Review Article

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Evidence for intravenous self-administration of mitragynine in fentanyl-dependent rats

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ABSTRACT

Introduction: Kratom (*Mitragyna speciosa* Korth) is currently used as an alternative for the self-treatment of pain and management of opioid dependence and withdrawal. Due to the opioid-like effect of the plant's active alkaloid, mitragynine (MG), the evaluation of its ability to maintain self-administration in animal models of opioid dependence appears to be great significance. **Objectives:** Here, the ability of MG to cross-substitute to the reinforcing effects of the synthetic narcotic fentanyl is investigated. **Methods:** Rats with implanted catheters were allowed to self-administer fentanyl (2.0 μg/kg/infusion) on a fixed-ratio 1 of schedule of reinforcement. **Results:** A significant increase in lever pressing indicated the successful acquisition of fentanyl self-administration. Next, rats were presented with saline or various doses of MG. The cross-substitution of MG at three unit doses (0.3, 1.0, and 3.0 mg/kg/infusion) maintained the lever-pressing responses of the fentanyl self-administration while extinction was evident following the substitution of saline for the fentanyl solution. **Conclusion:** The differences in the profile of MG cross-substitution tests to the baseline level observed during extinction suggest that MG has fentanyl-like reinforcing effects. However, a more thorough examination of its primary reinforcing effects will need to be determined in naïve rats to confirm the potential dependence producing effects of MG.

Keywords: Fentanyl, kratom, mitragynine, opioid, rats, self-administration

INTRODUCTION

Opioids continue to be used and abused for their analgesic and euphoric properties.[1] Abuse of opioids has become the leading cause of drug overdose in the United States (US), highlighting a growing crisis of the opioid overdose epidemic globally.[2] The synthetic opioid, fentanyl is one of the most potent pain-relieving opioids developed for the management and treatment of chronic pain.[3] Evidence indicates that there is an increase in the medicinal use and abuse of fentanyl, like other opioids (*i.e.,* heroin and morphine).[4,5] The striking increase in the number of people affected by opioid use disorder (OUD) has caused the US health providers to prescribe less opioids.[2,6] Unfortunately, when tougher controls are implemented on opioid-based prescriptions, there is a high possibility for patients on opioid treatment to seek alternative treatment or consider using herbal-based substances which claim to provide pain relief.[7]

Mitragyna speciosa (Korth and Rubiaceae) or kratom is a native medicinal plant of Southeast Asia.[8] Although many large-scale surveys have reported kratom's beneficial effects in reducing pain and managing opioid dependence and physical withdrawal,[9-11] enforcement agencies and health-care providers continue to report the negative effects of kratom use.[12,13] This has led to the argument about kratom's potential benefits and its perceived risk of abuse. Therefore, the study of kratom for abuse potential is of the high priority in relation to public health which has received much attention. Even though the increasing use of kratom creates concern over its abuse potential, the scientific evidence to support the anecdotal claims remains unclear.

Kratom's pharmacological effects, largely mediated through opioid receptors, are believed to be attributed to its constituent alkaloids. Mitragynine (MG) is the main active alkaloid which accounts for 66% of the total alkaloid extracted from the leaves of *Mitragyna speciosa* Korth.[8,14] The previous

studies have reported various pharmacological effects of MG which are similar to opioid, particularly morphine.[15-18] Due to this pharmacological similarity, the most behavioral studies conducted in assessing the abuse potential of MG have used morphine as the training or reference drug in self-administration, drug discrimination, and conditioned-place preference studies.[16,18-21] The previous self-administration studies suggested that MG has no reinforcing properties when MG was not self-administered in rats that were trained to self-administer methamphetamine[21] and morphine.[19]

Published literature in the field of drug abuse research has demonstrated a correlation between those drugs self-administered by laboratory animals and those that are abused and found to be addictive in humans.[22-24] Intravenous self-administration of drugs by experimental animals is the gold standard model to assess the dependence-producing effects of drugs which measure the reinforcing effects. A standard approach to assess the reinforcing properties of a new psychoactive drug is through a substitution procedure which provides a baseline level of self-administration responses.[23,25] The present study aims to evaluate MG for its ability to transfer responding previously maintained by presentation of fentanyl in rats. A cross-substitution experiment will be conducted in rats previously trained to self-administer intravenous injections of fentanyl. This study was designed to specifically investigate the efficacy of MG to support self-administration behavior in fentanyl-dependent rats.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats weighing between 280 and 320 g at the start of the study were used. All rats were maintained in a temperature-controlled room (21–22°C) under a 12-h light/dark cycle. Once surgically prepared with intravenous catheters, all rats were housed individually. The rats were allowed to recover for at least 5 days following surgery. The rats were provided with free access to food and water in the home cage throughout. At the start of the study, all rats were naïve and had no prior experience with operant conditioning procedures. The experimental procedures were performed in compliance with the requirements of protocols according to the local guidelines for the use of experimental animals and approved by Animal Ethics Committee, Universiti Sains Malaysia. [Reference number: USM/Animal Ethics Approval/2013/(90) (533)].

Drugs

MG was extracted, isolated, and verified from fresh leaves of *M. speciosa* as previously described[26] at the Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia. The purity of MG extracted was approximately 98% as confirmed by the high performance liquid chromatography and nuclear magnetic resonance analyzes. Fentanyl citrate (Pharmaniaga Logistics, Malaysia) was dissolved in physiological saline (NaCl 0.9%). MG was suspended in a vehicle of 20% (v/v) Tween-20 (polyoxyethylene sorbitan monooleate, Sigma-Aldrich Co., USA) and physiological saline (NaCl 0.9%) and was diluted to the desired concentrations before the experiment. The drug concentrations were expressed as a unit dose per infusion. Each infusion was delivered in a volume of 0.1 ml delivered over 5 s duration.

Apparatus

The experiment utilized standard two-lever operant conditioning chambers (Med-Associates, Vermont, USA). The operant panel of each chamber consisted of two fixed levers (MED-Associates, Vermont, USA) which were 4.5 cm wide, extending 2.5 cm from the wall, and located 7.0 cm above the floor. One lever was assigned as active and the other lever was assigned as inactive. Responses on the active lever resulted in simultaneous activation of the infusion pump that resulted in the delivery of drug. All presses on the inactive lever were recorded but did not result in drug delivery. The delivery of drug infusion was followed by termination of the house light. The house light was on during the entire session to signal drug availability and was turned off during the timeout period.

Surgical Procedures

Rats were implanted with a chronic silastic catheter into the external jugular vein, under surgical anesthesia, ketamine (80 mg/kg), and xylazine (10 mg/kg) through intraperitoneal injection. The procedures of catheter implantation surgery for rats were adopted as described by Schenk *et al*.[27] and Shoaib *et al*.[28] The catheter was connected to an L-shaped connector that was mounted on the rat's skull embedded in dental acrylate. Daily flushing with 0.9% physiological saline containing Baytril (enrofloxacin) (0.16 mg/kg/day) maintained the patency of the intravenous catheter. Once animals regained body weights above pre-operative weights, the self-administration session started.

Acquisition of Fentanyl Self-administration

The intravenous drug self-administration procedure was adopted from a previous study.[29] During 1-h sessions, rats were given the opportunity to press a lever for intravenous infusions of fentanyl at 2.0 μg/kg/infusion.[30,31] Responding was initiated using fixed-ratio 1 (FR-1) schedule when each infusion delivered 0.1 ml of fentanyl (2.0 μg/kg) to the rat over a period of 5 s. The drug infusion was followed by a 20 s timeout period in which responses on either lever were not recorded and no additional infusion could be earned by the rats.

The acquisition training was carried out until responses were considered stable when the rats displayed accurate discrimination between the active and inactive levers. Once the rats showed acquisition criteria; at least 80% of response accuracy on the active lever and stable intake of fentanyl (± 2 infusions) over 2–3 days under FR-1 schedule, the number of responses required to produce an infusion was gradually increased to FR-2 and FR-3. Only rats that satisfied the acquisition criteria and showed stable responding at FR-3 were used for subsequent cross-substitution tests.

Cross-substitution Tests

An extinction test was first conducted by substituting fentanyl solution with saline for three consecutive sessions. Following extinction testing, cross-substitution tests with MG were

conducted by substituting syringes containing fentanyl to those containing appropriate concentrations of MG. Each unit dose of MG (0.3, 1.0, and 3.0 mg/kg/infusion) was tested in three successive sessions in which the order of unit doses was randomly presented. These doses were based on the previous self-administration study of MG.[23] At least 3 days of testing with fentanyl were allowed to re-establish baseline responding between each dose. The diagram for the cross-substitution procedure in this study is summarized in Figure 1.

Statistical Analysis

Data from self-administration experiments in the form of number of infusions or total lever-responses were analyzed using repeated measures ANOVA. Bonferroni *post hoc* tests were used to specify differences between means. GraphPad prism 5.0 (Version 5.0, GraphPad Software, Inc., USA) was used for graphical presentation of the analyzed data. The data were expressed as means ± SEM. $P < 0.05$ was set as the threshold for statistical significance.

RESULTS

Acquisition of Fentanyl Self-administration

Of the 12 rats trained to self-administer fentanyl, only eight rats showed evidence of acquisition. The rats displayed gradual increases in the number of fentanyl-reinforced responses when the FR was progressively increased [Figure 2]. A two-way ANOVA for repeated measures revealed significant differences between lever responding in the active and inactive levers [$F(1,7) = 70.77$, $P < 0.0001$) with significant differences over session [$F(27,189) = 4.45$, $P < 0.0001$]. An overall significant difference emerged between responses on the active and inactive levers and training sessions [$F(27, 189) = 30.53$, $P < 0.0001$], reflecting acquisition of fentanyl self-administration. Over the course of the acquisition sessions, responses on the inactive levers declined to nominal levels [$F(27, 189) = 1.67$, $P = 0.027$].

As shown in Figure 2, rats self-administered approximately eight fentanyl infusions during each 1 h session. A one-way ANOVA for repeated measures on the total infusions revealed a significant increases in fentanyl intake over the training session [$F(27,189) = 16.77$, $P < 0.0001$] which stabilized toward the latter stages of training when the FR was increased over sessions [Figure 2].

Cross-substitution Tests

Substitution of saline for fentanyl produced a gradual decrease of self-administration responding over the three test sessions [Figure 3]. A one-way ANOVA for repeated measures indicated significant differences in the responding over the extinction sessions [$F(8, 40) = 4.73$, $P < 0.0001$]. Substitution of MG tested at three unit doses (0.3, 1.0, and 3.0 mg/kg/ infusion) did not show any significant decreases of responding when these MG doses were tested over the three-test days [$F(8,32) = 1.31$, $P = 0.273$; $F(8,32) = 0.87$, $P = 0.551$; and $F(8,32) = 1.27$, $P = 0.291$] [Figure 3]. There was a trend for responses to increase when the lower unit doses of MG were presented over the 3 days of cross substitution. The highest dose of MG tested appeared to suppress lever-press responses relative to fentanyl-maintained responses.

DISCUSSION

The present study demonstrated that cross-substitution of MG in three unit doses (0.3, 1.0, and 3.0 mg/kg/infusion) maintained lever-press responding in rats previously trained to self-administer fentanyl. The profile over the MG cross-substitution tests appeared different to the extinction-like behavior observed with saline substitution which suggests that MG within the dose range examined has fentanyl-like reinforcing effects. Because the experiment was not designed to specifically evaluate the primary reinforcing effects of MG, the future studies are warranted to confirm the potential dependence producing effects of MG in naïve rats.

In agreement with the previous reports,[30,31] fentanyl at a unit dose of 2.0 μg/kg/infusion was observed to sustain intravenous self-administration behavior in rats. The group of rats exhibited stable levels of responding on the active lever with a high degree of discrimination from the inactive lever, which was maintained following increases to the fixed ratio. Of significance was the observation that saline substitution

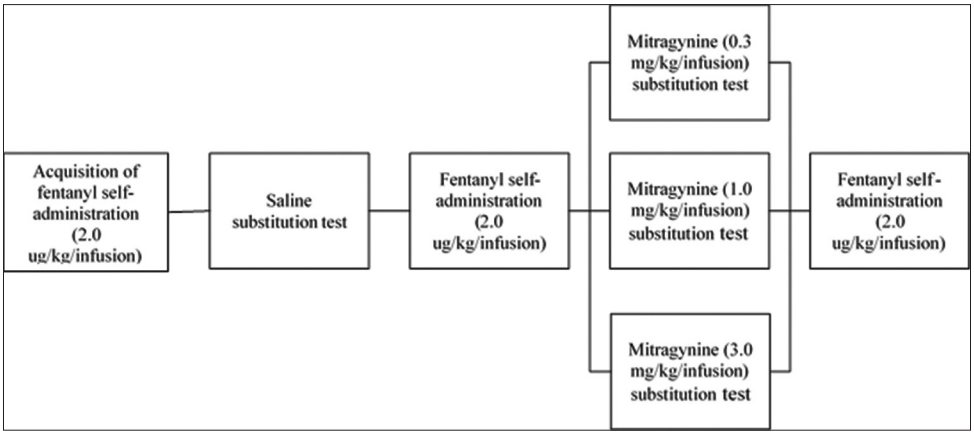

Figure 1: An outline of the experimental diagram of cross-substitution procedure. Following the acquisition of intravenous fentanyl self-administration, saline was first substituted for the fentanyl. Fentanyl self-administration was required after each substitution test to re-establish the baseline of behavior responding. Following saline substitution test, mitragynine substitution test was conducted per three sessions in a randomized order of unit doses

test resulted in decreases of responding on the active lever indicative of extinction. Thus, this provided an appropriate baseline of fentanyl-maintained responses to compare the effects of the psychoactive compound MG using the cross-substitution experimental design.

The present finding, however, contradict those reported by Hemby *et al*.[19] in which rats trained to self-administer morphine did not support self-administration of MG in doses that approximate to 0.07–0.43 mg/kg/infusion (assuming rats weigh approximately 350 g).[19,21] Besides the lower unit doses used in the Hemby *et al*.[19] study, another possible explanation for the discrepancy may be the different pharmacological action of the training drugs (fentanyl vs. morphine) which in part, may have attributed to the differential lipophilicity and binding affinities.[32] Fentanyl is known to have higher lipophilic properties[33] and greater binding affinity for mu-opioid receptors[34] compared to morphine which could partly facilitate the MG self-administration in the present study. Besides, the inconsistency of such findings also raises the possibility that several substitution test sessions rather than a single session may be more effective to engender and maintain MG self-administration in opioid-dependent rats. In another study that substituted with approximately similar doses of MG (0.03–3.0 mg/kg/infusion) but using methamphetamine as a training drug also showed no maintenance of responding when compared to substitution of both heroin and methamphetamine.[21] Other than the use of different class of training drug, the discrepancy of these findings could be due to the different substitution procedures used. The present study used cross-substitution procedure in which rats were represented with fentanyl between substitutions of each MG doses while Yue *et al*.[21] and Hemby *et al*.[19] constantly substituted the MG doses. However, the discrepancies of the substitution of MG between the present finding and those of Yue *et al*.[21] and Hemby *et al*.[19] are intriguing and warrant further detailed studies. Thus, while the present finding with a cross-substitution procedure suggests an

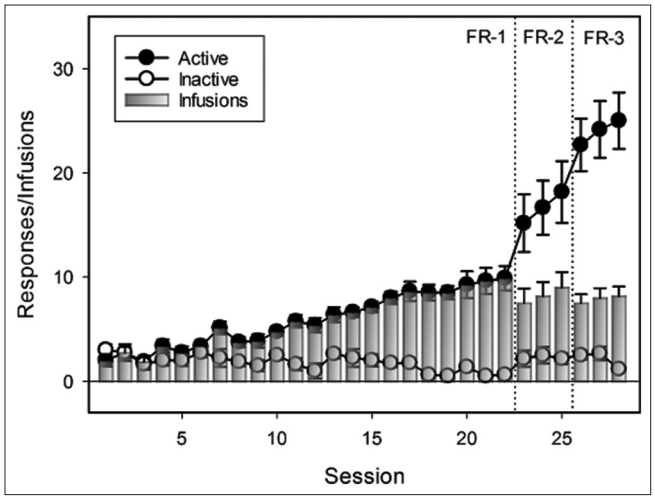

Figure 2: Acquisition of fentanyl self-administration under FR schedule of reinforcement. Each point represents number of lever responses on the active lever denoted by closed circles and inactive lever denoted by open circles on a dose of 2.0 $\mu\mu$g/kg/infusion fentanyl emitted during 1-h session (mean ± SEM, n = 8). The bars represent the total number of fentanyl infusions delivered during the session

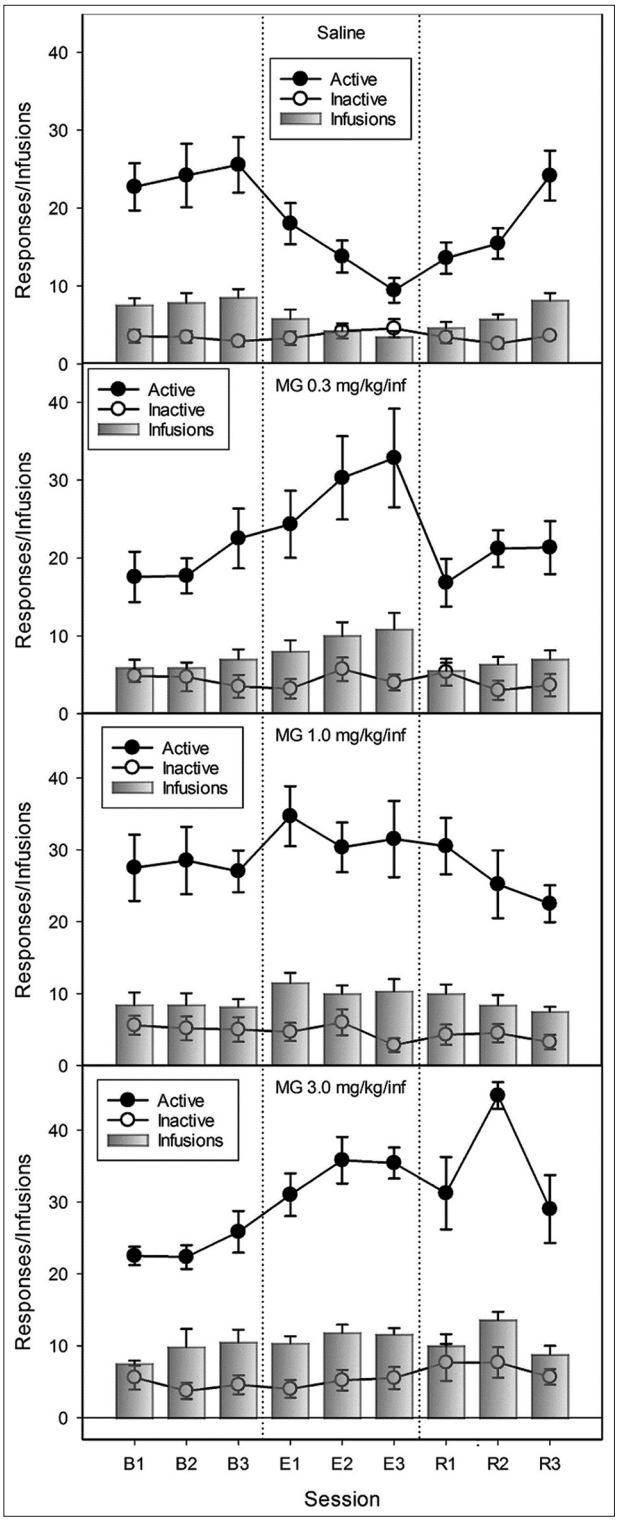

Figure 3: Cross-substitution tests of fentanyl (2.0 μg/kg/infusion) with saline and MG unit doses (0.3, 1.0, and 3.0 mg/kg/infusion). The mean number ± SEM of active and inactive lever responses are shown during stable responding over three sessions (n = 6). The bars represent the total number of infusions delivered during the 1-hr session. B1-B3 represents fentanyl self-administration session, E1-E3 represents saline or MG substitution test session, and R1-R3 represents re-establishment of fentanyl self-administration session

opioid-like reinforcing effect of MG, additional studies would add assurance to this conclusion.

The finding of the present study suggests that MG may have potential for abuse which corroborates with the previous studies which demonstrated dose-related rewarding effects of MG in a conditioned-place preference paradigm.[18,20] Furthermore, MG was also demonstrated to share similar subjective effects with opioid which further suggested its abuse liability.[16] Therefore, it is conceivable that the opioid-like effects of MG in particular the discriminative stimulus like effects[16] may play a role in supporting the self-administration of MG in fentanyl-trained rats.

It is also likely that the efficacy of MG to support fentanyl self-administration behavior was suggested through stimulation at mu-opioid receptors. Since the reinforcing effect of fentanyl is mediated through opioid receptors,[35] interactions with opioid systems in mediating some MG effects such as antinociception, rewarding properties, and the attenuation of opioid withdrawal symptoms[15,17,36] might suggest that the reinforcing effects of MG and opioids might be functionally linked. This would be in line with human reports that the most opioid users use kratom to manage their OUD[10,11,37] which highlights the therapeutic potential of MG or kratom as a substitution drug for opioids. Hence, the finding of cross-substitution effects of MG to the opioid fentanyl in this study may offer another insight into why kratom is routinely coused with other opioids.

CONCLUSION

This study reports on a small study to examine the reinforcing effects of the kratom constituent, MG in fentanyl-dependent rats. The results are interpreted to suggest abuse liability of MG when responding decreased during saline cross-substitution with fentanyl but not during cross-substitution of the MG doses. The dependence-producing potential of MG in cross-substitution tests for fentanyl self-administration suggests it may have similar reinforcing effects, and thus may provide a useful screening for further quantitative evaluation of the reinforcing properties of MG. Prospective behavioral studies investigating reinforcing properties of MG using oral consumption methods are also warranted from a face validity perspective, as kratom is generally consumed in the form of tea concoctions brewed by humans.

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