Pharmacological Reports
2012, 64, 299–304
ISSN 1734-1140

Antinociception by metoclopramide, ketamine and their combinations in mice

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Abstract:
Background: Metoclopramide is a centrally acting antiemetic and ketamine is a general anesthetic used with sedatives, tranquilizers and analgesics. Metoclopramide has analgesic effects and its combination with ketamine causes sedation and hypnosis. The contribution of metoclopramide to the analgesic effect of ketamine is not known. The purpose of the present study was to explore the analgesic effects of metoclopramide and ketamine alone or in combination in mice.
Methods: The up-and-down method was used to determine the median effective analgesic dosages (ED_{50}s) of metoclopramide and ketamine administered intraperitoneally (*ip*) either alone or concomitantly in male albino Swiss mice. Analgesia was measured by using a hot plate. The ED_{50}s of both drugs were analyzed isobolographically to determine the type of interaction between them. The analgesic effect of metoclopramide-ketamine combination (62.3 and 4.3 mg/kg, *ip*) was also monitored by the hot plate and acetic acid writhing methods.
Results: The analgesic ED_{50}s for metoclopramide and ketamine in mice were 30.15 and 2.15 mg/kg, *ip*, respectively. Concomitant administration of the drugs reduced their ED_{50}s to 10.17 and 0.68 mg/kg, *ip*, respectively. Isobolographic analysis of these ED_{50}s for both drugs revealed synergistic analgesic effect. Further, the combination of the drugs was effective analgesic as seen by the hot plate test and by another analgesic test paradigm, the acetic acid-induced writhings in mice.
Conclusions: The data suggest that the combination of metoclopramide and ketamine synergistically controls acute pain in mice. This combination could be used clinically for restraint and minor surgical interventions in mice.

Key words:
analgesia, hot plate, isobolographic analysis, ketamine, metoclopramide, writhing

Introduction

Metoclopramide is a centrally acting antiemetic and gastroprokinetic agent used in man [10, 32] and animals [2, 21] by antagonizing dopamine D_2-receptors. The drug also has serotonergic effects [12] and indirect cholinergic activity [4]. Sedation as a centrally induced depressant effect of metoclopramide has been reported in man [30] and chickens [3, 25]. Although the possible analgesic effect of metoclopramide is still under debate [16, 24], several studies have reported analgesic action of the drug in man [6, 20], rats [5] and mice [31].

Ketamine is an N-methyl-D-aspartate receptor antagonist used in man [32] and animals [2] as a general anesthetic agent usually in combination with sedatives, tranquilizers and analgesics. Ketamine is also a restraining agent in combination with sedative analgesics in animals [7, 35]. Further, ketamine possesses anticonvulsant activity in mice [37] and chicks [33]

and interests are increasing on the effects of subanesthetic doses of ketamine as it can be abused [17]. Recent studies reported the hypnotic effect of metoclopramide alone [3] or in combination with ketamine [25] in chicks. The latter two studies unequivocally demonstrated the central nervous system depressant action of metoclopramide in chicks [3, 25]. Further, it has been shown that the combination of metoclopramide with ketamine might be a potential restraining agent in the avian species, as metoclopramide at non-hypnotic doses of 5 and 10 mg/kg, subcutaneously (*sc*) given with ketamine at a non-hypnotic dose too (5 mg/kg, *sc*) induced loss of righting reflex in 83% and 100% of the chicks for 19 and 36 min, respectively [25]. However, in our previous studies, we did not attempt to measure potential analgesic effects of metoclopramide and ketamine either alone or in combination in chicks [3, 25]. The contribution of metoclopramide to the analgesic effect of ketamine is, therefore, not yet clear. The purpose of the present study was to further explore the analgesic effect of metoclopramide and ketamine combination in mice. The models of pain induction used were the hot plate and chemical-induced writhing, which are predictive of acute pain responses with involvement of central and peripheral systems, respectively [1, 9, 19, 23].

Materials and Methods

Male albino Swiss mice weighing 20–31 g were housed at a temperature of 20 ± 2°C and 10/14 h light/dark cycle, with water and food *ad libitum*. All experiments complied with institutional regulations addressing animal use, and the mice received proper attention and humane care. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul has reviewed and approved the protocol of this study.

The required doses of metoclopramide HCl (Yuhan Corp., Seoul, Republic of Korea) was dissolved in saline solution. Ketamine (50 mg/ml injectable solution, Yuhan Corp.) was further diluted in saline solution to obtain the desired concentrations of the drug. The volume of administration of each drug was at 10 ml/kg body weight given intraperitoneally (*ip*). Two experimenters simultaneously observed the responses of the mice during the experiments which were conducted between 9–11 a.m.

The up-and-down method [11] was used to determine the median effective analgesic dosages (ED_{50}s) of metoclopramide and ketamine (administered either alone, or concomitantly – metoclopramide followed immediately with ketamine) in mice. The initial dose of metoclopramide was at 20 mg/kg, *ip* whereas that of ketamine was at 2 mg/kg, *ip*. In the combination experiment, the initial dosages of metoclopramide and ketamine were 7.5 and 0.5 mg/kg, *ip*, respectively. We based our choices for metoclopramide and ketamine dosages on preliminary experiments in mice, as well as on previous studies [3, 8, 25]. Analgesia was measured by the thermal method using a hot plate (Panlab, S.I.U., Cornella, Spain) held at a temperature of 56°C. Mice were individually placed on the hot plate 15 min after the drug administration and the latency time to the first hind paw removal/licking and/or jumping response was measured [1, 23]. The cutoff point latency for the induction of analgesia was equal to or more than 6 s (i.e., positive analgesic response), and the maximum time allowed for the animal to stay on the hot plate was 20 s to prevent tissue damage.

The ED_{50}s of both drugs were subjected to isobolographic analysis to determine the type of interaction involved in the administration of metoclopramide and ketamine concomitantly [13, 25, 29, 36]. A straight line was drawn for the isobolographic analysis between isoeffective analgesic doses (ED_{50}) of metoclopramide and ketamine given to the mice either alone or in combination. The ED_{50} points of metoclopramide and ketamine given alone are represented on the x- and y-axes, respectively. The straight diagonal line indicates the line of additivity (zero interaction) at the ED_{50} values, and the location of the combination points on the left (below) and right (above) sides of the additive line indicates synergistic and antagonistic interactions, respectively [13, 25, 36]. The interaction index was calculated by the equation da/Da + db/Db [36]. Da and Db are the individual ED_{50}s of metoclopramide and ketamine for the induction of analgesia, respectively, whereas da and db are their combined ED_{50}s for causing analgesia. An interaction index of 1 means additivity (no interaction), < 1 synergy and > 1 antagonism [25, 36].

To further examine the potential analgesic effect of metoclopramide-ketamine combination (by doubling the ED_{50}s of both drugs given to mice), additional ex-

Tab. 1. Median effective doses (ED_{50}) of metoclopramide and ketamine administered *ip* alone or concomitantly for induction of analgesia in the hot plate test in mice

Variable	Metoclopramide	Ketamine	Metoclopramide and ketamine	
			Metoclopramide	Ketamine
ED_{50}	31.15 mg/kg	2.15 mg/kg	10.17 mg/kg	0.68 mg/kg
Range of the doses used	35 – 20 = 15 mg/kg	2.5 – 1.5 = 1 mg/kg	15 – 3.75 = 11.25 mg/kg	1.0 – 0.25 = 0.75 mg/kg
Initial dose	20 mg/kg	2 mg/kg	7.5 mg/kg	0.5 mg/kg
Last dose	35 mg/kg	2 mg/kg	15 mg/kg	1.0 mg/kg
Number of mice used	6 (○○X○○○)[a]	5 (X○○X○)[a]	5 (X○○○X)[a]	5 (X○○○X)[a]
Increase or decrease in the dose	5 mg/kg	0.5 mg/kg	3.75 mg/kg	0.25 mg/kg

[a]X – analgesia; O – no analgesia. The ED_{50}s were determined by the up-and-down method [11]

periments were conducted to measure the analgesic effect of the combination 15 min after the administration by the thermal (hot plate) and chemical methods [1, 23]. These experiments were conducted on separate groups of mice. In the thermal method, latency time to the first hind paw removal/licking and/or jumping response of the mouse was measured. In the chemical method, 0.1 ml of 0.1% acetic acid was injected *ip* 15 min after the administration of ketamine-metoclopramide combination, and the number of writhing responses during 30-min period after the acetic acid administration was recorded. In all the experiments, each animal was subjected to thermal or chemical analgesic tests only once. The data of analgesic responses were statistically analyzed by Student's unpaired *t*-test, and those of the chemical one were additionally subjected to Mann Whitney U-test [28]. The level of statistical significance was at $p < 0.05$.

Results

The ED_{50}s for metoclopramide- and ketamine-induced analgesia in mice, as determined by the up-and-down method, were 30.15 and 2.15 mg/kg, *ip*, respectively (Tab. 1). The metoclopramide-treated mice appeared to be docile and slightly sedated. However, concomitant administration of metoclopramide and ketamine markedly reduced the analgesic ED_{50}s for metoclopramide and ketamine in the mice to 10.17 and 0.68 mg/kg, *ip*, respectively (Tab. 1). Isobolographic analysis of these ED_{50}s for both drugs (either alone or in combination) revealed that combined administration of the drugs has a synergistic effect on the induction of analgesia in mice (Fig. 1). This synergistic effect was indicated by the location of the points representing the combined analgesic ED_{50}s of metoclopramide and ketamine below the diagonal line that connect their isoeffective analgesic doses (ED_{50}s) given alone (Fig. 1). Further, the calculated interaction index for analgesia was 0.65 indicating a synergistic interactions between both drugs (an index of < 1 indicates synergy).

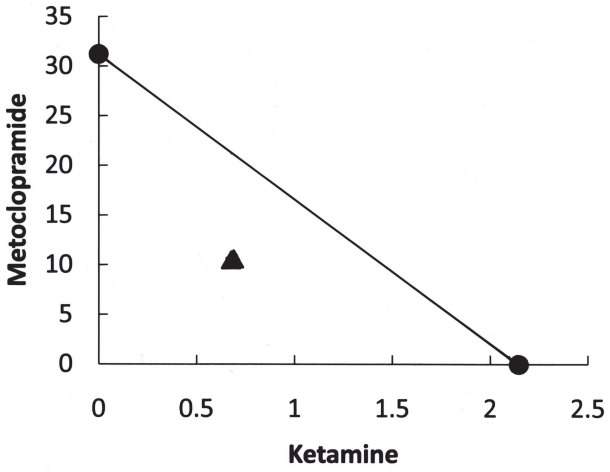

Fig. 1. Isobolographic analysis of the interaction of metoclopramide and ketamine at the analgesic ED_{50}s in mice. The diagonal line joining the individual ED_{50}s is the additivity line with no interaction, and the triangular point on the left (below) the additive line indicates synergistic analgesic interaction. Points on x- and y-axes represent isoeffective (analgesic ED_{50}) doses (mg/kg, *ip*) of ketamine and metoclopramide given alone, whereas the triangular point represents isoeffective combination of both drugs

Tab. 2. Metoclopramide (62.3 mg/kg) and ketamine (4.3 mg/kg) analgesia following concurrent administration in mice

Analgesic test	Saline-control	Analgesic combination
Hot plate (latency in s)	4.1 ± 0.6	8.0 ± 1.5*
Acetic acid writhings/30 min	10.5 ± 3.3 (median = 10)	1.6 ± 0.8* (median = 0.5)*

Values are the mean ± SE of 8 mice/test group. Analgesic tests were assessed in mice 15 min after the concurrent administration of the drugs *ip*. * Significantly different from the respective control value, $p < 0.05$

To further demonstrate the analgesic effect of metoclopramide and ketamine combination in two experimental paradigms of analgesia, the drugs were administered to mice at dosages of 62.3 and 4.3 mg/kg, *ip*, respectively (doubling the individual ED_{50}s). The combination significantly increased the latency time to the first pain response in mice, and decreased their writhing responses compared to respective control groups (Tab. 2).

Discussion

Metoclopramide either alone or in combination with ketamine produced significant analgesic effects (on the hot plate) in mice. This individual action of metoclopramide is in accordance with the reported analgesic effect of the drug in mice and rats [5, 31]. The pharmacological actions of metoclopramide are mediated centrally by antagonizing dopamine D_2 receptors [2, 10, 21, 32]. The drug also possesses serotonergic effects [12] and indirect cholinergic activity [4]. It has been suggested that the analgesic effect of metoclopramide is mediated through the release of prolactin hormone [31]. This action could also be mediated centrally as most of the pharmacological effects of metoclopramide such as anti-emesis and sedation are of central origin [2, 3, 10, 21, 25, 32]. The sedative and hypnotic effects of metoclopramide have been explored in chicks and it was found that its combination with ketamine produces synergistic hypnotic effect [25]. The studies showing the central depressant actions of metoclopramide in chicks, however, did not monitor any potential analgesic effect of the drug in this animal species [3, 25]. Hence, our present study further adds to and extends the sedative and hypnotic actions of metoclopramide in chicks to include analgesia too. It is also possible that sedation could contribute to the analgesic effect of the drug, as the metoclopramide-treated mice in the present study appeared to be docile and slightly sedated. However, several studies in man suspect that metoclopramide could be an analgesic agent alone [16, 24]. Such differences could be attributed to species variations, dosage employed and the experimental protocol for pain assessment [5, 6, 16, 20, 24, 31].

Ketamine acts *via* antagonizing N-methyl-D-aspartate receptors to induce analgesia and anesthesia in man and animals [2, 7, 32, 35]. Ketamine also induces analgesia by interacting with the supraspinal μ-opioid receptors [34]. In this context, ketamine enhances opioid-induced analgesic signaling by modulating phosphorylation in cells that endogenously express μ-opioid receptors [15]. Subanesthetic doses of ketamine induce analgesia [8], as found in the present study, modify motor performance in laboratory animals [18, 22, 26] and affect the mood in man [14, 17, 27].

The present study further explored the potential analgesic effect of metoclopramide in combination with ketamine as such an effect has not been reported before. In the present study, the analgesic effect of combined administration of metoclopramide and ketamine was found to be synergistic as substantiated by the isobolographic analysis of the individual and combined ED_{50}s of both drugs for the induction of analgesia, which yielded an interaction index of < 1 (0.65). This result further supports the notion of synergy between both drugs in inducing sedation and hypnosis in chicks [25]. However, it should be stressed that this type of interaction is only limited to the ratios of drug dosages used and the determined ED_{50} values in the present study; the conclusion cannot be extended to other combination levels [25, 29, 36]. The calculated individual ED_{50}s for metoclopramide and ketamine are within the analgesic dosage ranges reported by others in mice [8, 31]. Ketamine was also found to act additively with morphine or fentanyl as an analgesic combination in acute pain model (tail flick) in mice [8].

Further examination of the analgesic property of metoclopramide and ketamine combination by the hot plate and chemical methods, also confirmed the analgesic effectiveness of this combination at the dosages used (Tab. 2). The pain inducing models used in the present study vary from each other and they reflect the central (hot plate test) and peripheral (writhing

test) mechanisms involved in pain generation and recognition [1, 8, 9, 23]. Our data, therefore, suggest that the combination of metoclopramide and ketamine, at doses that could be used clinically in mice, controls the pain by both central and peripheral mechanisms. The limitation of the study was that the locomotor and central depressant effects of metoclopramide, ketamine and their combination were not tested in mice. Both drugs modify behavioral responses in several animal species [3, 18, 22, 25, 26] and man [14, 17, 27, 30].The use of metoclopramide with ketamine for restraint and minor surgical interventions in mice and other rodent species deserves further clinical exploitation.

Acknowledgment:
This study was supported by the College of Veterinary Medicine, University of Mosul, Iraq.

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Received: August 1, 2011; **in the revised form:** October 28, 2011; **accepted:** December 2, 2011.