Ciencia Latina Revista Científica Multidisciplinar
Septiembre-Octubre, 2023, Volumen 7, Número 5
https://doi.org/10.37811/cl_rcm.v7i5.7972

Evaluation of Analgesic Tolerance Induced by Metamizole in the Writhing Test Model

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ABSTRACT

Analgesic tolerance is defined as the need for increasing doses to achieve the intended effect. In this paper we present experimental data from the search for analgesic tolerance induced by metamizole in a pain model induced by 0.9% acetic acid known as abdominal constriction test or Writhing test. The aim of this study was to identify the development of analgesic tolerance to metamizole in the Writhing test model. Experimental: groups of Balb/C mice 6±2 were used. They were administered metamizole in different protocols, and subsequently underwent a pain stimulus with 0.9% acetic acid via intraperitoneal, evaluated for 30 minutes and counting time of latency, number of abdominal stretches in order to rate pain response, subsequently naloxone was administered to look for signs of withdrawal. Our results demonstrated that there is a tendency to develop analgesic tolerance to metamizole after its repeated administration.

Key words: *metamizole; writhing test; pain; tolerance.*

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Evaluación de la Tolerancia Analgésica Inducida por Metamizol en el Modelo de Prueba de Retorcimiento

RESUMEN

La tolerancia analgésica se define como la necesidad de dosis crecientes para lograr el efecto deseado. En este artículo presentamos datos experimentales de la búsqueda de tolerancia analgésica inducida por metamizol en un modelo de dolor inducido por ácido acético al 0,9% conocido como prueba de constricción abdominal o prueba de Writhing. El objetivo de este estudio fue identificar el desarrollo de tolerancia analgésica al metamizol en el modelo de prueba de Writhing. Experimental: se utilizaron grupos de ratones Balb/C 6±2. Se les administró metamizol en diferentes protocolos, posteriormente se les realizó un estímulo doloroso con ácido acético al 0,9% vía intraperitoneal, se evaluó durante 30 minutos y se contó tiempo de latencia, número de estiramientos abdominales para calificar la respuesta al dolor, posteriormente se les administró naloxona para buscar signos de abstinencia. Nuestros resultados demostraron que existe una tendencia a desarrollar tolerancia analgésica al metamizol después de su administración repetida.

Palabras clave*: metamizol; prueba de contorsión; dolor; tolerancia.*

Artículo recibido 19 agosto 2023

Aceptado para publicación: 23 setiembre 2023

INTRODUCTION

Pain is an unpleasant sensory and emotional experience related with potential or real tissue damage, or described in terms of such damage and if it persists, with no remedy available to alter its cause or manifestations, a disease in itself. (Pérez F.J., 2020; Ibarra, 2006), and it is one of the most common reasons of consultation in the medical hospital practice and outpatient visit. This is a protective mechanism of the body (Dray, 1997), since it allows to identify different disorders and appears every time there is a damaged tissue causing the individual to react reflexively in order to eliminate the painful stimulus (Guyton, 1977). When pain becomes chronic, it loses its protective function and turns into a physical and emotional load for the patient, causing a decrease in quality of life. There are different factors such as age, gender, genetic differences, among others, that may modify pain perception in the individual (Besson and Chaouch, 1987).

Nociception refers to the neural mechanisms through which noxious stimuli are detected (Julius and Basbaum, 2001). This series of mechanisms are integrated in all levels of the neuraxis, from the peripheral nerve, through the dorsal horn of the spinal cord, to the brain structure. Nociception involves the translation of noxious stimuli by the peripheral nerve endings, the transmission of information from the periphery to the central nervous system (CNS) and the endogenous modulation of transmission mechanisms (Besson and Chaouch, 1987; Basbaum, 1999). Nociception is a physiological phenomenon, while pain is a multidimensional experience which also involves cognitive and affective factors (Guyston, 2003)

The Writhing test model, also known as “abdominal constriction test”, is a model of visceral pain in which acetic acid is used as an irritant agent (Akman et al., 1996; Abdollahi, M. et al., 2003). For the Writhing test model, the level of integration of the response is spinal, which is mediated by Sherington’s reflex arc (Hudspith, et al., 2006). Although visceral pain has been less studied, it is known that abnormal widening or intense contraction of the smooth muscle of a hollow viscera is painful, as well as the distension of the liver or spleen capsules, the ligament traction and pleura and peritoneum inflammatory processes.

The reception of visceral nociceptive information in the spinal cord by the dorsal horns is less precise than the reception of somatic stimuli. It does it through several medullary segments

causing more vague and imprecise sensations. In humans, visceral pain is usually less located and it can be referred to a skin area with the same innervation (Cervero and Laird, 1999).

Analgesic tolerance

Tolerance is a pharmacological phenomenon defined as the need of higher doses in order to retain the effects of a medication, or as the reduction of the strength of a medication due to recurrent administration (Mayer et al., 1995). Tolerance has been a particular concern for opioid therapy, in which it manifests as the reduction of the intensity of the effect after the constant administration of the same dose of a medication or as the shift to the right of the agonist dose-response curve. The previously stated leads to the need of increasing the dose in order to reach the intended analgesic effect (Bhargava, 1994; Yaksh y Mark S., 2011). Consequently, the clinical utility of opioid analgesics in the treatment of chronic pain is limited, since a higher occurrence of adverse effects related to opioids is shown (Mercadante, 1999).

Metamizole

Metamizole, also known as dipyrone, belongs to the Non-steroidal anti-inflammatory drugs (NSAIDs) group and also to the pyrazolones group, together with phenylbutazone, oxyphenbutazone and aminopyrine. It is widely used in Europe, Asia and Latin-America and was reintroduced in Sweden (Groser et al., 2011).

Pharmacological properties of Metamizole

Metamizole is a drug with very good effectiveness when used as antipyretic and analgesic, and has weak anti-inflammatory activity (Groser et al., 2011)., besides, it contains spasmolytic properties because of its myorelaxant effect on smooth muscles. It shows a superior analgesic efficacy to that of salicylates and less risk of gastric mucosal lesions, in addition it has anticonvulsant properties (Laird et al., 1998; Doretto et al., 1998; Ergun et al., 2001., Duarte et. al. 2007). Metamizole is effective in therapy for musculoskeletal disorders, rheumatoid arthritis, osteoarthritis, and ankylosing spondyliti. Moreover, it intervenes in the persistence of ductus arteriosus to close the duct and helps in the treatment of dysmenorrhea. Besides, it is useful in the treatment of Bartter syndrome patients since the greatest harm that they suffer is because of the

action of prostaglandins in the kidney. Metamizole is prescribed for patients with hyperprostaglandin E syndrome and reduces the incidence of colon cancer (Groser et al., 2011).

Mechanisms of action of Metamizole

Metamizole exerts its analgesic and antiinflammatory effects at the peripheral level through an inhibitory action on the biosynthesis of prostaglandins, due to the non-selective inhibition of COX, mainly to the COX-1 and COX-3 isoforms (Weithmann and Alpermann, 1985; Cashman, 1996). At a central nervous system level, it was found that metamizole activates the neurons of the periaqueductal brain (PAG), producing a signal that inhibits the transmission of the nociceptive stimulus coming from the spinal cord. Metamizole has also been found to interfere with glutamate involvement in central nociception, and to inhibit prostaglandin production. In addition to metamizole actions at a supraspinal level on the neurons that send information to the cord to inhibit the transmission of the painful stimulus, it was demonstrated that it also has direct actions on the spinal neurons. Metamizole exerts its analgesic effects also by stimulating the peripheral nitric oxide synthase from L-arginina (Lorenzetti y Ferreira, 1985; Peñaranda Moren, M. 2006). Nitric oxide, being a gas, spreads through all the area near the injury and is capable of freely entering the interior of the cells, where it produces an increase in cyclic GMP, causing desensitization of the nociceptor.

Central analgesia induced by metamizole partially involves the activation of the endogenous opioid system in the PAG, the NRM and the spinal cord. Recent studies show some central encephalic and medulla action, where metamizole stimulates the liberation of endogenous opioids to exert its therapeutic effects without directly activating the opioid receptors (μ, κ y δ) (Vanegas and Tortorici, 2002). The former is suggested because naloxone, opioid antagonist, when systemically administered or microinjected in the previously mentioned CNS areas, reduces the analgesic effect of metamizole (Carlsson et al., 1986; Tortorici et al., 1996; Vazquez and Vanegas, 2000; Hernández and Vanegas, 2001; Navarro T., 2005).

Metamizole provokes a strong antipyretic effect, which is mainly based in its inhibition of prostaglandins in a central level. It also has an inhibition action in the synthesis or liberation of endogenous pyrogen interleukin-1. Not only is metamizole capable of diminishing body

temperature, but also protects from neuronal damage produced by high and prolonged fever, which has been demonstrated in rats subjected to experimental brain ischemia. Metamizole also has an anti-inflammatory effect, which derives from its inhibition of pro-inflammatory prostaglandins at a peripheral level, inhibition of neutrophil chemotaxis at the inflammation site and inhibition of the release of proinflammatory factors from macrophages (Watkins and Maier, 2003; Milligan y Watkins, 2009). In addition to its analgesic effect, metamizole has an antispasmodic effect through a direct inhibition on the peripheral smooth muscle, diminishing its excitability. This effect is partly also a consequence of an increase of nitric oxide, which stimulates the production of intracellular cyclic GMP, resulting in a relaxing effect in the smooth muscle. On the other hand, a metamizole inhibitory action on neurons innervating smooth muscle structure has been observed, inhibiting the release of mediators, namely, a presynaptic action. This antispasmodic effect has even been seen in the sphincter of Oddi. However, at the doses used therapeutically prevail its analgesic, antipyretic and antispasmodic effects over the anti-inflammatory effect (Feria, 2008).

Metamizole adverse reactions

The most significant adverse reactions are agranulocytosis and aplastic anemia, but with a risk rate of less than 1.1 cases per million. The risk of suffering aplastic anemia is lower than any other NSAIDs. Some other non-desired effects are: skin reactions, hypersensitivity, somnolence, arterial hypotension (especially when quickly administered via i.v.bolus). This is a restricted prescription drug in the United States due to the possibility of inducing agranulocytosis; however, in Mexico it is still used because multicenter studies have demonstrated that death associated to agranulocytes due to the use of metamizole is 30 times lower than death associated to gastrointestinal bleeding with other NSAIDs, such as indomethacin and diclofenac (Andrade et al., 1998; Arcila-Herrera et al., 2004).

Analgesic tolerance induced by metamizole.

In the past it was considered that the NSAIDs were not able to cause analgesic tolerance nor physical dependence (Sunshine and Olson 1994; Roberts and Morrow, 2001). In the evaluation of physical tolerance in two models of thermal nociception, the Tail flick and hot plate models, it

was found that this phenomenon can be developed after the constant administration of metamizole (Tortorici and Vanegas, 2000, Hernández-Delgadillo et al., 2003). In rats, microinjecting 150 μg of metamizole inside the PAG produced analgesia at the first administration. Nevertheless, when microinjected twice a day during two days, the same metamizole dose induced the development of analgesic tolerance, assessed in the two models of thermal nociception mentioned above. The i.p. administration of 1 mg/kg of naloxone precipitated withdrawal syndrome in rats being treated with metamizole (Tortorici y Vanegas, 2000). In another study, also evaluated in rat in the Tail flick model, it was shown that the development of tolerance to the analgesic effect produced by metamizole but administering 600 mg/kg via i.v. in a repeated administration protocol of twice a day for 11 days (Hernández-Delgadillo et al., 2003). In both studies, the nociceptive stimulus used was of thermal nature.

General objective

- To assess the development of Analgesic tolerance by metamizole in the Writhing test model.

Specific objectives

- To evaluate the effect of the dose, the route of administration, dosing interval and type of pain induced on the development of tolerance to the analgesic effect of metamizole in Balb/c mice.
- To evaluate the participation of the endogenous opioid system in metamizole acute analgesia.
- To evaluate the development of physical dependence by repeated administration of metamizole.

METHODOLOGY

Animals

The experiments were carried out in Balb/c mice, both females and males with an average weight of 30 g, from the biotherium of the D. In Medical and Molecular Pharmacology from the Faculty of Human Medicine and Health Sciences of the Universidad Autónoma de Zacatecas. The animals were kept in a special room under room temperature, alternating light-dark cycles each 12 hours (light was turned on at 7:00). All animals had access to water and ad libitum food. Behavioural experiments were made between 8:00 and 15:00 in temperature-controlled conditions (22±2 °C). At the end of the evaluation, the animals were culled in a CO_2 chamber. The experiments were

made according to the Ethical Guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983), and established in the Mexican Norm entitled Technical Specifications for the Production, Care and Use of Laboratory animals (NOM-062-ZOO-1999).

Drugs and reagents

The drugs used in this project were: metamizole sodium hydrate (Aventis Pharma Laboratories, Mexico), naloxone hydrochloride (Sigma Chemical Co., USA), sodium pentobarbital (Norvet Laboratories, Mexico). Other compounds used were acetic acid (Sigma Chemical Co., USA) and ethyl ether (Norvet Laboratories, México). The drugs were dissolved in sterile isotonic saline solution (Pisa Laboratories, Mexico), and administered via the intraperitoneal route (i.p.).

Intraperitoneal administration (i.p.)

For the i.p. administration the animals were laid in the supine position, immobilizing them by making a skin fold along the spine and holding the tail between the fingers of the left hand. The injection was carried out with a 1 ml Plastipak® disposable syringe with a 27G gauge hypodermic needle, 13 mm long, at a volume of 1 mL/Kg body weight for the rat, and 0.1 mL of weight for the mouse.

Antinociceptive evaluation

The analgesic effect of metamizole was evaluated by using the experimental model of pain: the abdominal constriction test or “Writhing test” evaluated in Balb/C mice. A chemical nociception was induced, generating visceral pain.

Abdominal constriction test or “Writhing test”

Abdominal constriction test is a model of visceral pain. The mouse was placed in a 20 cm diacrylic tube (Plexiglas) 20 cm in diameter and 30 cm in height to allow it to adapt to the new environment. Once 30 minutes passed, the mouse was moved from the cylinder for the i.p. administration of acetic acid 0.6% (0.1mL/10 g body weight). The mouse was placed again inside the cylinder. Immediately afterwards, the evaluation on the number of abdominal constriction (back arching, body stretching and extension of the extremities) during a period of 30 minutes. Nociception was assessed as the number of abdominal stretches and as the latency to the first stretch. A decrease in the number of stretching, as well as an increase in the latency to the first stretch are indicators

of analgesia. The i.p. administration of metamizole was done 30 minutes before the i.p. administration of acetic acid (Akman et al., 1996; Gawade, S. 2012).

Assessment of the development of physical dependence

In order to determine whether constant administration of metamizole induced the development of physical dependence in the animals, gradual weight loss over repeated administrations was considered as the reliable indicator of this phenomenon. Additionally, physical dependence was assessed in all groups of repeatedly treated animals as abstinence precipitated by administration of i.p. 1 mg/Kg of naloxone two hours after completion of the antinociceptive evaluation of the last administration. The assessment was performed according to the method proposed by Way (1969). After the administration of naloxone, the number of jumps and shakes during a period of 30 minutes were evaluated as indicative parameters of physical dependence to opioid.

Experimental design

Experimental groups were divided according to the nociception experimental model, since different species were required in each one.

Abdominal constriction test or Writhing test

The evaluation in the experimental model of Abdominal constriction was carried out in male mice, administering metamizole via i.p.

Dose-response curves (DRCs)

The CDRs of the analgesic effect of metamizole administered via i.p. were determined. In 7 independent groups, doses from 31 up to 1000 mg/Kg were tested with logarithmic increments of 0.25 (31, 56, 100,178, 316, 562 and 1000 mg/Kg). Each control group received a 0.9% saline solution. The former had the purpose of selecting the doses to be administered in the repeated administration studies. The selected doses must be higher than the effective dose 50 (DE_{50}) so that they are high enough to allow observing a decrease in the analgesic effect if tolerance development is induced.

Determination of lethal dose 50 (DL_{50})

The CDRs of death induced by high doses of metamizole administered via i.p. were determined. Four independent groups of mice were injected with doses of 1800, 2300, 3100 and 5600 mg/Kg.

The latency of death was also determined in the animals that died. The former with the purpose of determining the DL_{50}, to consider that the daily accumulated dose of metamizol in the studies of repeated administration was not near toxic doses.

Participation of the endogenous opioid system in acute metamizole analgesia

To determine whether the acute analgesic effect of metamizole is mediated by the activation of the endogenous opioid system, two independent groups of animals were administered 1 and 3 mg/kg i.p. of naloxone, 10 minutes before the administration of 500 mg/kg i.p. of metamizole. Two additional groups were administered saline solution, one of which received saline solution again after 10 minutes, while the other group was administered 500 mg/kg i.p. of metamizole. Immediately after, the analgesic effect was evaluated in the abdominal constriction model.

Repeated administration of metamizole

With the aim of determining the development of tolerance to the analgesic effect of metamizole in five independent groups of animals for each dose, and once the metamizole doses to be used in the studies of repeated administration (500 and 1000 mg/kg i.p.) were selected, the doses were administered repeatedly according to the following protocols:

Group 1. It was administered with i.p. saline solution once a day (8:00 h) for 18 days. On day 19, it was administered again and 30 minutes after it was assessed in the abdominal constriction model.

Group 2. It was administered with i.p. saline solution once a day (8:00 h) for 18 days. On day 19, it was administered with metamizole 500 mg/kg i.p., afterwards the antinociception in the abdominal constriction model was assessed.

Group 3. It was administered with i.p. 500 mg/kg metamizole once a day (8:00 h) for 18 days. On day 19, it was administered metamizole 500 mg/kg i.p. again and 30 minutes later antinociception was evaluated.

Group 4. It was administered with i.p. 500 mg/kg metamizole twice a day (8:00 and 20:00 h) for 9 days. On day 10, it was administered 500 mg/kg metamizole again and 30 minutes later antinociception was assessed.

Group 5. It was administered with i.p. 500 mg/kg metamizole three times a day (8:00, 15:00 and 22:00 h) for 6 days. On day 7, it was administered i.p. 500 mg/kg metamizole again and 30 minutes later antinociception was assessed.

The repeated administration protocols described in the previous five groups were repeated for the 1000 mg/kg i.p. dose of metamizole. It is important to mention and note that all animals received the same number of administrations (18), varying the administration interval (once, twice or three times a day) and the administration period (18, 9 or 6 days).

In addition to the repeated administration protocols described above for the 500 mg/kg of metamizole, four additional groups were also evaluated. They were injected the same dose of metamizole but for a total of 36 administrations according to the following protocols:

Group 1. It was administered i.p. with saline solution three times a day (8:00, 15:00 and 22:00 h) for 12 days. On day 13, the same saline solution was administered and after 30 minutes the abdominal constriction model was evaluated.

Group 2. It was administered i.p. with saline solution three times a day (8:00, 15:00 and 22:00 h) for 12 days. On day 13, i.p. metamizole 500 mg/kg was administered and after 30 minutes antinociception was evaluated in the abdominal constriction model.

Group 3. It was administered with i.p. 500 mg/kg metamizole three times a day (8:00, 15:00 and 22:00 h) for 12 days. On day 13, i.p. metamizole 500 mg/kg was administered again and after 30 minutes antinociception was evaluated.

Group 4. It was administered with i.p. 500 mg/kg metamizole twice a day (8:00 and 20:00 h) for 18 days. On day 19, i.p. metamizol 500 mg/kg was administered and after 30 minutes antinociception was evaluated.

Precipitation of withdrawal syndrome

In every mice of every experimental group described in the previous section (repeated administration of metamizole) the development of physical dependence was evaluated through a precipitated withdrawal syndrome with the administration of i.p. 1 mg/kg naloxone, two hours after having finished the antinociceptive evaluation in the abdominal constriction model.

Data analysis

The presented results show the standard error ± of the mean of 8 animals per group regarding the experiments performed in mice. The statistical tests used were according to the number of groups and the number of factors involved. The following tests were performed: Student's t-test, Analysis of Variance (ANOVA) on ranks or Kruskal-Wallis test followed by Dunnett's or Trukey's test. In every test, a significant statistical difference was considered when $p<0.05$. The statistical procedures were performed with the Sigma Stat program (Jandel Scientific, 2.03 version). The dose-response curves were adjusted with the Win-nonlin program (Pharshight Co., 2.1 version) with the purpose of calculating the DE_{50} and the 95% confidence interval.

RESULTS

Abdominal constriction model

Determination of DE_{50}

In order to select the metamizole doses to be used in the repeated administration protocols, a dose-response curve (DRC) to the antinociceptive effect of i.p. metamizole was performed by evaluating it as the number of stretches during 30 minutes. and as the latency to the first stretch, expressed in seconds. Figure 1 shows that antinociception induced by metamizole, both as the number of stretches (Panel A) and as latency to the first stretch (Panel B) is dose dependent. Antinociception was evaluated as a decrease in the number of stretches or as an increase in the latency to the first stretch, in relation to the control group (i.p. saline). For the parameter of number of stretches, the response is statistically significant as from the 316 mg/kg dose, while for the latency to the first stretch the analgesic effect was observed as from the 178 mg/kg dose.

In order to determine the effective dose 50 (DE_{50}) with its 95% confidence limit, the data shown in Panel A and B in figure 1 was transformed into percent maximum possible effect (%MPE). Such data was adjusted to a sigmoid curve using the Win-nonlin program (Parsight Co., version 2.1) and is shown in figure 2. The data obtained from such adjustment shows that considering the number of stretches the DE_{50} was 118±50 mg/kg, while for the latency to the first stretch, the DE_{50} was 300±69 mg/kg. As can be observed, the value of the DE_{50} is a function of the indicator of antinociception or of the evaluated parameter.

Determination of DL_{50}

The DL_{50} of i.p. metamizole was determined by obtaining the data of death percentage and by making an adjust to a sigmoid curve with the Win-nonlin program (Pharsight Co., version 2.1). The data is shown in figure 3. The DL_{50} was 2160±215 mg/kg of i.p. metamizole. The inset shows the latency of death, a decrease can be seen when the dose increases. The value of DL_{50} must be considered so as not exceed the cumulative dose in repeated administration protocols.

Based on the DE_{50} 118±50 mg/Kg y 300±69 mg/Kg, which was determined considering the number of stretches and latency to the first stretch, respectively, as well as the value of DL_{50} 2160±215mg/Kg of i.p. metamizole, the doses of 500 and 1000 mg/kg were selected to be administered in the repeated administration protocols.

Involvement of the endogenous opioid system in acute metamizole analgesia

The involvement of the endogenous opioid system was assessed with two different naloxone doses: 1 and 3 mg/kg i.p. 10 minutes before the administration of 500 mg/kg metamizole i.p. Figure 4 shows that the antinociceptive effect of metamizole is not modified in the presence of naloxone, suggesting that the endogenous opioid system does not mediate the acute analgesic effect of metamizole, evaluated as a decrease in the number of stretches. In the case of participation, an increase in the number of stretches as a consequence of the nociceptive stimulus in presence of metamizole and naloxone should be noted, since the latter blocks the action sites of endogenous opioids.

Development of tolerance to the analgesic effect of metamizole

The development of analgesic tolerance was evaluated using different repeated administration protocols. Two experimental groups were administered 0.9% saline solution once a day for 18 days (1/d/18d) and on day 19 they were administered saline or 500 mg/kg metamizole i.p. and evaluated according to the description of the model; the third group was administered 500 mg/kg metamizole i.p. and on day 19 they received metamizole 500 mg/kg i.p. The fourth group received it twice a day for 9 days (2/d/9) and to the last group it was administered 3 times a day for 6 days (3/d/6/d). It must be noted that each experimental group received 18 doses of 500 mg/Kg metamizole i.p. (panel A). Additional groups of animals received the same dose of metamizole

(500mg/kg i.p.) but in protocols of 2/day/18 days and 3/day/12 days, for a total of 36 administrations (panel B). Figure 5 shows a tendency to the development of analgesic tolerance to metamizole depending on the administration interval when metamizole is administered 18 times; however, even if the number of administrations were doubled no development of tolerance is observed, only a tendency depending on the administration interval, which shows that the number of administrations is important but not determinant for the development of analgesic tolerance.

Since only a tendency to develop analgesic tolerance was observed with metamizole doses of 500 mg/kg i.p. it was decided to proceed with the evaluation of 1000 mg/kg metamizole in the same repeated administration protocols of 1/d/18d, 2/d/9d and 3/d/6d. Results are shown in figure 6, where partial tolerance development with doses of 1000 mg/kg administered 3 times a day for 6 days can be observed (panel B).

Development of physical dependence

In order to accomplish the objective of identifying the withdrawal syndrome after the implementation of the metamizole repeated administration protocols, by identifying the described signs of physical dependence such as hyperexcitability, hyperactivity, micturition, diarrhea, weight loss, lacrimation, hopping, among others, 1 mg/kg naloxone i.p. was administered after the evaluation with acetic acid. The animals were observed during 30 minutes, as previously described in the material and methods sections. The results show that none of the animals administered repeatedly with metamizole 500 and 1000 mg/kg i.p. in any of the three repeated administration protocols (1/d/18d, 2/d/9d y 3/d/6d) showed any signs indicative of development of physical dependence evaluated as precipitated withdrawal syndrome. Chart 1 shows the absence of hopping in all evaluated animals. Likewise, figure 7 shows that there was no weight loss in the referred animals, suggesting the absence of development of withdrawal syndrome.

DISCUSSION

Tortorici and Vanegas (2000) argue that there is indeed development of analgesic tolerance to metamizole. Their experiments were performed in rats administered in the PAG and two thermal nociception models were used: the Tail flick and hot-plate models. In the models performed in

male mice, the development of partial tolerance to metamizol was observed dependent on the dose and administration interval, since when 500 mg/kg i.p. was used, a tendency to development of analgesic tolerance to metamizole was obtained by observing an increase in the number of stretches in relation to the administration interval, that is, as the administration interval decreases, the number of stretching increases.

It is important to note that every mouse received 18 administrations, and that the used model was chemical nociception, unlike Totorici's group, whose model was thermic nociception.

Likewise, Hernández-Delgadillo et al. (2003) showed, with the Tail flick model, that repeated administration of metamizole induces antinociceptive tolerance. In addition, it is shown that there is a tendency to develop analgesic tolerance to metamizole administered via i.v. in a protocol of twice a day for 5 days with a 600 mg/kg metamizole dose. A study of tendency was also performed, in which it was observed that in the 21st administration there could be a development of analgesic tolerance to metamizole in their conditions and evaluated in the Tail-flick model. It was identified that it depends on the number of metamizole administrations. In the study performed for this paper, when the administrations were incremented from 28 to 36 there was no statistical difference, which demonstrates the tendency to develop analgesic tolerance to metamizole.

In regard to the dose and administration interval, Bhargava (1994) mentions that the dose is a factor that has a key role in the development of analgesic tolerance to metamizole. In this work a statistical difference that sustains a partial development of analgesic tolerance to metamizole was found in the protocol of 3 times a day for 6 days with the 1000 mg/kg i.p. in mi

Physical dependence is a phenomenon described as part of collateral effects of opioids, and it refers to the series of complex alterations in the organism balance, which manifest when a medicine is suspended (by deletion) or when an antagonist is administered (naloxone). Repeated administration of opioids leads to the development of physical dependence, which is exhibited as described above as withdrawal syndrome in the antecedents. Bhargava (1994) and Williams et al. (2001) and Christie, M. J. (2008) outline that physical dependence is not present in acute exposure. Heishman et al. (1989), Kim et al. (1990) and Azorlosa, J. L., et. al. (1994), mention

that physical dependence is only present after a few days of repeated administration of opioids, namely, physical dependence is a phenomenon that appears further in time in relation to the other collateral effect (development of analgesic tolerance). In this paper it was found that in the Writhing test model, there is a partial development of analgesic tolerance, but physical dependence is not present, which explains the previously stated regarding the delay of physical dependence onset.

In regard to the participation of the endogenous opioid system in analgesia of metamizole, it is known that μ, δ, and κ opioids have a crucial role in the endogenous modulation of pain transmission and they are widely distributed in the PAG, NMR and the spinal cord (Millan, 2002). The endogenous opioid system acts through enkephalins, dynorphins and β-endorphins. Its effect is antagonized by the non-specific antagonist: naloxone, as described by Basbaum and Fields (1984). It is know that metamizole, according to Vanegas and Tortorici (2002), exerts its action in the CNS level, enabling the PAG neurons and producing a signal that inhibits the transmission of the nociceptive stimulus coming from the spinal cord.

Analgesia produced by metamizole partially involves the activation of the endogenous opioid system in the PAG and, in the NRM and the spinal cord, as well as some central action: brainstem and medullary, where metamizole stimulates the liberation of endogenous opioids to exert its analgesic action without directly activating the opioid receptors (μ, δ and κ). This suggests, as stated by Carlsson (1986), Tortorici (1996), Vasquez and Vanegas (2000), and Hernández and Vanegas (2001), that an antagonist of receptors μ, δ and κ, as naloxone administered systemically or micro-injected in the areas of the CNS (PAG and NRM) reduces the analgesic effect of metamizole.

In the results obtained in this work, a participation of the endogenous opioid system was not found, since naloxone 1 and 3 mg/kg i.p. was administered acutely with the 500 mg/kg metamizole i.p. Dose and evaluated as the number of stretches in the Writhing test models, without obtaining any differences between the group with metamizole nor between groups 1 and 3 mg/kg naloxone and metamizole. In every case, only the antinociceptive effect of metamizole was found. It would be expected that in the case of finding participation of the endogenous opioid

system when blocking the receptor sites μ, δ and κ with naloxone, an increase in the number of stretches existed. It is worth mentioning that this phase was only performed in the Writhing test model.

Vasquez and Vanegas (2000), conducted experiments indicating that by administering 100 μg/0.5 μL metamizole, in the PAG and NRM, and subsequently 0.5 μg/0.5 μL naloxone, and when evaluating with the hot-plate model and counting the latency of paw withdrawal, it was possible to observe that the animals presented a reverse effect of the antinociceptive effect when injecting naloxone, which speaks of the inhibition of the spinal circuit, NRM and PAG, in the liberation of endogenous opioids. In consequence, it reaffirms the action mechanism of metamizole in the CNS.

Bhargava (1994) argues that different conditions modify the response to analgesic tolerance such as the route of administration (subcutaneous, intraperitoneal, intravenous, intrathecal) in the PAG and NRM, the frequency of injection (once, twice or three times a day), number of dose, drug exposure period, the species (rat, mice, guinea pig, rabbit or monkey) and the type of stimulus (thermal, mechanical and chemical). The previous in addition to conditions such as type of model, namely, it will not be the same since in our study for the Writhing test model it was decided to use the 1000 mg/kg dose based on the male results, and consequently a tendency to develop analgesic tolerance was observed, which confirms that in respect to the type of model, the development of analgesic tolerance shows differences.

In mice, a tendency to the development of analgesic tolerance to metamizole was found, in both genders, using the 1000 mg/kg i.p. dose. There was a statistical difference that shows a partial development of analgesic tolerance. In this sense, the interval of administration, the dose, as well as the metamizole's administration via, have a key role in the development of analgesic tolerance; the previously stated is supported by Kayan and Mitchell (1969), and Dafers and Ober's (1989) work.

Physical dependence was not demonstrated because, even though Saelens (1971); Tortorici and Vanegas (2000) mention that the withdrawal syndrome is characterized by a general state of hyperexcitability accompanied by hyperactivity, diarrhea, weight loss, micturition, lacrimation, among others, which are used as parameters of the degree of physical dependence, in this work during the repeated administration protocols it was not observed any weight loss, nor hopping as indicators of withdrawal model for the formaldehyde model.

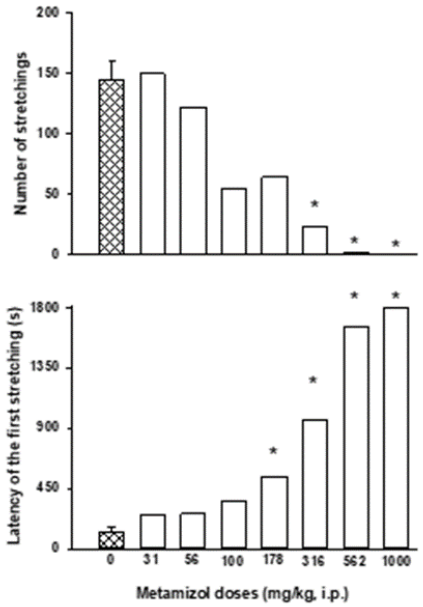

Fig. 1 Dose-response curve of the antinociceptive effect produced by metamizole, evaluated as number of stretches and as latency to the first stretch in the Writhing test model in male mice. Each bar graph represents the mean standard error ± of 8 determinations. Wilcoxon's ANADEVA followed by Dunnet's test *p<0.05.

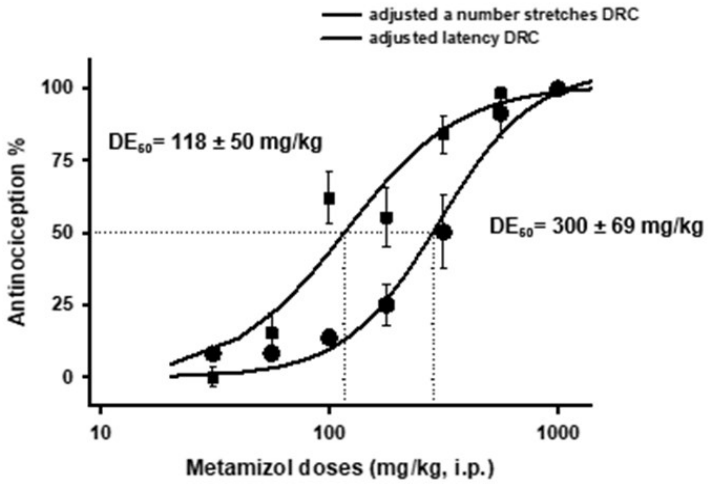

Figure 2. Adjusted DRCs of the antinociceptive effect of metamizole expressed as percentage of maximum possible effect (%MPE) or percentage of antinociception from the data shown in panel A and B in figure 3. The continuing curved line represents the DRC for the number of stretches and the dashed curved line represents the adjusted CDR for the latency to the first stretch. The points represent the data obtained experimentally. The dashed lines show the approximate points of the DE_{50}. The DE_{50} were 118±50 mg/kg and 300±69 mg/kg i.p. considering the number of stretches and latency to the first stretch, respectively. Each point represents the mean ± standard error of 8 mice.

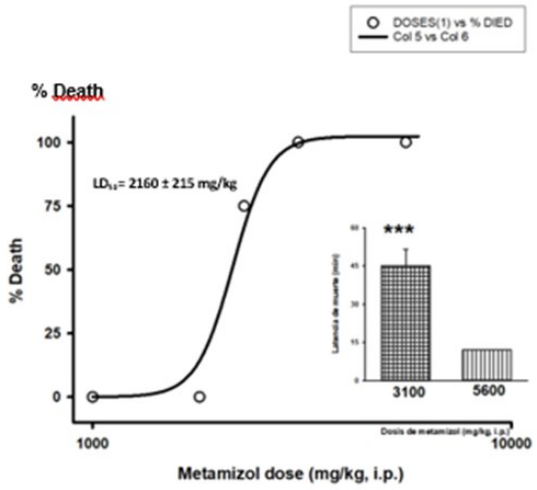

Figure 3. Adjusted DRC of percentage of death for metamizole evaluated in male mice. The DL_{50} was 2160±215 mg/Kg i.p. The inset shows the latency of death. Each graph bar represents the mean ± standard error of 8 animals. Kruskal-Wallis's ANOVA followed by Dunn's *p<0.05.

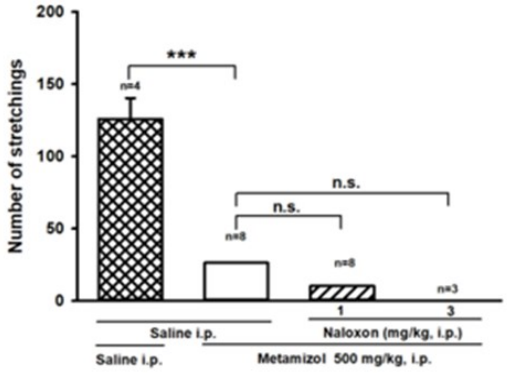

Figure 4. Effect of naloxone 1 and 3 mg/kg i.p. on the antinociception induced by 500 mg/kg metamizole i.p., evaluated in the Writhing test model in male mice. Each graph bar represents the mean ± s.e. of 8 determinations ***P<0.001, n.s., not significant, Wilcoxon's ANOVA, followed by Tukey's test.

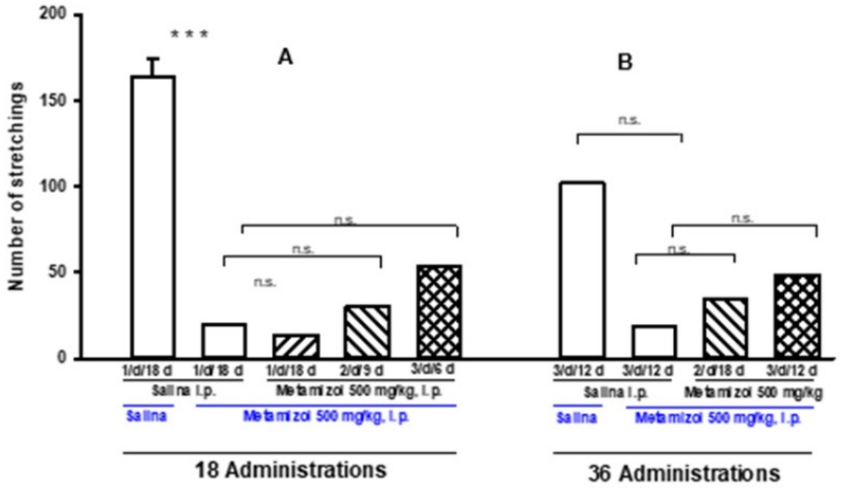

Figure 5. Metamizole antinociceptive effect, evaluated as the number of stretches in the Writhing test model in the different repeated administration protocols with 500 mg/kg metamizole i.p. for 18 (panel A) and 36 administrations (panel B) ***P<0.001, n.s. non-significant, Wilcoxon's ANOVA followed by Tukey's test.

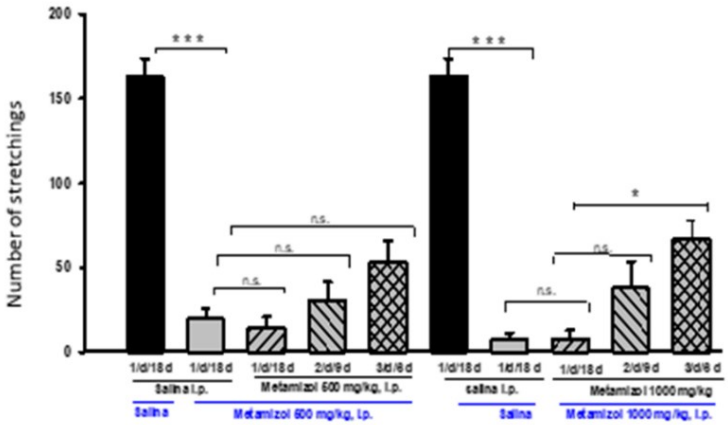

Figure 6. Antinociceptive effect of i.p. 500 and 1000 mg/kg metamizole administered once a day for 18 days, twice a day for 9 days and 3 times a day for 6 days evaluated in male mice ***p<0.001, n.s. non-significant, Wilcoxon's ANOVA, followed by Tukey's test.

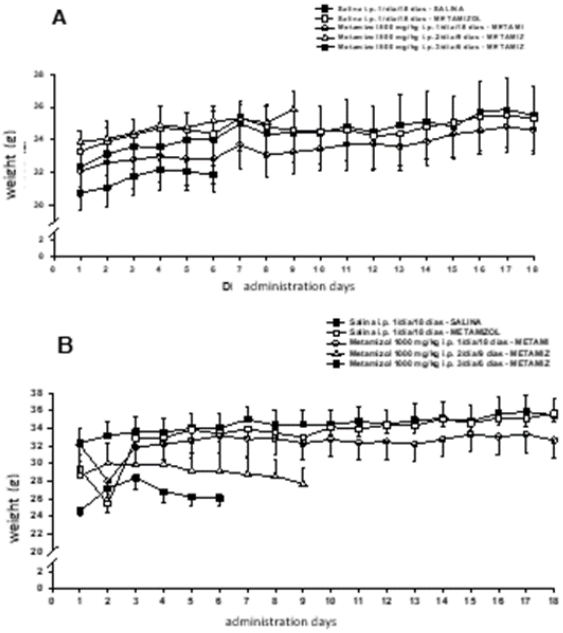

Figure 7. Weight male mice administered with 500 mg/kg i.p. (Panel A) and 1000 mg/kg i.p. (panel B) 1, 2 and 3 times a day during 18, 9 and 6 days respectively. Each point corresponds to the mean ± s.e. of 8 values.

PRE-TREATMENT AND TREATMENT	Number of HOPS	
18 repeated administrations	**500 mg/kg**	**1000 mg/kg**
Saline 1/d/18 d - Saline	0	0
Saline 1/d/18 d - Metamizole	0	0
Metamizole 1/d/18 d - Metamizole	0	0
Metamizole 2/d/9 d - Metamizole	0	0
Metamizole 3/d/6 d - Metamizole	0	0
36 repeated administrations		
Saline 3/d/12 d - Saline	0	0
Saline 3/d/12 d - Metamizole	0	0
Metamizole 2/d/18 d - Metamizole	0	0
Metamizole 3/d/12 d - Metamizole	0	0

Table 1. Precipitated abstinence, evaluated as number of hops. Each group was integrated by 8 experimental animals.

CONCLUSION

In consequence, we can conclude that in the case of male mice there is a tendency to develop analgesic tolerance to metamizole in the repeated administration protocol, excepting the 3 times a day for 6 days protocol with the 1000 mg/kg i.p. dose, where there is partial analgesic tolerance to metamizole, since statistically there is a significant difference.

Participation of the endogenous opioid system was not observed in the Writhing test model via i.p. In addition, there is not a development of physical dependence in male mice in the Writhing test model via i.p.

No observamos la participación del sistema opioide endógeno en el modelo de Writhing test en la vía i.p.

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