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MAGNESIUM SULPHATE INCREASES KETAMINE ANALGESIC EFFECT IN A RAT MODEL OF INCISIONAL PAIN

Rasha Bakheet Abd-ellatief*

Lecturer of Pharmacology- Faculty of Medicine- Assiut University.

***Corresponding Author: Rasha Bakheet Abd-ellatief**
Lecturer of Pharmacology- Faculty of Medicine- Assiut University.

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ABSTRACT
Effective control of postoperative pain is an important concern for both the patient and the physician. Optimization of analgesia with reduction of side effects lead to better outcomes. Unfortunately, this issue remains a challenge. The aim of this study is to determine whether a low dose of systemic magnesium sulfate can synergize the analgesic effect of ketamine in rats and whether this combination can decrease the side effects of ketamine. Adult male Wister rats were assigned into five groups. Group I was kept as normal animals and received no treatment. The surgical animals (incisional pain model was done by a 1-cm planter incision) were divided into four groups (n=10 for each group), group II: received saline, group III: received i.p ketamine 5mg/ kg, group IV received s.c. magnesium sulphate 5mg/kg, group V received i.p ketamine 5mg/ kg combined with s.c. magnesium sulphate 5mg/kg. Mechanical hyperalgesia and allodynia were assessed by analgesimeter and von Frey apparatus pre- and post-drug administration. Also, the rotarod apparatus was used to verify the effect of ketamine-magnesium combination on motor impairment. Single administration of ketamine or magnesium sulphate didn't produce any significant antinociceptive effect. However, significant antinociception was revealed when they were concomitantly administered. Also, this combination didn't produce any motor impairment. In conclusion, sole administration of ketamine and magnesium sulfate at the assigned doses didn't inhibit nociception in rats, but their combination produced synergistic antinociception with no sedative effects.

KEYWORDS: Magnesium, ketamine, incisional pain, rats.

INTRODUCTION
Proper postoperative pain relief is one of the primary concerns for both the surgeon and the patient. Patient usually worries about the amount of pain he will experience after surgery, and surgeon knows the relation between postoperative pain control, the clinical outcome and patient well-being.[1] Different types of NMDA receptors are involved in nociception and may have different roles in spinal hypersensitivity and chronic pain. Hence, NMDA receptors' antagonists were investigated in different experimental models of pain. However, despite their clinical efficacy in treatment of pain, side effects limit their clinical use.[2] Magnesium is an antagonist of the glutamate subtype of NMDA receptors, it blocks their induced current in a voltage-dependent manner.[3] However, data concerning the effect of magnesium on pain is discordant. Many authors have reported its influence on pain intensity; however, others reported that it lacks any effect on pain.[4] Ketamine is a non-competitive NMDA receptor blocker, that has analgesic properties in sub-anesthetic doses. Because of its sedative and motor impairment effects, it's not used as a sole analgesic for postoperative pain.[5] Some literatures reported that magnesium can potentiate the analgesic effect of ketamine, while others reported that it may interacts in an opposing manner.[6] Because magnesium and ketamine block NMDA receptors by different mechanisms of action, we have to hypothesize that magnesium may amplify the antinociceptive effect of ketamine. Therefore, the objective of this study is to determine whether a low dose of systemic magnesium sulfate can synergize the analgesic effect of ketamine in rats. Furthermore, this study investigated whether this combination can decrease the side effects of ketamine in terms of sedation and ataxia.

MATERIALS AND METHODS
Chemicals and drugs
Ketamine (Sigma-Tec Pharmaceutical Industries, Egypt-S.A.E.), magnesium sulphate (EIPICO, Egypt).

Animals
Adult male Wistar rats weighing 180-200 gm, obtained from the animal house facility, Faculty of Medicine, Assiut University (Assuit, Egypt). The animals were housed in stainless-steel cages, in groups of three and had free access to food and water. Animals were kept under controlled environmental conditions (22± 3) °C

and on a 12h light-dark cycle. All animal procedures were conducted in compliance with the protocol approved by our local ethics committee in accordance with the ethical guidelines of the international association for the study of pain.[7]

Surgery

Surgery was performed as described by Whiteside et al., 2004.[8] Rats were anesthetized with 50mg/kg pentobarbital sodium. After antiseptic preparation of the left hind paw. A 1cm longitudinal skin incision was made with a no 11 blade through skin and fascia of the planter foot. The incision was made 0.5cm form the proximal edge of the heel and extended toward the toes. The plantaris muscle was elevated with a curved foreceps and incised longitudinally. The skin was opposed with a single suture of 5-0 nylon and the wound was covered with an antibiotic ointment.

Experimental protocol

Normal control group was separated (Group I) (n=10). After measurement of the pre-drug pain thresholds, rats with surgery were divided into four groups (n=10 for each group), group II: received saline, group III: received i.p ketamine 5mg/ kg, group IV received s.c. magnesium sulphate 5mg/kg, group V received i.p ketamine 5mg/ kg combined with s.c. magnesium sulphate 5mg/kg.

Behavioral testing

Mechanical hyperalgesia was assessed by measuring paw withdrawal threshold (PWT) in different groups 30 min, 60 min, 90 min and 120 min after injection. PWT were expressed in grams using an analgisemeter (Ugo Basil Analgesimeter biological research apparatus, model 24880, Comerio-Va-Italy).When the rat displayed pain by withdrawal of the hind paw or vocalization, the pedal was immediately released and the nociceptive pain threshold read on a scale (A cut-off was 250gm to avoid tissue injury).[9]

To assess tactile allodynia, paw withdrawal thresholds were again measured in different groups 30 min, 60 min, 90 min and 120 min after injection by the use of an automated von Frey apparatus (model EVF3, Bioseb, France). A non-flexile filament was applied to the mid plantar surface of the hind paw, withdrawal thresholds to punctate mechanical stimulation were automatically recorded when the paw was reflexly flexed followed by a clear flinch response after paw withdrawal. We used 50 gm as a maximum cut-off value to prevent tissue damage. Three thresholds were taken per test and averaged.[10]

Furthermore, to verify whether the administration of ketamine-magnesium combination induces motor impairment, the rotarod apparatus (Treadmill for Rats 7700, Ugo basile biological research apparatus, Comerio-Va-Itally) was used. Preliminary experiments were performed, rats were trained on a rotarod cylinder for 2 consecutive periods of 45 seconds, rats that fell-off were excluded. Rats then injected with saline, ketamine, magnesium and their combination and tested on the rotarod at 30, 60, 90, 120 min post-treatment. The results are expressed as percentage of animals that remained on the rotarod for 45sec.[11]

Statistical analysis

Statistical analysis was done using the computer program "prism" and "SPSS". The quantitative data were presented in the form of mean ± standard error of mean (S.E.). One way analysis of variance (ANOVA) was done to compare between the studied groups, followed by student's t-test to compare between significant groups. The difference was insignificant at $p>0.05$, significant at $P<0.05$ and highly significant at $P< 0.01$.

RESULTS

Administration of ketamine or magnesium sulphate alone at the assigned doses didn't produce any significant changes of hyperalgesia and allodynia in comparison to saline-treated group. But, administration of their combination caused a time dependent reversal of the mechanical hyperalgesia and allodynia which reaches its maximum effect on 15 min and continues up to 30 min compared to saline-treated and individual-drug treated animals.

On the rotarod, no significant difference in motor impairment was observed between combined ketamine-magnesium sulphate and ketamine-treated rats along 120 min observation period. Neither the administration of ketamine alone, nor the co-administration of magnesium sulphate with ketamine caused any significant decrease in the time the rats spent on the rotarod during the observation period.

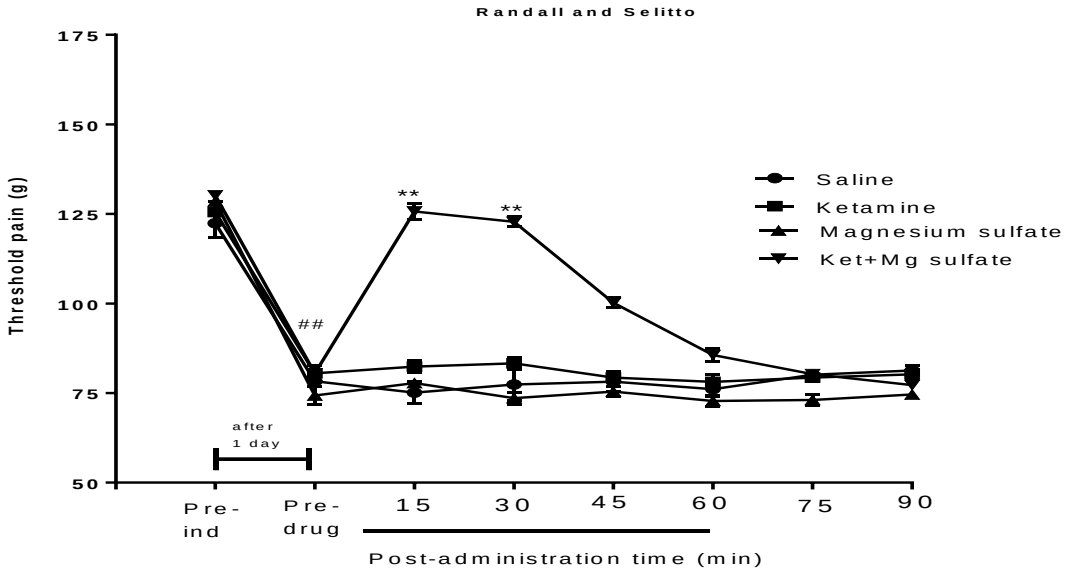

Fig 1: The effects of ketamine, magnesium sulfate and their combination on Randall-Selitto test. Points indicate mean ±S.E.M. for n=10 in each group. ## and ** indicate highly significant difference compared to pre-incision group and ketamine-treated group respectively.

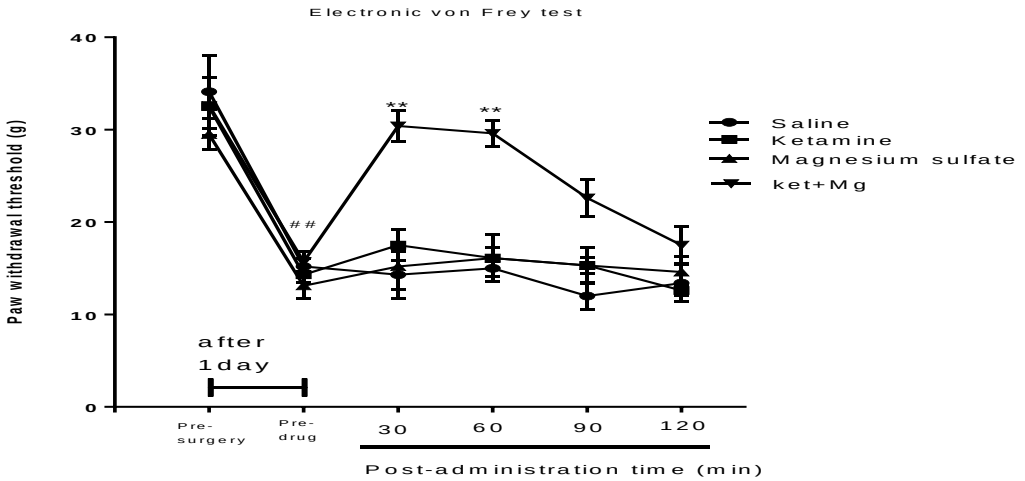

Fig 2: The effects of ketamine, magnesium sulfate and their combination on electronic von Frey test. Points indicate mean ±S.E.M. for n=10 in each group. ## and ** indicate highly significant difference compared to pre-incision group and ketamine-treated group respectively.

Table 1: Effect of various treatment on time spent on rotarod in groups of rats (n=10). No significant difference was observed between groups.

Treatment	Time spent on rotarod(s)
Saline	121.3±1.1
Ketamine 5 mg/kg i.p	125.1 ±0.9
Magnesium sulfate 5 mg/kg sc	122.1±0.7
Ketamine 5 mg/kg i.p +Magnesium sulfate 5 mg/kg sc	124.2±1.3

DISCUSSION

Post-operative pain is an important health-care issue, 50% of patients suffer from pain during the first days after surgery. Although, proper management of this pain facilitates patient's recovery and offers a better clinical outcome, the individual variability for pain sensation, side effects of analgesics and the specific mechanisms of post-operative pain make its optimum treatment remains mysterious.[12] Recently, neurophysiological and pharmacological models of incisional pain become increasingly important for the exploration of the pathophysiology of incisional pain as well as the evaluation of new therapeutic strategies.[13] These animal

models have advanced our knowledge and emphasize a comprehensive overview of the mechanisms involved in the processing of postoperative pain.[14]

The present study demonstrates that surgical incision of the planter surface of the rat hind paw caused a significant mechanical hyperalgesia and allodynia as measured by Randall-Selitto and electronic von Frey. This hypersensitivity is in accordance with previous studies conducted by Whiteside et al., 2004[8] and Brenann et al., 1996.[15] Manifestations of postoperative pain include primary and secondary hyperalgesia. Primary hyperalgesia occurs due to peripheral sensitization of nociceptors at the site of tissue injury, while secondary hyperalgesia is due to central sensitization (occurs only to mechanical stimuli and applied to uninjuried tissues surrounding the wound).[16] The neurochemical and electrophysiological mechanisms that subserve postoperative pain still unclear.[17] Pogatzki et al., 2001[18] have attributed mechanical hyperalgesia to the peripheral sensitization of Aδ and C nociceptors. These fibres increase their receptive field (RF), more fibres become activated and spontaneous activity of fibres occur. This converts the Aδ mechanically insensitive to mechanically sensitive fibres. Other studies attributed this pain to the increase in the spontaneous activity of spinal dorsal horn neurons after skin incision.[19] Additional studies showed that skin incision caused an increased lactate concentration and low PH which cause ischemic pain.[20]

Zahn et al, 1998[21] concluded that the role of AMPA receptors in post-operative pain can't be excluded in hyperalgesia and spinal sensitization as their antagonists produce decrease in pain behaviour. Vadivelu et al., 2010[1] have reported that activation of NMDA receptors by skin incisions leads to clinical hyperalgesia (immediate), central sensitization, synaptic plasticity and long term modifications (persistent pain). NMDA receptors are present in the spinal cord and supraspinal areas, their activation leads to excessive neuronal Ca^{++} ion influx and long term potentiation of pain that increases the excitatory postsynaptic potentials involved in chronic pain.[22] These findings must promote us to assess NMDA-receptor antagonists in experimental models of incisional pain.

To date, many studies have been conducted to optimize pain management in the perioperative period. However, combinations of two analgesics or more couldn't relieve pain in many clinical trials.[23] This study tries to explore the type of interaction between two NMDA antagonists, ketamine and magnesium sulfate in an incisional model of pain. This study revealed that although each of these agents was not an effective antinociceptive agent against incisional pain in the assigned doses when given alone, their combination resulted in a synergestic inhibition of nociception. At the employed doses, their combination yields a significant increase in pain threshold. In harmony with this finding, a study conducted by Savic Vujouvic, 2015[5] reported that either magnesium sulfate (5mg/kg) or ketamine (5mg/kg) didn't elicit any analgesic effects in the tail immersion test. However, their concurrent administration caused a significant elongation of tail immersion latency. Such synergism between ketamine and magnesium sulfate is expected as each of them blocks NMDA receptors by a distinct mechanism. In neuronal tissues, magnesium sulfate is a potent voltage-gated physiological and pharmacological blocker of NMDA receptors.[24] After nociceptive peripheral stimulation, magnesium modulates the release and action of glutamate, substance P and CGRP in the spinal cord. Stimulation of NMDA receptors by glutamate is very important for both acute and chronic pain.[25] Kroin et al., 2000[26] showed that intrathecal magnesium sulfate can potentiates morphine analgesia in an incisional model of pain. Srebro et al., 2014[27] have reported that nitric oxide pathway may be involved in the antihyperalgesic effect of magnesium sulfate against inflammatory pain. Ketamine is a non-competitive NMDA- receptor antagonist. However, it interacts with many biological systems and has multiple pharmacological effects. Ketamine is an opioid receptors agonist,[28] activator of the monoaminergic descending inhibitory pathway,[29] modulator of proinflammatory cytokines production[30] and causes adenosine release through its interaction with the purinergic system.[31] Although many studies have reported that NMDA-receptor antagonists cause CNS side effects,[32] our study revealed that magnesium sulfate and ketamine at the assigned doses didn't cause motor impairment, somnolence or dizziness. This is supported by previous findings that low doses of magnesium sulfate or ketamine don't induce adverse effects in rats.[33,34] Here, we have to add that no pharmacokinetic interaction between magnesium and ketamine has been reported.[35] Furthermore, as Savic Vajovic et al, 2015[5] have reported a discrepancy in the antinociceptive effects of ketamine and magnesium sulphate by changing their doses or their order of administration, differences in doses and order of magnesium sulfate and ketamine administration must be considered.

CONCLUSION

This study demonstrates that ketamine/magnesium sulfate combination could exhibit a synergestic inhibition of nociception in a rat model of incisional pain without motor impairment.

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