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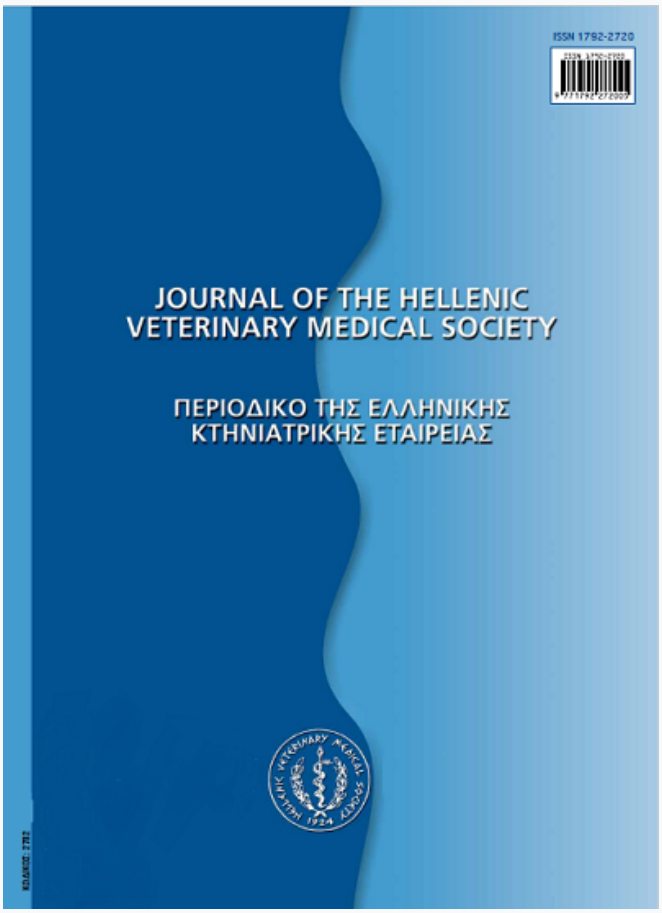

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IRENE SYMEON, ALEXIA POLISSIDIS, EVANGELOS BALAFAS, MARIANNA STASINOPOULOU, PAVLOS ALEXAKOS, CHRYSA VOYIATZAKI, NIKOLAOS KOSTOMITSOPOULOS

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Research article
Ερευνητικό άρθρο

Evaluation of the effects of tramadol on analgesic response and locomotor activity on two different strains of laboratory mice

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Εκτίμηση της δράσης της τραμαδόλης στην αναλγησία και στην κινητικότα σε δύο διαφορετικά είδη εργαστηριακών μυών

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ABSTRACT. Analgesia of laboratory animals consists an important component in experimental studies where painful stimuli or procedures may take place. When choosing analgesics, the severity of pain along with the response to medication is among the factors that determine the choice of agent. Tramadol is a known synthetic opioid analgesic used to treat main acute or chronic pain including perioperative pain. The purpose of this study was to evaluate the analgesic response as well as the effect on locomotor activity in two different strains of mice after the intraperitoneal (i.p.) administration of tramadol. Subjects were 11-13 week-old male C57BL/6J (n=39) and BALB/cJ (n=38) mice, randomly assigned to receive either saline, tramadol 10 mg/kg or tramadol 40 mg/kg. Analgesia was measured using the hot-plate test, 30 or 60 minutes after drug administration while the open field test was used in order to assess locomotor activity. Both strains exhibited a significant increase of hot-plate latencies after administration of tramadol 40 mg/kg while the same dose induced significantly greater analgesia in BALB/cJ as compared with the C57BL/6J mice. BALB/cJ mice presented a dose-dependent decrease in locomotor activity following tramadol administration whereas C57BL/6J mice receiving 40 mg/kg tramadol showed hyperactivity. In conclusion, the lower dose of tramadol (10 mg/kg) has insufficient antinociceptive effects on acute thermal pain for both strains. The highest dose of tramadol used in this study (40 mg/kg) was greater than the one required for BALB/cJ mice, as they were under sedation for at least 60 minutes after drug administration. The same dose of tramadol appeared to be effective on C57BL/6J

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mice as latency times on the hot plate were significantly increased. Despite this fact, it is not a suitable choice as an analgesic, especially postoperatively, as it causes hyperactivity to this strain. Special concern should be given to the fact that tramadol's analgesic and behavioral effects depend not only on its dosage, but also on the strain in which it is administered.
Keywords: *tramadol, mice, locomotor activity, analgesia*

ΠΕΡΙΛΗΨΗ. Η αναλγησία αποτελεί θέμα ιδιαίτερης σημασίας για τα ζώα εργαστηρίου τα οποία πρόκειται να υποβληθούν σε επώδυνους πειραματισμούς. Η επιλογή ενός αναλγητικού παράγοντα εξαρτάται μεταξύ των άλλων και από το μέγεθος και τη διάρκεια του επώδυνου ερεθίσματος αλλά και από την αποτελεσματικότητά του στον συγκεκριμένο οργανισμό. Η τραμαδόλη συμπεριλαμβανομένου του περιεγχειρητικού πόνου αποτελεί ένα γνωστό συνθετικό οπιοειδές αναλγητικό που χρησιμοποιείται τόσο για τον οξύ όσο και για τον χρόνιο πόνο. Σκοπός της μελέτης ήταν η εκτίμηση της αποτελεσματικότητας του συγκεκριμένου αναλγητικού σε δύο διαφορετικά είδη εργαστηριακών μυών μετά από ενδοπεριτοναϊκή χορήγηση. Τα ζώα που χρησιμοποιήθηκαν ήταν αρσενικά, ηλικίας 11-13 εβδομάδων, των φυλών C57BL/6J (n=39) και BALB/cJ (n=38). Τα ζώα χωρίστηκαν τυχαία σε ομάδες στις οποίες χορηγήθηκαν είτε φυσιολογικός ορός, είτε τραμαδόλη 10 mg/kg ή τραμαδόλη 40 mg/kg. Η αναλγησία μελετήθηκε με την υποβολή των ζώων στη δοκιμή της θερμαινόμενης πλάκας, για 30 και 60 λεπτά μετά τη χορήγηση της τραμαδόλης. Προκειμένου για τη δοκιμασία της θερμαινόμενης πλάκας, τα ζώα της φυλής BALB/cJ στα οποία χορηγήθηκε τραμαδόλη στη δοσολογία των 40 mg/kg εμφάνισαν καλύτερο επίπεδο αναλγησίας από τα αντίστοιχα ζώα της φυλής C57BL/6J. Η κινητικότητα των ζώων μελετήθηκε με τη δοκιμή ανοικτού πεδίου. Τα ζώα της φυλής BALB/cJ παρουσίασαν δοσοεξαρτώμενη μείωση της κινητικότητάς τους, ενώ τα ζώα της φυλής C57BL/6J παρουσίασαν έντονη υπερκινητικότητα στη δόση των 40 mg/kg τραμαδόλης. Συμπερασματικά χορήγηση τραμαδόλης στη δόση των 10 mg/kg δεν έχει καμμία επίδραση στο επίπεδο αναλγησίας για τα ζώα και των δύο φυλών. Προκειμένου για τη δόση των 40 mg/kg τραμαδόλης αυτή φαίνεται να έχει καλά αποτελέσματα για τα ζώα της φυλής BALB/cJ, ενώ για τα ζώα της φυλής C57BL/6J δεν θεωρείται κατάλληλη λόγω της προκαλούμενης υπερκινητικότητας στη δοκιμή ανοικτού πεδίου. Θα πρέπει να επισημανθεί ότι κατά τη διαδικασία επιλογής της τραμαδόλης ως αναλγητικού παράγοντα θα πρέπει να λαμβάνεται υπόψη όχι μόνο η δοσολογία αλλά και η φυλή του ζώου στο οποίο αυτή θα χορηγηθεί.
Λέξεις ευρετηριασμού: *τραμαδόλη, εργαστηριακός μυς, κινητικότητα, αναλγησία*

INTRODUCTION

Tramadol is a centrally acting synthetic analgesic drug with antinociceptive effects. It binds to the ***μ***-opioid receptors with weak affinity while inhibiting the neuronal uptake of norepinephrine and serotonin. Tramadol is a well known drug for the treatment of intermediate or severe pain, with lower incidence of adverse effects than other opioids such as respiratory depression, sedation or nausea (Heavmer, 1997; Nolan AM, 2000). Its pharmacokinetics have been studied in many animals including rats and mice (Flecknel, 2009). Even though its antinociceptive effects are based both on opioid and non opioid mechanisms, it does not cause tolerance or physical dependence (Miranda and Pinardi, 1998). It has been proven that tramadol exhibits dose-dependent and time-dependent antinociceptive effect on acute thermal pain in mice using the models of nociception most applied, the hot plate and the tail flick test (Mattia et al., 1993; Bannon and Malmberg, 2007; Aydin et al., 2012).

The distinct response to thermal pain after analgesia administration may vary depending on the animal strain. In a study during which 11 inbred strains of mice were submitted to 3 assays of thermal nociception, the results presented significant interstrain differences (Mogil et al., 1999). Strain responsiveness depends on the genotype as well as on the interaction between genes and environmental factors like stress-induced antinociception (SIA) (Mogil et al., 1999). These differences are generally reflected in the analgesic response to various opioids such as morphine (Korostynski et al., 2006). Belknap et al. showed in their study that DBA/2J and C3H/HeJ mice exhibited stronger analgesic response to morphine than C57BL/6J mice by using the hot plate test (Belknap et al., 1990).

Locomotor activity can be used in order to assess behavioral patterns as well as signs of pain and discomfort (Flecknell and Liles, 1991; Jansen van't Land and Hendriksen, 1995). Administration of opioids, like trama-

dol, can affect locomotor activity in rodents. In their study, Murphy et al. observed differences in locomotor activity in three different mouse strains after morphine administration (Murphy et al., 2001). Furthermore, in a study conducted on rats, higher doses of tramadol and administration for a longer period had greater impact on the decrease of locomotor activity (Szkutnik-Fiedler et al. 2012).

The aim of this study was to evaluate the effects of tramadol on acute thermal pain and locomotor activity in C57BL/6J and BALB/cJ mice.

MATERIALS AND METHODS

The study was performed in the Laboratory Animal Facilities of the Biomedical Research Foundation, Academy of Athens. The competent Regional Veterinary authority approved the experimental protocol in accordance to Greek legislation (Presidential Decree 56/2013, in compliance with the European Directive 2010/63).

Animals

Mice were housed in groups of 8 or 9 individuals, under positive pressure in polysulfone type III individual ventilated cages (Sealsafe®, Tecniplast, Buguggiate, Italy) under specific pathogen-free (SPF) conditions, with 70 air changes per hour and constant room environmental conditions (12:12 hour light: dark cycle (0700-1900), temperature 22±2°C, a light intensity of 300 Lux measured 1m above the floor in the middle of the room, a positive air pressure of 0.6 Pa and relative humidity 45±10%. The mice were fed irradiated pellets (2918 Teklad Global 18% Protein Rodent Diet, Harlan Laboratories, Indianapolis, USA) and had access to tap water *ad libitum*. The cage bedding comprised corncob granules (REHOFIX®, J. Rettenmaier & Söhne Co., Rosenberg, Germany) and the cages and their bedding were changed once a week. In each cage a mouse house was placed. All mice in the facility were screened regularly by using a health monitoring program, in accordance to the Federation of European Laboratory Animal Science Associations' recommendations, and were free from a wide range of pathogens (Mähler et al., 2014).

Pharmaceutical compound

The analgesic used in this study was tramadol hydrochloride (Tramal®, 100 mg/amp, Grünenthal, Aachen, Germany), and was injected intraperitoneally (i.p.). Tramal 0.1 mL was diluted in 0.9 ml saline, and mice received intraperitoneally 0.02 ml solution for every 10 g of their weight. The administration of the substances was performed by an experienced animal technician between 0900 and 1200 hours.

Treatment groups

In our study, subjects were 11-13 weeks-old male C57BL/6J (n=39) and BALB/cJ (n=38) mice. Mice of each strain were randomly divided into 6 groups. Group A (n=12), group B (n=13), group C (n=13), group D (n=13), group E (n=13) and group F (n=13). C57BL/6J mice were randomly allocated in groups A, C and E. BALB/cJ mice were divided in groups B, D and F. Animals were treated with saline (groups A and B), 10 mg/kg tramadol (groups C and D) or 40 mg/kg tramadol (groups E and F).

Data collection

One week prior to the experimental study as well as on the day of the experiment, animals' body weight was recorded. All animals were subjected to the same experimental procedure. The room in which the behavior tests were conducted had constant temperature of 22°C. In order to acclimatize the animals, the cages were transferred to the room 30 minutes before the commencement of any procedure. In our study, prior to the onset of the experiments, there was a week handling period in order to minimize the stress of the animals during the procedures.

Hot-plate test

The surface of the hot-plate apparatus (Stoelting Co., IL, USA) was heated up to 52.0oC. An open-top acrylic glass cylinder was placed on the aluminum plate to prevent the animals' escape. Between the cylinder glass and the aluminum surface, a cardboard was placed in order to avoid heating of the animal before timing started. Each mouse was placed on the hot-plate and monitored by two observers. The animals were observed until they presented a nociceptive response or until the cutoff time was reached. The responses that were measured were: licking, lifting or fluttering a hindpaw and jumping. Front paw licking or lifting is not a reliable sign of discomfort because it is a common grooming response and may have no relation to experiencing pain (Bannon and Malmberg 2007). A cut-off time of 30 seconds was imposed to prevent tissue damage. When one of the responses was observed, or when cut-off time had passed, the mouse was removed from the hot-plate and the chronometer of the apparatus was stopped. The animals were exposed at the hot-plate twice. The first time was before the i.p. injection, in order to have a baseline reaction, while the second time was after the i.p.

injection in order to record the substance's effect on the animal's reflexes. Thirty-eight mice were tested 30 minutes and 39 mice were tested 60 minutes after treatment. The hot-plate responses of each mouse in the drug-induced antinociception were converted to percent of maximal possible effect (%MPE) according to the following formula:

%MPE = [(test latency-baseline latency)/ (cutoff latency-baseline latency)] x100%.

Open field test

Locomotor activity was assessed in a transparent plexiglass box (40 x 40 x 35 cm) immediately after the saline or drug administration. Testing was performed between 0900 and 1300 hours. The apparatus was designed in such way that two animals could be observed simultaneously. Each animal was placed in the center of the box and was allowed to explore the arena freely for 30 min. Distance travelled (cm), velocity (cm/sec), and time spent at the center of the field (sec), were measured with an overhead camera and specialized video tracking software (Ethovision XT8.5, Noldus). The testing chamber was cleaned between trials with 70% ethanol.

Statistical analysis

All statistical analyses were conducted using a computerized statistical software package (SPSS Version 20.0, SPSS Inc., Chicago, IL, USA). Results have been compared using multi-factor analysis of variance. A p-value less than 0.05 was considered statistically significant. All data are presented as mean or percentage ± standard error (SEM). The first step in the statistical analysis was to evaluate the normal distribution of data (Kolmogorov-Smirnov test and P-P plots). For the hot-plate test, dose-response relationships as well as time course of tramadol antinociceptive effects were analyzed in C57BL/6J and BALB/cJ mice. For this purpose a 3 (Treatment) x 2 (Strains) x 2 (Time point) analysis of variance (ANOVA) was calculated. For the open field test, the ambulatory distance, the time spent in the center of the apparatus as well as the velocity were analyzed using repeated measures ANOVA with post hoc analysis.

RESULTS

Hot-Plate Test

There was a significant Treatment x Strains interaction [$F(2,64)=8.28$, $p<0.005$] while a significant main effect of strain was observed on the highest dose of tramadol (40 mg/kg) [$F(1,64)=15.00$, $p<0.0001$]. For BALB/cJ mice there was a statistically significant difference between vehicle and tramadol 40 mg/kg ($p<0.0001$) as well as between tramadol 10 mg/kg and tramadol 40 mg/kg ($p<0.00001$). More specifically, tramadol 40 mg/kg showed significantly higher % MPE compared to vehicle and tramadol 10 mg/kg for animals tested 30 min (100% ± 0.00 vs 10.63% ± 10.21 and 31.79% ± 13.72 respectively) as well for those tested 60 min (90.40% ± 7.56 vs 7.69% ± 4.41 and 22.38% ± 13.88 respectively) after injection. There was no statistically significant difference ($p>0.05$) between vehicle and tramadol 10 mg/kg for both time points (10.63% ± 10.21 vs 31.79% ± 13.72, 30 min post injection; 7.69% ± 4.41 vs 22.38% ± 13.88, 60 min post injection). The same phenomenon was observed for C57BL/6 mice where tramadol 40 mg/kg subgroup exhibited statistically greater % MPE values both 30 min ($p<0.05$) and 60 min ($p<0.05$) after injection in comparison with vehicle (37.43% ± 13.71 vs 6.40% ± 5.21, 30 min post injection; 37.97% ± 11.81 vs 8.67% ± 8.67, 60 min post injection) and tramadol 10 mg/kg (37.43% ± 13.71 vs 6.31% ± 3.55, 30 min post injection; 37.97% ± 11.81 vs 26.06% ± 9.66, 60 min post injection) subgroups. There was also no statistically significant difference ($p>0.05$) between vehicle and tramadol 10 mg/kg (6.40% ± 5.21 vs 6.31% ± 3.55, 30 min post injection; 8.67 % ± 8.67 vs 26.06 % ± 9.66, 60 min post injection).

Furthermore, a significant main effect of treatment was observed for both BALB/cJ ($p<0.0001$) and C57BL/6J ($p<0.05$) mice. BALB/cJ and C57BL/6J showed statistically significant difference for tramadol 40 mg/kg ($p<0.0001$) with BALB/cJ mice exhibiting higher % MPE than C57BL/6J mice (100% ± 0.00 vs 37.43% ± 13.71, 30 min post injection; 90.40 % ± 7.56 vs 37.97% ± 11.81, 60 min post injection). No statistically significant difference ($p>0.05$) was observed on % MPE between BALB/cJ and C57BL/6J for vehicle (10.63% ± 10.21 vs 6.40% ± 5.21, 30 min post injection; 7.68% ± 4.41 vs 8.67% ± 8.67, 60 min post injection) as well as for tramadol 10 mg/kg (31.79% ± 13.73 vs 6.31% ± 3.55, 30 min post injection; 22.38% ± 13.88 vs 26.06% ± 9.66, 60 min post injection). Finally, % MPE in C57BL/6J mice showed an increment by tramadol in hot plate test from 30 min to 60 min after injection while for BALB/cJ mice % MPE decreased from 30 min to 60 min after injection. All estimated values are presented in Figure 1.

Open Field Test

Examining the distance traveled during the six 5 minute periods of the 30-minute open field test, three way repeated measures ANOVA with post hoc analysis showed significant interaction of Treatment x Time periods x

Strains [F(10,350)=6.63, p<0.001]. There was a significant main effect of treatment [F(2,70)=5.75, p=0.05] as well as a significant main effect of strain [F(1,70)=141.035, p<0.001]. Multiple comparisons (Bonferonni correction) showed that in both strains the ambulatory distance for vehicle and tramadol-treated animals was significantly reduced during the test with the exception of C57BL/6J mice treated with the higher dose of tramadol which exhibited significantly increasing traveled distance over time [statistically significant difference (p<0.001) only between the first and the following periods]. BALB/cJ mice in tramadol 10 mg/kg and tramadol 40 mg/kg subgroups covered significantly less distance (p<0.001) compared to vehicle subgroup (Figure 2).

For all five-minutes periods, it was observed that C57BL/6J mice were more hyperactive covering greater distance in the open field apparatus but only for tramadol 40 mg/kg this difference was statistically significant [F(5,120)=16.320, p<0.001] (Figure 3).

Furthermore, concerning the time spent in the center of the apparatus, we observed a significant Time periods x Strains interaction [F(5,355)=6.886, p=0.001]. There was a significant main effect of Treatment [F(2,71)=8.782, p<0.001] as well as a main effect of Strain [F(1,71)=11.082, p=0.001]. Tramadol in either concentration further reduced the time that BALB/cJ and C57BL/6J mice spent on the central square compared to vehicle but only for BALB/cJ there was a statistically significant difference between vehicle and tramadol 10 mg/kg (p<0.05) as well as between vehicle and tramadol 40 mg/kg (p<0.01). C57BL/6J mice spent longer resting time in the center than BALB/cJ exhibiting statistically significant difference between strains for the animals receiving tramadol 10mg/kg (p<0.001) or tramadol 40mg/kg (p<0.001) but not for the ones receiving vehicle (p>0.05) (Figure 4).

Lastly, as far as the animals' velocity is concerned three-way repeated measures showed significant interaction of Treatment x Time periods x Strains [F(10,355)=5.425, p<0.001]. Velocity exhibited the same pattern as ambulatory distance, which was reducing over time for all treatment groups in both strains [statistically significant difference (p<0.001) only between the first and the following periods] with the exception of C57BL/6J mice receiving tramadol 40 mg/kg which increased their speed starting from the third five-minute period. Multiple comparisons showed that for BALB/cJ mice there was a statistically significant difference between vehicle and tramadol 10 mg/kg (p<0.01) as well as tramadol 40 mg/kg (p<0.001) but not between tramadol 10 mg/kg and tramadol 40 mg/kg (p>0.05), with the vehicle group moving faster (Figure 5). In addition, for

Figure 1. Histograms showing the comparison of percent of maximal positive effect (%MPE) during hot plate test between vehicle (VEH) (groups A, B), tramadol (TRM)10 mg/kg (groups C, D) and tramadol (TRM) 40 mg/kg (groups E, F) in C57BL/6J and BALB/cJ mice for two different time points (30 and 60 min). Bars indicate mean±SEM.

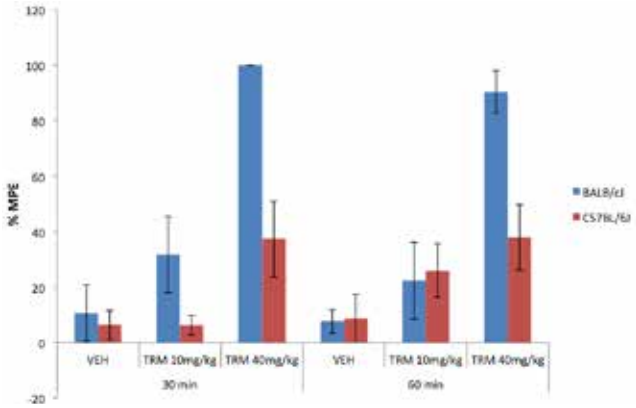


Figure 2. Diagrams showing locomotor activity observed during the open field test for BALB/cJ mice after vehicle (VEH) (group B), tramadol (TRM) 10 mg/kg (group D) and tramadol (TRM) 40 mg/kg (group F) administration. Values expressed as mean±SEM.

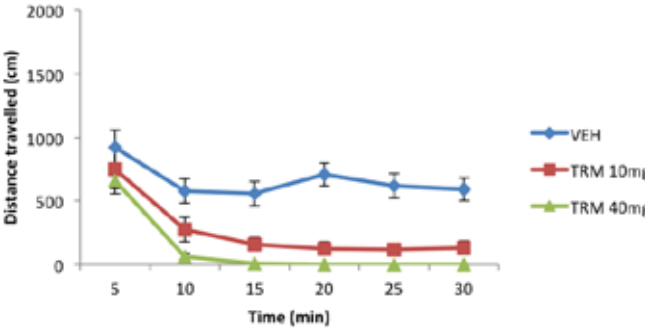


Figure 3. Diagrams showing locomotor activity observed during the open field test for C57BL/6J mice after vehicle (VEH) (group A), tramadol (TRM) 10 mg/kg (group C) and tramadol (TRM) 40 mg/kg (group E) administration. Values expressed as mean±SEM.

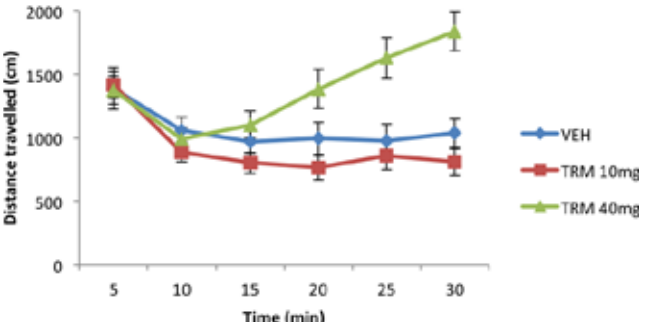


Figure 4. Histograms showing the comparison of time spent in the central square of the open field arena during the open field test by mice of all groups. Bars indicate mean±SEM

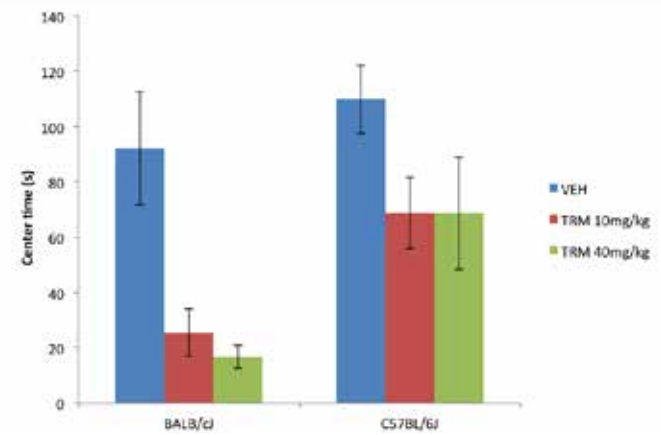


Figure 5. Diagrams showing velocity observed at BALB/cJ mice during the open field test after vehicle (VEH) (group B), tramadol (TRM) 10 mg/kg (group D) and tramadol (TRM) 40 mg/kg (group F) administration. Values expressed as mean±SEM

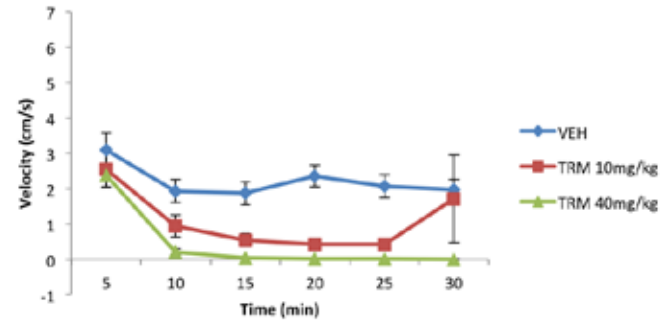
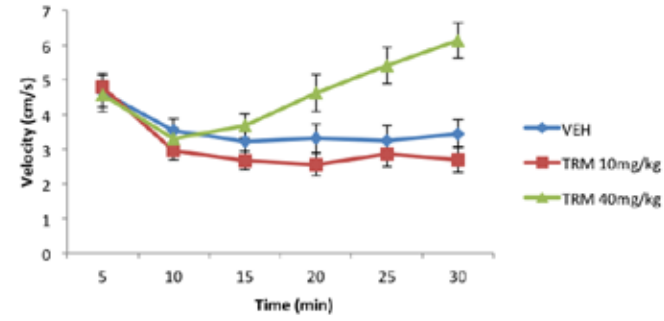


Figure 6. Diagrams showing velocity observed at C57BL/6J mice during the open field test after vehicle (VEH) (group A), tramadol (TRM)10 mg/kg (group C) and tramadol (TRM) 40 mg/kg (group E) administration. Values expressed as mean±SEM.



C57BL/6J mice there was statistically significant difference between tramadol 40 mg/kg and tramadol 10 mg/kg ($p<0.01$) with then animals of the higher dose moving faster than the animals receiving vehicle or tramadol 10 mg/kg (Figure 6). For every treatment group C57BL/6J mice exhibited increased velocity compared to BALB/cJ mice.

DISCUSSION

Laboratory animals can experience pain and distress during and after various types of experimental procedures. In general, the prevention of pain and therefore the timely administration of analgesics, whenever pain is expected, are of highest importance. The assessment of pain and distress in order to reduce it is equally significant. Different analgesic regimens are available to effectively treat pain without interfering with the objectives or the results of the experimental protocol. Choosing the analgesic drug and the appropriate dose depends on the animal's species. The purpose of the present study was to evaluate the effects of tramadol on acute thermal pain and locomotor activity in two different strains of mice.

Tramadol is used in order to treat moderate or severe pain, both acute and chronic. Its effectiveness is based upon two mechanisms: it binds to the μ-opioid receptor and inhibits the reuptake of serotonin and norepinephrine, acting as an atypical opioid analgesic (Duthie,1998; Ide et al., 2006). It is suggested that tramadol addresses pain through the activation of prosynaptic a2-adrenoceptors. This monoaminergic modulation along with the opioid activity results in the analgesic effects of tramadol (Duthie, 1998). Tramadol has been tested on various animal species such as dogs, goats, rats and mice. The recommended dose for mice is 5 mg/kg injected subcutaneous (s.c.) or intraperitoneally (i.p.) but the duration of action is uncertain (Flecknel, 2009).

Various studies have indicated that tramadol is also effective on acute thermal pain with dose- and time- related antinociceptive effects in mice (Mattia et al.,1993; Aydin et al., 2012). Additionally, in a study conducted on Kunming mice, where the hot plate test was used, i.p. administration of tramadol 32 mg/kg produced a maximal analgesic effect after 15 minutes (Zhang et al., 2011), while on ICR mice, tramadol produced a maximal analgesic effect at the dose of 75 mg/kg by i.p. injection, and the effect persisted for approximately 2 hours (Mattia et al., 1993). Nevertheless, i.p. administration of tramadol 10 mg/kg 30 min prior to surgery, followed by 10 mg/kg i.p. every 12h for 60 h showed no effect on heat hyperalgesia or mechanical weight bearing on rats which were followed for 6 days after

surgery (McKeon et al., 2011). In the current study only the dose of 40 mg/kg tramadol had statistically significant antinociceptive effects both on BALB/cJ and C57BL/6J mice. The group of tramadol 10 mg/kg exhibited greater latencies compared to the vehicle (control) group but this difference was not statistically significant. This leads to the conclusion that tramadol 10 mg/kg may be an insufficient dose for the treatment of acute thermal pain in these strains. Furthermore, BALB/cJ mice appeared to be sedated after administration of the higher dose of tramadol. A study conducted on Sprague Dawley rats showed that 60 min after i.p. administration of 12.5 mg/kg tramadol increased latency in the hot plate test, whereas greater doses (25 and 50 mg/kg) caused sedation and affected motor function (Cannon et al., 2010).

Mogil et al. concluded that genotype affects performance of mice in various models of nociception, including the hot plate test. BALB/cJ mice exhibited greater latency times (almost double) than C57BL/6 mice on the hot-plate (53^{o}C) without use of analgesia (Mogil et al., 1999). Same observations were made after the administration of sustained-release buprenorphine (bup-SR) with SWR/J mice being more sensitive to acute thermal pain than BALB/cJ mice (Carbone et al., 2012). There were also no statistically significant differences between the %MPEs of the two strains after the administration of tramadol 10 mg/kg. There were statistically significant differences between C57BL/6J and BALB/cJ only for the tramadol 40 mg/kg groups, where BALB/cJ mice exhibited much higher %MPEs. It was also observed that the two strains exhibited different behaviors during the hot-plate test. C57BL/6J mice appeared to be more aggressive and more stressed, trying to step on their tiptoes and place their rear paws on the glass cylinder possibly to avoid the heat. No attempts to escape from the apparatus (jumping) were observed. Nearly all BALB/cJ mice treated with 40 mg/kg tramadol reached cut-off time without moving and were removed from the apparatus.

The open-field test is the most common way to assess locomotion and other behaviors such as anxiety in rodents (Hall and Ballachey, 1932; Gould et al., 2009). In this study the open-field test was used, and three parameters of locomotor activity were examined: the distance travelled, the velocity, and the time spent in the center of the apparatus. In the tramadol treated groups it was observed that C57BL/6J moved significantly faster than BALB/cJ mice, and also covered greater distance in the apparatus but the difference was not statistically significant. Carola et al. showed in their study that BALB/cJ mice exhibited lower overall locomotor activity than C57BL/6J mice in the open field test, whereas the parameters measured suggested that the two strains present different mechanisms and behaviors in order to cope with stress and anxiety (Carola et al., 2002). The time spent in the center of the open-field apparatus is mainly a measure of anxiety-related behavior It has been shown that BALB/cJ mice appear to be more stressed than C57BL/6J according to locomotor activity measurements using the open field test (Carola et al., 2002; O'Leary et al., 2013). In our study C57BL/6J mice spent more time in the center of the apparatus compared to BALB/cJ mice in all groups, but there was no significant difference between the control groups. Tramadol administration reduced the time spent in the center in all groups, and had a greater impact on BALB/cJ than on C57BL/6J mice.

Tramadol appeared to cause a dose-and time-related reduction of ambulatory distance and velocity in BALB/cJ mice. It was also observed that they spent less time in the center of the apparatus compared to C57BL/6J mice, which suggests higher stress levels. A study conducted on Wistar rats showed that although tramadol 5 mg/kg in single administration did not cause changes in locomotor activity compared to the control group, the higher doses administered (10 and 20 mg/kg) leaded to dose-depended decrease in locomotor activity (Szkutnik-Fiedler et al., 2012).

The most important finding of the open field test was the great increase of ambulatory distance and velocity of C57BL/6J mice in the subgroup treated with 40 mg/kg tramadol. It seems that the highest dose of tramadol caused hyperactivity in C57BL/6J mice almost immediately after drug administration. Liang et al. showed that locomotor activity of Kunming mice was not affected by tramadol treatment (1-16 mg/kg i.p.), suggesting that tramadol did not appear to possess psychomotor-stimulating effects in mice (Liang et al., 2006), assumption which contrasts to the results of the current study. The aforementioned data concur well with another study conducted on female CD1 mice that were injected s.c. with tramadol 20 mg/kg once daily for 48 hours after surgery. On the first post operative day locomotor activity was greatly increased, while nighttime activity was reduced (Rätsep et al., 2013). Likewise, single i.p. injection of intermediate doses of tramadol in rats slightly increased their locomotor activity reaching the maximum effect 20-30 minutes after administration, while higher doses reduced activity (Tzschentke et al., 2002).

CONCLUDING REMARKS

The lower dose of tramadol (10 mg/kg) has insufficient antinociceptive effects on acute thermal pain for both strains. The highest dose of tramadol used in this study

(40 mg/kg) was greater than the one required for BALB/cJ mice, as they were under sedation for at least 60 minutes after drug administration. The same dose of tramadol appeared to be effective on C57BL/6J mice as latency times on the hot plate were significantly increased. Despite this fact, it is not a suitable choice as an analgesic, especially postoperatively, as it causes hyperactivity to this strain. Therefore, we recommend that when planning an experiment that includes administration of drugs and especially analgesics, one should take under consideration the strain of the animal, and also the potential side effects before confirming the choice of drug and selecting the most effective dose. It should be also underlined that although treatment of pain is of major significance, when signs of pain are observed, the prevention of pain and therefore the timely administration of painkillers whenever pain is expected are of highest importance.

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CONFLICT OF INTEREST

The authors of this article declare that they do not have any conflicting interests.

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