

## Evaluation of the antinociceptive effect of xylazine and its interaction with metoclopramide in the acute pain model in mice

Kh.A. Shaban<sup>1</sup>, M.H. Ibrahim<sup>2</sup> and G.A. Faris<sup>3</sup>

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, Mosul University, Mosul, Iraq  
Email: <sup>1</sup>akhalid18@yahoo.com, <sup>2</sup>munaalzubaidy77@yahoo.com, <sup>3</sup>ghadafaris2018@gmail.com

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### Abstract

The study was designed to qualitative and quantitative evaluation of the antinociceptive effect of metoclopramide and xylazine each alone or as a concomitant administration in mice. Adult albino Swiss mice weighing 20-30 mg used in all experiments. By using the hot plate test, the individual analgesic dose (ED<sub>50</sub>) of metoclopramide and xylazine detected depending on the up and down method. Isobolographic analysis used to evaluate the type of interaction between two drugs at the ratio 0.5:0.5 of individual ED<sub>50</sub> for each drug at the level of antinociception effect. Simultaneously administration of the double dose of individual ED<sub>50</sub> and low doses (sedative, non-analgesic doses) of both drugs, also evaluated at the level of central and visceral analgesia using a hot plate and writhing response test respectively. The individual ED<sub>50</sub> of xylazine and metoclopramide was 10.8 and 25.6mg/kg IP respectively. A synergistic interaction at the level of analgesia explored between two drugs at ratio 0.5:0.5 which represented as decreased in ED<sub>50</sub> of metoclopramide and xylazine by 58.75 and 58.15% respectively. The animal suffered from only slight sedation and docile. Simultaneously IP administration of xylazine and metoclopramide at double dose of ED<sub>50</sub> for each drug-induced significant increase in latency time of thermal response, as well as a significant decrease in writhes number, which induced by acetic acid in comparison with control groups. The percentage of analgesia at sub analgesic doses of a concomitant administration of both drugs was 100% in comparison with each drug alone. These results suggested safe and good use of both drugs in veterinary medicine.

**Keywords:** Xylazine, Metoclopramide, Analgesia, Hot plate, Acetic acid

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### تقييم التأثير المسكن للألم للزيبلازين وتداخله مع الميتوكلوبرومايد في نموذج الألم الحاد في الفئران

خالد احمد شعبان، منى حازم البراهيم و عادة عبدالمنعم فارس

فرع الفلسفة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

صُمِّمَت الدراسة لتقييم الفعل المسكن لعقار الميتوكلوبرومايد والزيلازين تقميما كميما ونوعيا عند إعطائهما كل لوحده أو معا للحيوان نفسه (الفئران). استخدمت فئران بيضاء سويسرية بالغة تزن 20-30 غم في كل التجارب. باستخدام اختبار الصفيحة الساخنة تم تحديد الجرعة الفاعلة المسكنة الوسطية جم 50 لعقار الزيبلازين والميتوكلوبرومايد كل لوحده اعتمادا على طريقة الصعود والنزول في الجرعة واستخدم تحليل الايزوبولوجرافي لتقييم نوع التداخل الدوائي بين العقارين عند نسبة 0,5:0,5 من الجرعة الفاعلة المسكنة الوسطية لكل عقار على مستوى التسكين من الألم. فضلا عن تقييم إعطاء العقارين بالجرع المضاعفة للجرعة المسكنة الوسطية لكل عقار وبالجرع المسدرة (غير المسكنة) على مستوى التسكين من الألم المركزي والحشوي باستخدام اختبار الصفيحة الساخنة واختبار التلوي على التوالي. كانت الجرعة الفاعلة المسكنة الوسطية للميتوكلوبرومايد والزيلازين 10,8 و 25,6 ملغم/كغم من وزن الجسم بالحقن داخل الخلب على التوالي. كان التداخل بين العقارين تأزرا عند نسبة 0,5:0,5 من الجرعة الفاعلة الوسطية لكل عقار والذي تمثل بالانخفاض

المعنوي في قيمة الجرعة لعقار الزيلازين والميتوكلوبرومايد وبنسبة ٥٨,١٥ و ٥٨,٧٥% على التوالي. ولم تعاني الحيوانات المعاملة بالعقارين معا بهذه الجرعة أي آثار جانبية فيما عدا التسدير البسيط وقلّة الحركة. أدى حقن العقارين معا في الخلب بالجرع المضاعفة لكل عقار الى زيادة معنوية في مدة بقاء الحيوان على الصفيحة الساخنة فضلا عن الانخفاض المعنوي في عدد مرات التلوي المحدث بحامض الخليك مقارنة مع مجموعة السيطرة. كانت النسبة المئوية للتسكين عند الجرع تحت المسكنة ١٠٠% مقارنة مع إعطاء كل عقار لوحده. اثبتت النتائج استخدام جيد وأمين لهذين العقارين في مجال الطب البيطري.

## Introduction

Metoclopramide is a benzamine derivative (1), used in humans and animals (2,3), as centrally acting antiemetic and reduces stomach movement. These effects related to its centrally antagonizing of dopamine D<sub>2</sub>-receptors antagonist, and serotonergic effects 5HT<sub>3</sub> (4). Indirect cholinergic action also involved in this mechanism (5). The analgesic effect of metoclopramide was reported recently in humans (6,7), dogs (8), rats and mice (9). The mechanism of antinociception effect by metoclopramide remain is unknown yet. Xylazine is one of alpha-2 adrenergic receptor agonists, widely used in veterinary practice as sedative, analgesic and muscle relaxant (10,11). Xylazine also can be used as pre-anesthetic with ketamine to produce balance anesthetic (12,13). Different combinations of xylazine with other analgesic were evaluated in different animal models to improve the quality of analgesic properties and minimizing the side effects. Combination of xylazine with dipyrone (14) or tramadol (15) in mice and in chicks (16) or with chlorpromazine in sheep (17) and chicks (18), produced safe and effective analgesia at low doses of each drug (synergistic antinociceptive effect).

However, the combination of xylazine and metoclopramide was not evaluated in previous studies, so the purpose of current study was to qualitative and quantitative evaluation of the analgesic effect of metoclopramide and xylazine each alone or as a combination. the type of interaction between two drugs also evaluated in mice by using two models of acute pain induction, hot plate (central) and writhing response (peripheral) (19,20).

## Materials and methods

### Animals

All experiments were carried out by using male and female albino Swiss mice weighing 20-30 g. The animals were housed in the animal house of the college of veterinary medicine, University of Mosul. They were maintained under standard conditions with 10/14 hrs. light/dark cycle, at 22±2°C room temperature. Food and water available at the laboratory. The scientific committee of the department of physiology, biochemistry and pharmacology at the college of veterinary medicine has reviewed and approved the protocol of this study. The

doses of metoclopramide (pure powder Vainkunth Co., India) and xylazine (20 mg/ml injectable solution, Holland) were dissolved in physiological saline solution at volume of injection 5ml/kg body weight, IP

### Methods

By using the up-and-down method (21) the median effective analgesic doses of metoclopramide and xylazine (alone or as a combination) were detected in mice by using a hot plate (thermal method). The initial dose was determined based on experience and previous studies (22,23). The increasing and decreasing in the later dose of each drug was at a constant value. The analgesic effect was assessed by placing the mice on a hot plate (Heidolph Me, Germany, Hei-standard Co. at 56°C), the time between placing the mice on the hot plate and licking of fore or hind paw or/and jumping off from the surface was recorded as the response in seconds. Only mice that response to nociceptive response within 30 seconds was used in this study, the cut off time was 30 seconds to avoid any thermal injury to the paw (19), animals were used only once. The response was measured before (baseline) and at 15 minutes after injected of each drug doses.

### Analgesic interaction between metoclopramide and xylazine

In order to evaluate the type of interaction between metoclopramide and xylazine. The first animal was simultaneously injected with initial dose of metoclopramide and xylazine at 12.8 mg/kg i.p and 5.4 mg/kg i.p respectively at ratio 0.5:0.5 of ED<sub>50</sub> for each drug. The type of interaction was detected by isobolographic analysis and Y interaction index (24-26). A straight line drawled between the ED<sub>50</sub> of metoclopramide (pointed on x axis) and that for xylazine (pointed on y axis). The ED<sub>50</sub> value of combination (0.5:0.5) also pointed, when this value detects under the straight diagonal line, the interaction is synergistic, while falling this value on/and above the line, the interaction are additive and antagonized respectively. The addition interaction index was calculated by using equation:  $Y = \frac{da}{Da} + \frac{db}{Db}$ .

Where: (Da and Db) are individual ED<sub>50</sub> of metoclopramide and xylazine respectively, while the (da and db) represent the combination doses that produce the same effect (analgesic). When the value of Y =1 its additive

(no interaction),  $Y < 1$  synergism (supra additive) and  $Y > 1$  its antagonism (sub additive), this is an interaction index.

**Acetic acid inducing writhing reflex (chemical test) and hot plate (thermal test) at double doses of ED<sub>50</sub> of metoclopramide and xylazine**

Mice were used in this experimental divided into four groups (5 animals for each group), group I and III injected with normal saline while group II and IV injected with metoclopramide and xylazine at 51.2mg/kg b.w i.p and 21.6mg/kg b.w i.p respectively (double dose of ED<sub>50</sub>).

Animals in group I and II placed on hot plate before and 15 minute after injection of normal saline and drugs for calculation of latency time, while group III and IV were received normal saline and drugs 15 minute before injected with 1% acetic acid at volume 0.1 ml/10g b.w i.p to induce writhing response and calculate number of writhing which recorded within 30minute after injection of acetic acid (27).

**Estimation percentage of analgesia of pain in subanalgesic doses of metoclopramide and xylazine each alone or in combination by thermal method (hot plate)**

In this experimental, mice divided into three groups, each group consist of 5 animals, group I injected with metoclopramide 10 mg/kg b.w i.p, group II injected with xylazine 2mg/kg b.w i.p and group III injected with

metoclopramide and xylazine at 10 mg/kg b.w i.p and 2mg/kg b.w i.p respectively. animals placed on hot plate before and 15 minutes after injection of drugs for calculate of latency time (19).

**Data analysis**

The data for latency time (thermal test) were statistically subjected to unpaired t-test, while number of writhing were analyzed by Mann Whitney u-test, and for percentage of analgesia (hot plate) by fisher test (28). The level of significant was  $P < 0.05$ .

**Results**

In the present study the dose that produce 50% of analgesic effect in mice for metoclopramide and xylazine induced was 25.6 and 10.8 mg/kg, respectively by using up and down method (Table 1), while concomitant injected mice with a combination of metoclopramide and xylazine at ratio 0.5:0.5 of ED<sub>50</sub> for each, was markedly reduced the value of ED<sub>50</sub> to 10.56 and 4.52 mg/kg, respectively (Table 1). The mice that injected with metoclopramide and xylazine were suffered from signs of slight sedation and docile.

Table1: Determination of median effective doses of metoclopramide and xylazine

Variable	Metoclopramide	Xylazine	Metoclopramide and Xylazine at ratio 0.5:0.5	
			Metoclopramide	Xylazine
ED <sub>50</sub>	25.6 mg/kg	10.8 mg/kg	10.56 mg/kg	4.52 mg/kg
Range of doses used	20 - 30 mg/kg	10 - 20 mg/kg	9.6 - 12.8 mg/kg	4.15 - 5.4 mg/kg
Initial dose	20 mg/kg	20 mg/kg	12.8 mg/kg	5.4 mg/kg
Last dose	30 mg/kg	10 mg/kg	12.8 mg /kg	5.4 mg/kg
Number of mice used	5(OXOOX) <sup>a</sup>	5(XOXXX) <sup>a</sup>	5(XOXOX) <sup>a</sup>	5(XOXOX) <sup>a</sup>
Increase or decrease in dose	5 mg/kg	5 mg/kg	3.2 mg/kg	1.25 mg/kg
Percentage of decrease in ED <sub>50</sub>			58.75%	58.15%
Y value			0.83	0.83

<sup>a</sup>x - analgesia; o - no analgesia. The ED<sub>50</sub> were determined by the up and down method.

By using Isobolographic analysis, pointed the value of ED<sub>50s</sub> for each alone, then detected the situation of ED<sub>50</sub> value for a combination, this value was fallen down under the diagonal line that connect the ED<sub>50s</sub> for each drug alone that revealed a synergistic antinociceptive interaction in mice (Figure 1).

Furthermore, calculate the Y value from equation of interaction index, it was lower than 1 (0.83) that indicated the synergism interaction ( $Y < 1$ ).

In second experiment we evaluated the peripheral (chemical test of acetic acid) and central (thermal test) antinociception effect for further examine the synergistic

interaction between metoclopramide and xylazine given as combination (doubling of ED<sub>50</sub> for each drug), whereas significantly increased the latency time of thermal response and significant decreased in writhing number of acetic acids in compares with control groups (Table 2).

Evaluation of analgesia percentage of pain by administration of sub analgesic (sedated) dosages of metoclopramide (10 mg/kg i.p) and xylazine (2 mg/ kg i.p) each alone or in combination in mice. whereby was analgesia percentage of pain 0% when administered each drug alone while analgesia percentage of pain 100% when administered two drugs in combined form (Table 3).

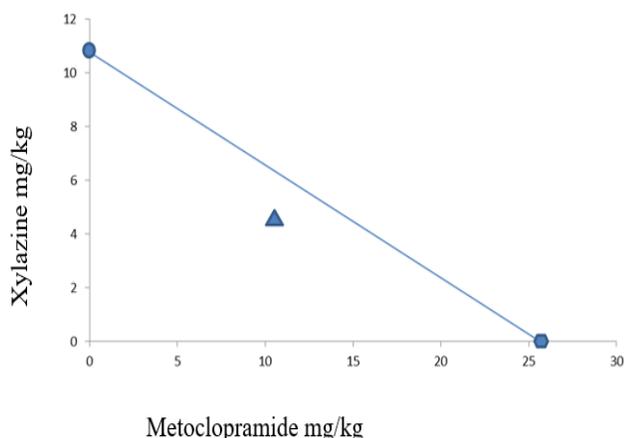


Figure 1: Isobolographic analysis of interaction between metoclopramide and xylazine at ratio 0.5:0.5 in mice. The diagonal line joints the individual ED<sub>50</sub>s of metoclopramide and that of xylazine, the triangle points which represent 0.5:0.5 of ED<sub>50</sub> combination of two drugs, located below the diagonal line, indicate synergism interaction. xylazine at dose 10.8mg/kg IP, metoclopramide at dose 25.6 mg/kg IP, xylazine and metoclopramide at ratio 0.5:0.5.

Table 2: Metoclopramide and xylazine analgesia following combined administration in mice

Analgesic test	Saline control	Analgesic combination
Hot plate (latency in seconds)	0.2 ± 0.2	11.6 ± 0.8 *
Acetic acid (Writhing /30 minutes)	43.5 ± 3.27	0.0 ± 0.0 *

Values are Mean ± SE of 5 mice / group. Analgesia evaluated in mice 15 minutes after administration of both drugs.

\* Significantly different from the respective control value at P < 0.05.

### Discussion

In this study we explored the antinociceptive effect of xylazine alone or as a combination with metoclopramide in mice. Xylazine is an alpha-2 agonist which induced analgesia by inhibit NE and dopamine reuptake in CNS, which in turn produce CNS depression (10,28). Metoclopramide is well known as antiemetic, but recently reported as analgesic in man (6,7), dog (8), rat (9) and mice, alone or as a combination with other drugs as ketamine (3), tramadol (29) and diphenhydramine (30).

Table 3: Percentage of analgesia for each drug alone or as a combination by using hot plate test

Drug and dose	Latency time (base line)	Latency time (after 15 minutes of injection)	percentage of analgesia (Hot plate test)
Metoclopramide 10 mg/kg IP	8.8±1.24	7.8±0.73	0%
Xylazine 2 mg/kg IP	8.2±1.01	7±0.70	0%
Metoclopramide 10 mg/kg IP and Xylazine 2 mg/kg IP	6.7±0.42	15.85±0.45 *	100% *

\* Significantly different from each drug alone (P < 0.05).

Dependent on up and down method (21), ED<sub>50</sub> for analgesia of metoclopramide and xylazine was evaluated using hot plate test, as a thermal test for detecting central pain (31), while visceral pain was detected using writhing test as chemical test (32). ED<sub>50</sub> of xylazine and metoclopramide was 10.8 and 25.6 mg/kg, IP respectively. Dependent on up and down method, this value was in agreement with previous reports for xylazine (13) and that for metoclopramide (3). The type of drug combination may be additive, synergism or antagonism (23-25).

The present study revealed that the type of interaction of xylazine and metoclopramide as a combination was synergistic at fixed ration 0.5:0.5 of individual ED<sub>50</sub> for each at the level of analgesia, we supported this finding by calculate the value of Y interaction index, which was lower than 1 (23-25), this finding similar to the previous studies

of other combinations as ketamine with metoclopramide (3), xylazine with tramadol (15) and metoclopramide with diphenhydramine (30) in mice, as all of them was synergistic interaction at level of analgesia. The synergistic interaction between xylazine and metoclopramide may be attributed to effect of each on different receptors (pharmacodynamics). Xylazine acts as strong central alpha 2 agonist via stimulating central α<sub>2</sub> pre-receptors leading to decrease release NE and dopamine in CNS (10,28), which induced synergistic analgesic effects with other drugs as tramadol (15) and dipyrone (14), while metoclopramide act as analgesic by effect on D<sub>2</sub>-receptors as antagonist, which related with opioid system without interaction with opioid receptors (33), metoclopramide also increase the level of prolactin hormone which related with opioid system (34,35). Other suggested mechanisms for analgesic effect of

metoclopramide that correlate with its interference with transport of Ca<sup>+</sup> cross the cell membrane (35,36), as well as act as serotonin agonist (4).

Pharmacokinetic may be suggested as another mechanism for explain the synergistic interaction between metoclopramide and xylazine, as metoclopramide metabolized in liver by cytochrome p450 2D6 (37), in addition metoclopramide inhibit same enzyme, this may prolong the duration of action of drug in the body (38-40). Xylazine also metabolized in the liver by cytochrome p450 3A (41) which inhibited by xylazine and then the duration of action of xylazine and metoclopramide well be increased. This suggested mechanism may also explain the visceral antinociceptive effect of the same combination at double ED<sub>50</sub> of each drug, this visceral analgesic effect in agreement with that previous studies as a combination of ketamine with metoclopramide (3) and metoclopramide with diphenhydramine (30) in mice. A good and safe analgesic effect without overt sedative effect also reported in this study using sedative non-analgesic doses of both drugs as a combination. This additional support the synergistic effect between two drugs by mechanism which may be similar to that explain above. All results in the present study especially the synergistic combination of both drugs at sedative doses not reported previously.

## Conclusion

In conclusion the present study suggests that the synergism combination between xylazine and metoclopramide at level of central and visceral analgesia in mice. The combination of both drugs at low doses produce good and safe analgesia, which can be useful in veterinary medicine.

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## Conflict of interests

The authors declares no conflict of interest

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