Synergism of the analgesic activities of tramadol with α_2 adrenoreceptor agonist xylazine in mice

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(Received December 29, 2011; Accepted September 16, 2012)

Abstract

The present study was undertaken to evaluate the antinociceptive interaction between tramadol and xylazine. The antinociceptive effect of intraperitoneal (I.P) administration of the drug alone or in combination was evaluated using the mouse hot-plate test. Administration of tramadol in fixed dose (20mg\kg) I.P with different doses of xylazine (0, 2, 4, 8) mg\kg I.P significantly referred synergism of the antinociceptive effect of tramadol depending on the dose of xylazine. There was an increased the antinociceptive maximum possible effect (%MPE) from (27.08%) to (85.4%). The effective dose that produce 50% antinociceptive (ED50) were evaluated for each drug alone or in combination. The present study found that the administration of tramadol and xylazine markedly reduced the median effective dose (ED50) of both drugs for antinociceptive effect in mice. The result of this study demonstrated that there was synergism (super-additive) interaction between tramadol and xylazine.

***Keywords*:** Tramadol, Xylazine, Antinociception, Opioid, α2- adrenoreceptor, Isobolographic analysis.
Available online at http://www.vetmedmosul.org/ijvs

تأزر الفعالية المسكنة للترامادول مع متقبل الفا-٢ الادرينالي الزايلزين في الفئران

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الخلاصة

تهدف الدراسة الى تقييم التأثير المسكن للتداخل الدوائي بين الترامادول والزايلازين. حيث تم اجراء الدراسة عن طريق قياس الفعل المسكن لكل من الترامادول والزايلازين المعطاة في الخلب باستخدام اختبار الصفيحة الساخنة في الفئران. ادى اعطاء الترامادول بجرعة ثابتة (٢٠ ملغم/ كغم) بالخلب، مع جرعات مختلفة من الزايلازين (٠، ٢، ٤، ٨ ملغم/كغم) بالخلب، الى تقوية الفعل المسكن للترامادول معنويا وبالاعتماد على زيادة جرعة الزايلازين. وقد لوحظ ان هناك زيادة في النسبة المئوية للتسكين (%MPE) من (٢٧,٠٨%) الى (٨٥,٤%). كما وتم قيا س الجرعة المسكنة الوسطية (الجم ٥٠) عند اعطاء كل دواء لوحده او عند اعطا ئهما معا، حيث توصلت الدراسة الى ان اعطاء الترامادول مع الزايلازين قد سجل انخفاضا ملحوظا في الجرعة المسكنة الوسطية للدوائين كلاهما في الفئران. لقد اظهرت النتائج ان التداخل بين الترامادول والزايلازين هو تأزري.

Introduction

Xylazine and tramadol are both widely used to treat moderate and severe pain (1,2). Xylazine is an α_2 adrenergic receptor agonist that produces sedation, analgesia, and muscle relaxation with administrated parentrally to animals (3). The mechanism of action of xylazine to produce antinociceptive is effected by its activation presynaptic α_2 receptor, thus lead to decrease the amount of noradrenaline and dopamine, therefore lead to depression of the brain (4,5).

Tramadol is a centrally acting synthetic analgesic drug with both opioid and non opioid properties (6,7). The antinociceptive effect of tramadol result from dual mechanism of action, first; the affinity of tramadol for μ-opioid receptor and the second; tramadol has inhibition of

the presynaptic reuptake of norepinephrine and serotonin. The inhibitory effect contributing to analgesic effect of tramadol by inhibiting pain transmission in the central nervous system (7,8).

In case of different analgesics combination, the method of achieving one or more therapeutic aim will be managed. This will result in facilitate the compliance of patient, prescription will be more simplified, the efficacy will have more improved without increasing side effects of analgesic drugs combination (9).

The α_2-agonists have an significant hypnotic interaction with others anesthetics and an analgesic interaction with opioid (10). However, the analgesic interaction of xylazine–tramadol have not been evaluated. Therefore the present study was to evaluate the effect of selective α_2 adrenoceptor agonist xylazine on tramadol antinociceptive effect and evaluation the type of interaction of these two drugs, using a hot-plate test in mice. Hot-plate test is a pain assessment method used in experimental animal to measurement the pain by using a thermal stimulus.

Materials and methods

Animal

Fifty five male albino mice weighing 25-30 g were used. The animals were housed at 22±2°C on a 12 h light\dark cycle and fed with standard diet and water ad libitum. The mice were obtained from animal care in Collage of Dentistry, University of Mosul, Iraq.

Measurement of analgesic activity

The median analgesic dose of tramadol and xylazine either individually or in combination were studied on mouse by Hot-plate test: Mice were placed on a hot-plate maintained at 55±1C^0. The response latency (analgesic activity) was evaluated on the basis of either fore or hind paw lick or jump reaction, following contact with the hot-plate (11).The ED50 for 2 drugs were determine according to Dixon table (12).

Evaluation the effect of different doses of xylazine on tramadol analgesia

The animals were divided randomly into 5 groups, each group consisted of six animals. The mice were injected with fixed dose of tramadol hydrochloride 5% (Mepha Ltd Aesh-Basel Swit-sland) at 20mg\kg IP and different doses of xylazine 2% (Holland, Castenray, Interchemra) (0,2,4,8 mg\kg IP). Xylazine in different doses were injected after 15 min of tramadol administration. The analgesic activity was evaluated according to the above (11) and the percentage of antinociceptive maximal possible effect (MPE) was calculated according to the formula (13)**:**

%MPE= Test latency - predrug latency/cut off time - predrug latency × 100.

MPE: Percentage of antinociception maximal possible effect. Test latency : sec after drug treatment.
Predrug latency : second before drug treatment at zero time.
Cut off time : 30 second.

Antinociceptive interaction between tramadol and xylazine

In the present study determined the median effective dose (ED50) of tramadol and xylazine each one alone by up and down method for antinociceptive activity (11). The interaction between 2 drugs were evaluated simultaneous administration of fixed ED50 ratio of tramadol with different ED50 ratio of xylazine, and performing an isobolographic analysis for the different combination as described by Tallarida et al (14).

In the present study fixed ratio were selected by combining the ED50 of tramadol and xylazine (1:1, 1:0.5) respectively. The ED50 point of tramadol is represent X axis while ED50 of xylazine is represent in Y axis. A diagonal line is drawn to join the isoeffective dose on the axis. Doses of drug combination producing the same effect are then plotted. Point falling on the diagonal line represent zero interaction (additively),while those located above and below are antagonistic and synergistic, respectively (15).

The interaction index was calculated using the following equation: (Y=a\A+b\B)

Where A and B are individual ED50 of tramadol and xylazine respectively and (a,b) are the combination doses that produce the same effect. An interaction index, if Y<1, it is supra-additive (Synergy); and if Y> 1 its sub-additive (antagonism).

Statistical analysis

The data were expressed as mean±SD, difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at P<0.05. (16)

Results

Antinociceptive effect of different doses of xylazine with tramadol in hot plate test

Systemic I.P administration of different doses of xylazine and tramadol produce a dose dependent antinociceptive effect in mice tested by hot-plate test assay. The latency time reaction was significantly increased represented by increasing the latency time from (12.5) sec in group treated with tramadol alone in comparison with others treated group with tramadol and xylazine together at

(20+4 mg\kg IP) and (20+4 mg\kg IP) to (19.75) (26.5)sec respectively. The increased in latency time proportional with increased the dose of xylazine (table 1). The percentage of pain inhibition, maximal possible effect (MPE), was significantly increased from (39.58 %) in low dose of xylazine to (85.4%) in high dose when fixed the dose of tramadol table (1) at P< 0.05.

Table 1: Antinociceptive effects of tramadol (fixed dose) alone and in combination with xylazine in the hot-plate test in mice.

Treatment groups	Latency time (seconds)	% Pain inhibition (%MPE)
Tramadol 20mg\kg IP + normal saline IP	12.5±3.1	27.08
Tramadol 20mg\kg IP+ Xylazine 2mg\kg IP	15.5±4.4	39.58 *
Tramadol 20mg\kg IP+ Xylazine 4mg\kg IP	19.7±3.5 *	57.08 * a
Tramadol 20mg\kg IP+ Xylazine 8mg\kg IP	26.5±6.2 *a b	85.40 * a b

Value are mean ± SE 6 mice /group, * Significantly different from the control group at P<0.05, a. Significantly different from tramadol 20mg\kg + xylazine 2mg\kg group at P<0.05, b. Significantly different from tramadol 20 mg\kg + xylazine 4mg\kg group at P<0.05.

Interaction of tramadol and α_2 adrenoreceptor:

The ED50 for tramadol and xylazine antinociceptive determined were 19.4 and 3.2 mg\kg IP respectively Table (2,3). However when coadministration Tramadol and xylazine reduced the ED50 for tramadol produce antinociceptive in the mice to (11.18) and (7.86) mg\kg IP after used in ratio (1:0.5) and (1:1) respectively Table (4, 5). The antinociceptive activity induced by coadministration of fixed ratio ED50 fraction of tramadol and xylazine was examined by isobolographic analysis after I.P administration. The ED50 values for the combination were shown in (table 6) and the type of interaction were shown in (figure 1and 2).

Table 2: Determination of median effective doses (ED50) of tramadol for antinociceptive effect in mice.

Variable	Result
ED50	19.4 mg\kg IP
Range of the doses used	18-20 mg\kg IP
Initial dose	20 mg\kg IP
Last dose	20 mg\kg IP
Number of mice used	5 (XOOXX)
Increase or decrease in the dose	2 mg\kg IP

The isobolographic analysis for combination tramadol and xylazine showed a synergistic antinociceptive interaction between two drugs, and the Y calculated from equation is appear lower than 1(Y<1), thus this indicated that interaction is synergy. Table (6).

Table 3: Determination of median effective doses (ED50) of for xylazine antinociceptive effect in mice.

Variable	Result
ED50	3.2 mg\kg IP
Range of the doses used	1 - 4 mg\kg IP
Initial dose	1 mg\kg IP
Last dose	4 mg\kg IP
Number of mice used	6 (OOXOOX)
Increase or decrease in the dose	1 mg\kg IP

Table 4: Determination of median effective doses (ED50) of interaction tramadol and xylazine in ratio (1:0.5).

Variable	Result for tramadol	Result for xylazine
ED50	11.18 mg\kg IP	0.23 mg\kg IP
Range of the doses used	10.4-19.4 mg\kg IP	0.1- 1.6 mg\kg IP
Initial dose	19.4 mg\kg IP	1.6 mg\kg IP
Last dose	13.4 mg\kg IP	0.6 mg\kg IP
Number of mice used	7 (XXXOXOX)	7 (XXXOXOX)
Increase or decrease in the dose	3 mg\kg IP	0.5 mg\kg IP

Table 5: Determination median effective doses (ED50) of interaction tramadol and xylazine in ratio (1:1)

Variable	Result for tramadol	Result for xylazine
ED50	7.86 mg\kg IP	1.28 mg\kg IP
Range of the doses used	7.4- 19.4 mg\kg IP	1.3 - 3.2 mg\kg IP
Initial dose	19.4 mg\kg IP	3.2 mg\kg IP
Last dose	7.4 mg\kg IP	1.2 mg\kg IP
Number of mice used	7 (XXXOXXX)	7 (XXXOXXX)
Increase or decrease in the dose	3 mg\kg IP	0.5 mg\kg IP

Table 6: The ED50 values for the interaction of tramadol and xylazine in ration (1:0.5, 1:1).

Parameters	Gp1	Gp2	Gp3	Gp4
Tramadol+ xylazine	T alone	X alone	T: X	T:X
Ratio	-	-	1:0.5	1:1
number on mice	5	6	7	7
ED50 mg\kg	19.4	3.2	19.4:1.6	19.4:3.2
$ED50_{mixing}$ mg\kg	-	-	11.18:0.23	7.86:1.28
Y(interaction index)	-	-	0.647	0.805

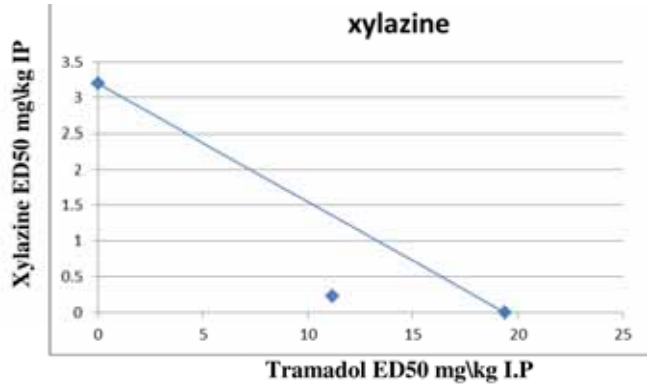

Figure 1: Isobolographic representation of the interaction between tramadol and xylazine in a ratio 1: 0.5 fixed potency ratio at the 50% level of response (ED50). The interaction of tramadol with xylazine demonstrates synergy.

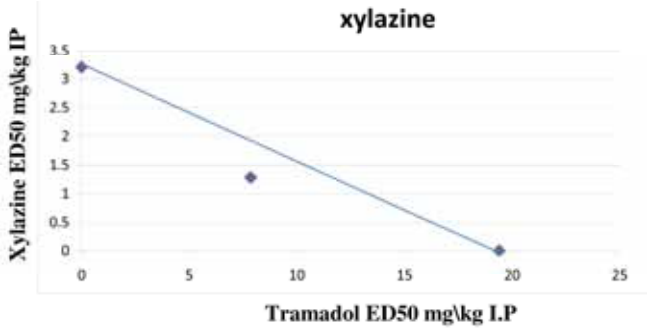

Figure 2: Isobolographic representation of the interaction between tramadol and xylazine in a ratio 1: 1 fixed potency ratio at the 50% level of response (ED50). The interaction of tramadol with xylazine demonstrates synergy.

Discussion

Many reports have been published on analgesic combination such as Ketoprofen with acetaminophen, Tramadol with acetaminophen (17,18). It has also been demonstrated that the combination of drug acting on different receptor may produce super or sub additive interaction in antinociceptive effects (15).

The results demonstrated that a synergism of the analgesic activity of opioid (tramadol) and α_2 adrenergic agonist (xylazine). The synergism antinociception effect between tramadol and xylazine may be because tramadol has weak opioid agonist with antinociceptive effects through its action on μ-receptor or by inhibiting the neuronal reuptake of both noradrenaline and serotonin (7). Many study demonstrated that tramadol has the antinociceptive effect through acting on descending noradrenergic pathway and this play a role in analgesic properties of the non opioid, stimulating this pathway produces antinociceptive effect from the activation of the spinal α_2 adrenergic receptor by noradrenergic neuron (19,20).

The α_2 adrenoreceptor agonist xylazine is widely used in veterinary practice as a sedative, analgesic and muscle relaxant (20). The analgesic effect of xylazine is produced by centrally activation of presynaptic α-$_2$ adrenoceptor, causing decrease in the release and turnover of noradrenaline and dopamine (4,5). Also, xylazine has other properties similar to that of opioid (2). Xylazine and other α_2 agonist such as detomidine, and medetomidine were potential the analgesic properties of opioid (10,22).

The alpha$_2$- agonist can induce analgesia by acting at three different locations: in brain and brain stem, spinal cord and in peripheral tissues. Alpha$_2$-adrenergic and opioidergic system have common effector mechanisms in locus coeruleus, probably through a common transduction mechanism, responding a supraspinal site of action (23). In the spinal cord, the analgesic action of alpha$_2$- agonist is likely related to activation of descending medullospinal noradrenergic pathway or to the reduction of spinal sympathetic outflow at presynaptic ganglionic sites. There is also significant interaction between opioid and alpha$_2$-agonists at the spinal cord level which contribute to the usefulness of this class of agent in the clinical setting (24).

The current results indicate that xylazine can synergistic the analgesic properties of opioid. The synergistic activities of α2 adrenoreceptor agonist could be measurement both in reduction the amount of opioid needed to reach particular level of analgesia with a fixed dose of the opioid. This result agreement with previous study suggested that the administration of fentanyl like opioid potentiate the analgesic properties of α_2 adrenoreceptor agonist (10).

A synergistic interaction between α_2 adrenoreceptor agonist and opioid has been demonstrated (25). The antinociceptive effect of spinal α_2 adrenoreceptor agonist seem to be a direct action on dorsal horn neuron on supraspinal and spinal sites and activate descending inhibitory system (26). Activation of α_1 and α_2

adrenoreceptor either located pre and\or post synaptic level are involved in the control of spinal antinociception (27).

Drugs that act at α2 adrenoreceptor and opioid receptor as agonist inhibit pain transmission in the spinal cord when administration together, agonists activating these receptor interact in synergistic manner (22). Previous study suggested that the administration of synergistic receptor pair α2 adrenoreceptor and opioid receptor on nociceptive fibers can produce analgesic synergy, this may be a result of interaction between neuronal G -protein coupled receptors (28). α2 adrenoreceptor coupled to sensitive inhibitory G –protein that causing inhibition of adenylase cyclase activity which result in decrease formation of CAMP, is an important consequence of α2-adrenoceptor activation (29).

Conclusion

The administration tramadol with α_2 adrenoreceptor xylazine demonstrated synergy in antinociceptive effect, because two drugs have the same pathway inhibited the pain transmission through acting on α_2 adrenoreceptor and opioid receptor.

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