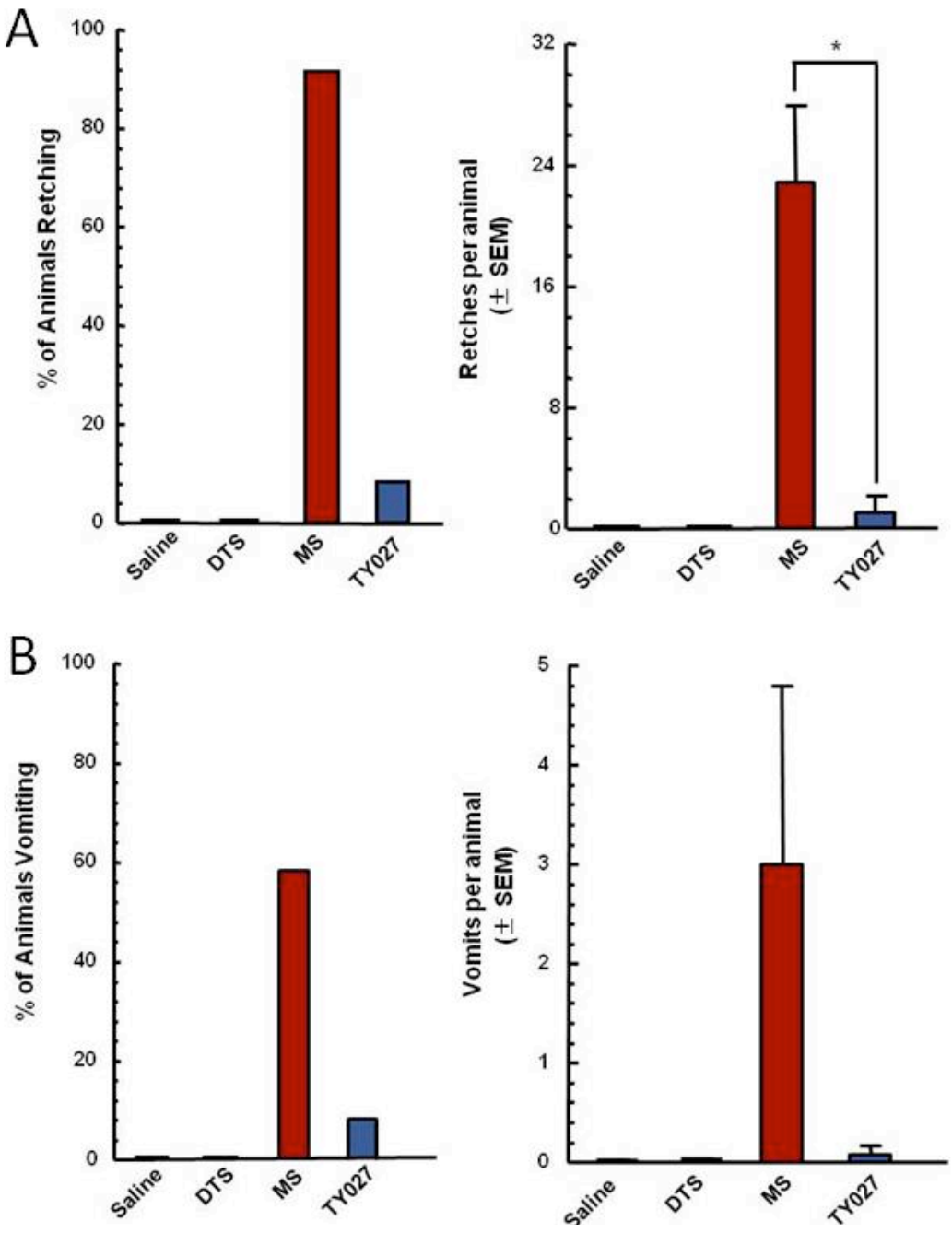

Figure 2: A. MS reliably induced retching in all ferrets (count = 25.0 ± 5.0, $p < 0.001$) while TY027 administration resulted in one animal retching. B. MS induced vomiting (count = and 5.0 ± 3.0, $p<0.05$) in 58% of animals, while a single TY027 animal vomited once.

Opioid Induced Constipation

The effect of vehicle, MS, or TY027 on gastric transit was evaluated in rats 15 minutes after administration of a nutritive meal supplement marked with ^{51}chromium (^{51}Cr). Vehicle treatment resulted in transit of 56.2 ± 13.1% of the total ^{51}Cr through the small intestine, which correlated to a geometric center of 4.8 ± 0.8. MS (1 mg/kg) resulted in significant slowing of gastric transit, with only 20.6 ± 9.3% of ^{51}Cr in the small intestine (geometric center = 2.5 ± 0.7, $p < 0.0001$). Gastric transit after treatment with TY027 (3 mg/kg) was not significantly different from vehicle ($p > 0.05$, gastric emptying of 52.0 ± 11.8% of ^{51}Cr, geometric center = 4.2 ± 0.8).

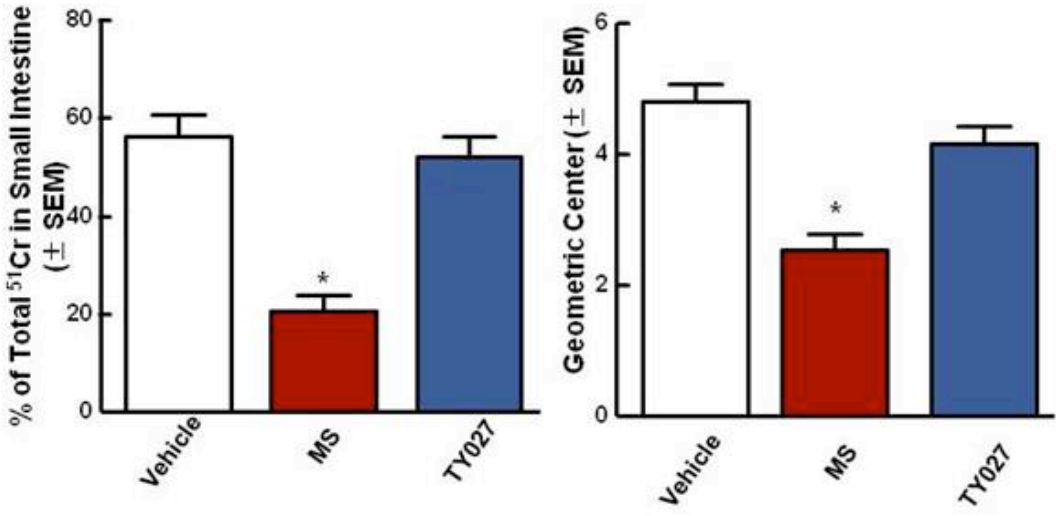

Figure 3: MS significantly slowed gastric transit compared to vehicle ($p < 0.0001$), while TY027 was not significantly different from vehicle ($p > 0.05$).

Discussion

The present study presents a novel bifunctional peptide, TY027, which lacks the constipating, emetic, or rewarding properties of other μ-agonists, such as morphine.

Previous work had already demonstrated TY027 to be an effective agent in relieving thermal hyperalgesia and tactile allodynia, an effect that was μ-opioid receptor mediated. Despite its action on μ-opioid receptors, TY027 did not produce conditioned place preference. The conditioned place preference paradigm has been used to model the rewarding properties of various drugs, and has been reliable in indentifying drugs that have abuse potential in humans (32-34). This indicates that TY027 is unlikely to produce rewarding effects in humans.

This finding builds upon evidence linking the opioid-NK-1 systems, and particularly, that disrupting NK-1 signaling can reduce the rewarding effects of opioids (25, 27). However, a recent human study in opioid abusers found that acute administration of the NK-1 antagonist aprepitant *increased* the euphoria and subjective 'high' of oxycodone administration (31). The authors speculate that part of this may be due to aprepitant's inhibitory action on the enzyme P450 3A4, the same enzyme used to metabolize oxycodone. Thus, administration of aprepitant would have likely resulted in increased plasma AUC for oxycodone. Despite this, the authors argue that none of the other parameters changed (time to peak effect, duration of effects), and rule out enzyme inhibition as the primary cause for their findings. However, this finding runs contrary to the hypothesis, and evidence presented here, that NK-1 antagonism blunts the rewarding effects of opioids. Clearly, more work is needed to determine the interaction between the opioid-NK-1 systems, particularly with respective to rewarding effects.

Nausea and vomiting are common side effects of opioids, and can cause significant distress to the patients. Morphine reliably caused retching and vomiting, while administration of TY027 only induced retching/vomiting in a single animal. This finding

is not surprising, as there is an FDA approved NK-1 antagonist for the treatment of nausea and vomiting (28). Morphine resulted in significant slowing of gastric transit, while TY027 did not result in significant inhibition of gastric transit. This means that TY027 lacks the constipating effects of traditional opioids, including morphine.

TY027 is a rationally designed, bifunctional compound with μ-opioid agonist and NK-1 antagonist activities. It displayed excellent analgesic activities, and was active after both systemic and central administration. Additionally, it displays a lack of side effects common to other opioids, such as nausea/emesis, constipating, and reward liability. TY027 represents a novel analgesic with an improved side effect profile and a lack of rewarding effects that may one day be beneficial to patients in the clinical setting suffering from both acute and chronic pain states.

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