EXPLORE THE SYNERGISM OF SEDATIVE DOSE MEDETOMIDINE WITH TRAMADOL TO INDUCE ANALGESIC EFFECTS IN RABBITS

Mahmood. B. Mahmood

Department of Pharmacology, College of Veterinary Medicine, University of Duhok , Duhok, Iraq

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Key word: Medetomidine, Tramadol, Analgesia , Rabbits

ABSTRACT

The study was designed to evaluate the analgesic effects of Medetomidine and Tramadol in rabbits, to detect the best dose (as onset and duration) for antinociceptive in this model, also evaluate the antinociceptive effect as sedative doses in these drugs as a mixture by using electrical stimulator. Administration of Medetomidine alone at 200 µg/kg B.W. (I.M) and Tramadol alone at 2 mg/kg B.W. (I.P) were the best doses for relief pain induced by electrical stimuli. There was increased in the voltage change for pain (15, 30, 45, 60, 75, 90 minutes) in comparison with control and other doses for each drug. Administration of Medetomidine at 50 µg/kg B.W. (I.M) with Tramadol at 0.5 mg/kg B.W. I.P) significantly referred to synergism of the antinociceptive effect which induced analgesia in 100 % of the rabbits in comparison with other groups for each drug alone (at the same analgesic doses) without any overt side effects and without differences in glucose, glutathione, ALT and AST level in animals. The data of this study demonstrated the mixture of Medetomidine and Tramadol at low doses (subanalgesic doses) had a typical synergistic effect (super-additive) for inducing good and safe analgesia as well as its skeletal muscle relaxation in rabbits.

INTRODUCTION

Medetomedine(Domitor)® is a potent and highly specific alpha 2 –adrenoceptor agonist that induce sedation, analgesia and muscle relaxation, and anxiolytic, as well as decrease in the anesthetic requirements of injectable and inhalant agents in wild animals, dogs and cats (1; 2; and 3). Its widely used to treat moderate and severe pain (4; 5) the drug also had clinical uses in small ruminants such as sheep and goat (6; 7; 8; and 9) horses (10) camel (11) buffalo (12) and birds (13 ; 14) and chicks (15 ) The mechanism of action of Medetomidine to induce
antinociceptive on presynaptic alpha 2 – adrenoceptors in peripheral and central nervous system (CNS) led to decrease catecholamine release and turnover and subsequently inducing depression of the brain (16).

Tramadol is mostly commonly used in human and animals (17; 18; and 19). The antinociceptive effect of Tramadol were resulted from dual action, first, it binding for Mu-opioid receptor in CNS and second, it inhibited the presynaptic reuptake of norepinephrine and serotonin (20). It’s used for reduction of the mostly signs of aneuralgia, depression, anxiety (21) and treat post-surgical chronic pain in dogs, ruminant and laboratory animals as rat and mice (22; 23 and 24). The inhibitory effects were contributed as analgesic effect of Tramadol by inhibiting pain reflex in the CNS (25 and 26).

Because widely used of these drugs in veterinary medicine as sedative and analgesics agents alone or as combination, there for,

The aim of study to evaluate the best analgesic dose, the maximal analgesic effect response by synergistic modulation of sub analgesic dose of Medetomidine with Tramadol, the analgesic effect estimate through electrical stimulator of pain indication in rabbits and the efficacy and efficiency improved checking adverse effects of analgesia of synergism (27). Electrical stimulator test is a pain assessment method used in animals to measurement the pain by using a voltage frequency set points.

MATERIALS AND METHODS

Animals:

Sixty four of both sexes rabbits weighing 1-2 kg were used. The animals were obtained from animal house of Veterinary Medicine, University of Dohok, The animals were housed at 20 ± 1°C, 12 h light / dark cycle and fed standard diet (put origin or type) and water and Libitum

Experiments:

First Experiment: Detection the best analgesic dose of Medetomidine and Tramadol in Rabbits.

A- Detection the best analgesic dose of Medetomidine in rabbits:
The animals were randomly divided into 4 groups; each group consisted of 4 animals. First group of animals (control group) were injected with physiological saline (I.M), whereas; the other groups (2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th}) were injected with Medetomidine (1 µg/mL, Domitor, Orion corporation, Turku, Finland) at 50, 100, 200 µg/kg (B.W) I.M respectively.

**B- Detection the best analgesic dose of Tramadol in rabbits:**

The animals were randomly divided into 4 groups; each group consisted of 4 animals. First group of animals (control group) were injected with physiological saline (I.P), whereas; the other groups (2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th}) were injected Tramadol (50 mg/ml, Mepha, Ltd Aesh-Basel, Swits-land) at 0.5, 1 and 2 mg/kg of body weight I.P, respectively.

The voltage that induced pain via electrical stimulation by electrical stimulator device (100- Bio science, England) which is specific for muscle stimulator after modified to induce pain in rabbit (15) was measured at time zero (pre-treatment), 15, 30, 45, 60, 75, 90 minutes of injection with different analgesic drugs (Medetomidine and Tramadol) and record the changes in voltage (pain threshold) with the same drugs.

**Second Experiment: The effect of sedative dose of Medetomidine or Tramadol to induce analgesia in Rabbits.**

The rabbits were divided to the 4 groups each group has 4 animals:

1- First group (control) injected with Physiological saline I.M.

2- Second group injected with Medetomidine at 50 µg/kg B.W I.M.

3- Third group injected with Tramadol at 0.5 mg/kg B.W I.P.

4- Forth group injected with Medetomidine at 50 µg/kg I.M with directly injected with Tramadol at 0.5 mg/kg I.P.

After injection the animals with drugs according to above groups, detect the onset of sedation (minute), the duration of sedation (minute) and the duration of analgesia after 30 minutes of injection (calculate the percentage of analgesia in each group).
In preliminary experiments those doses were observed to induce mild sedation in rabbits but not induce any relief of pain (analgesia) after 30 minutes of injection of any drug alone (the voltage that cause pain to each animal was determined before injection of any drug and then repeated the pain test by using electrical stimulator device after 30 minutes of injection at the same voltage of each animal as previously clarify.

After 30 minutes of injection of each animal groups were sacrificed to obtain blood samples by anticoagulant tubes to be used in measuring the glucose level, AST and ALT by using kits after that opening the abdominal wall of the animals, the liver exercised and used in measuring of glutathione level by using alternative Elman’s method (28) to evaluate the glutathione level in the liver tissue.

**Statistical analysis:**

The parametric data were subjected to analysis of variance, followed by the least significant difference test, and the non-parametric data (percentage data) were analysis by Fisher Exact probability (29; 30; 31 and 32). The level of significance was $P < 0.05$.

**RESULTS**

**First Experiment: Detection the best analgesic dose of Medetomidine and Tramadol in Rabbits.**

**A- Detection the best analgesic dose of Medetomidine (I.M) in rabbits:**

Medetomidine at 200 $\mu$g/ kg B.W (I.M) was caused significantly increased in pain voltage in animals at time (15, 30, 45, 60, 75, 90) minutes compared with time zero within same group and with control group and groups at 50 ,100 $\mu$g/ kg B.W (I.M), respectively at the same periods (table 1), whereas; the dose at 50 $\mu$g/ kg B.W.(I.M) was failed to induce analgesia in rabbits in all period of the test(Table 1).

Table 1 investigate the best dose of Medetomidine at 200 $\mu$g/ kg B.W (I.M) to induce analgesia at time (15, 30, 45, 60, 75, 90) minutes of injection compared with other groups.
Table 1: Detection the best onset of analgesia and the best analgesic dose of intra muscular injection of Medetomidine in Rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Voltage in time zero (pre treatment)</th>
<th>Voltage after 15 min. of inj.</th>
<th>Voltage after 30 min. of inj.</th>
<th>Voltage after 45 min. of inj.</th>
<th>Voltage after 60 min. of inj.</th>
<th>Voltage after 75 min. of inj.</th>
<th>Voltage after 90 min. of inj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Physiological salt solution)</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Medetomidine 50µg/kg IM</td>
<td>2.5 ± 0.24</td>
<td>2.6 ± 0.24</td>
<td>2.6 ± 0.24</td>
<td>2.5 ± 0.24</td>
<td>2.6 ± 0.24</td>
<td>2.6 ± 0.24</td>
<td>2.2 ± 0.29</td>
</tr>
<tr>
<td>Medetomidine 100µg/kg IM</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.5</td>
<td>3.3 ± 0.7</td>
<td>4.7 ±0.6</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>*(a, c, d, e)</td>
<td>4.7 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6 ± 0.5</td>
<td>*(a, c, d, e)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Medetomidine 200µg/kg IM</td>
<td>2.1 ± 0.5</td>
<td>8.4 ± 0.8</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>*(a, b, c)</td>
<td>8.1 ± 0.8</td>
<td>*(a, b, c)</td>
<td>8.8 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*(a, b, c)</td>
<td></td>
<td>*(a, b, c)</td>
<td>8.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*(a, b, c)</td>
<td>8.2 ± 0.8</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.5 ± 0.7</td>
</tr>
</tbody>
</table>

Value are presented as the mean ± SE of 4 rabbits/group

* : Significantly different than the control group at same times, P < 0.05.
*a : Significantly different than the 50 µg / kg of Medetomidine , P < 0.05.
*b : Significantly different than the 100 µg / kg of Medetomidine , P < 0.05.
*c : Significantly different than the time zero at same dose , P < 0.05.
*d : Significantly different than the time 15 at same dose , P < 0.05.
*e : Significantly different than the time 30 at same dose , P < 0.05.
*f : Significantly different than the time 45 at same dose , P < 0.05.
*g : Significantly different than the time 60 at same dose , P < 0.05.
*h : Significantly different than the time 75 at same dose , P < 0.05.

B- Detection the best analgesic dose of Tramadol (I.P) in rabbits:

Tramadol at 2 mg \( \text{kg B.W (I.P)} \) was rabidly induce analgesia than other doses (0.5 , 1 mg \( \text{kg} \)) B.W. (I.P) until 90 minutes after injection (last time of experiment) (Table 2). It (2 mg/ kg) was caused significantly increased in voltage that caused pain in animals at (15, 30, 45, 60, 75, 90) minutes of injection compared with control group at the same periods and with the time zero (before injection) in same dose (Table2), also significantly increased compared with Tramadol at 0.5 mg/ \( \text{kg} \) in (15, 30, 45, 75, 90) minutes of injection at the same periods also in 15, 90 minutes of injection compared with Tramadol at 1 mg \( \text{kg} \) at same periods (Table 2).
Table 2 investigated the best dose of Tramadol at 200 mg\kg B.W (I.P) to induce analgesia (increasing pain threshold) at (15, 30, 45, 60, 75, 90) minutes of injection compared with the other groups.

Table 2: Detection the best onset of analgesia and the best of analgesic dose of intra peritoneal injection of Tramadol in Rabbits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Voltage in time zero (pre treatment)</th>
<th>Voltage after 15 min. of inj.</th>
<th>Voltage after 30 min. of inj.</th>
<th>Voltage after 45 min. of inj.</th>
<th>Voltage after 60 min. of inj.</th>
<th>Voltage after 75 min. of inj.</th>
<th>Voltage after 90 min. of inj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Physiological salt solution)</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Tramadol 0.5 mg\kg I.P</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>3.5 ± 0.4</td>
<td>3.9 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>2.8 ± 0.5</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Tramadol 1 mg\kg I.P</td>
<td>2.3 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>4.2 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Tramadol 2 mg\kg I.P</td>
<td>2.3 ± 0.4</td>
<td>4.1 ± 0.6 *a b c</td>
<td>4.4 ± 0.3 *a c</td>
<td>5.0 ± 0.5 *a c</td>
<td>4.5 ± 0.6 *a c</td>
<td>4.5 ± 0.2 *a c</td>
<td>4.1 ± 0.3 *a b c</td>
</tr>
</tbody>
</table>

Value are presented as the mean ± SE of 4 rabbits/group

*: Significantly different than the control group at same times, P < 0.05.
*a*: Significantly different than the 0.5 mg / kg of Tramadol, P < 0.05.
*b*: Significantly different than the 1 mg / kg of Tramadol, P < 0.05.
*c*: Significantly different than the time zero at same dose, P < 0.05.
*d*: Significantly different than the time 15 at same dose, P < 0.05.
*e*: Significantly different than the time 30 at same dose, P < 0.05.
*f*: Significantly different than the time 45 at same dose, P < 0.05.
*g*: Significantly different than the time 60 at same dose, P < 0.05.
*h*: Significantly different than the time 75 at same dose, P < 0.05.

Second Experiment:

The effects of sedative dose of Medetomidine and Tramadol to induce analgesia and in glutathione concentration in liver, glucose level and AST, ALT activities in Rabbits.

Administration of Medetomidine at 50 µg \kg B.W (IM) and Tramadol at 0.5 mg \kg B.W (I.P) both of them induce sedation in rabbits without causing analgesia after 30 minutes of injection. There were significant no difference (p ≥(0.05)) difference between groups in onset and duration of sedation (Table 3). The clinical signs of sedation were represented as dropping of head, hair erection, ataxia, urination, defecation, salivation lacrimation, depress and
decumbency. whereas; Medetomidine with Tramadol together at 50 µg / kg B.w (I.M) with 0.5 mg \ kg B.w (I.P) were leds to induce analgesia at (100 %), respectively, in rabbits compared with control group and with groups of Medetomidine alone and Tramadol alone without significantly affecting on onset and duration of sedation (Table 3).

Table 3: Effect of sedative dose of Medetomidine and Tramadol to induce analgesia

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sedative onset(min)</th>
<th>Sedative duration (min)</th>
<th>% of analgesia after 30 (min) of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ( Physiological saline)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0%</td>
</tr>
<tr>
<td>Medetomidine 50 µg/kg I.M</td>
<td>1.5 ± 0.5</td>
<td>20 ± 0.6</td>
<td>0%</td>
</tr>
<tr>
<td>Tramadol 0.5 mg/kg I.P</td>
<td>3.7 ± 0.7</td>
<td>17.8 ± 0.7</td>
<td>0%</td>
</tr>
<tr>
<td>Medetomidine 50 µg /kg, I,M + Tramadol 0.5mg/kg, I.P</td>
<td>3.2 ± 1.3</td>
<td>16.7 ± 1.3</td>
<td>100 % *</td>
</tr>
</tbody>
</table>

All drugs used in this study as alone or combined (mixture) at the above doses have not significantly effects in glutathione level in the liver and in glucose level, AST, ALT activities in plasma of rabbits after 30 minutes of injection (Table 4).
Table 4: The sedative effect dose of Medetomidine or Tramadol alone and together in glutathione concentration in liver or glucose level, ALT and AST activity in plasma. after 30 minutes of injection in rabbits

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glutathione in liver (Micromol / g)</th>
<th>Glucose (mg/100 ml)</th>
<th>ALT activity (i.u/L)</th>
<th>AST activity (i.u/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Physiological saline)</td>
<td>3.81±1.5</td>
<td>199.05±4.5</td>
<td>5.61±1.6</td>
<td>212.65±8.5</td>
</tr>
<tr>
<td>Medetomidine 50 µg/kg IM</td>
<td>2.29±0.8</td>
<td>193.20±6.5</td>
<td>6.82±2.1</td>
<td>229.65±9.9</td>
</tr>
<tr>
<td>Tramadol 0.5 mg/kg IP</td>
<td>3.67±15</td>
<td>213.20±8.8</td>
<td>9.09±0.9</td>
<td>218.±7.8</td>
</tr>
<tr>
<td>Medetomidine 50 µg/kg IM + Tramadol 0.5 mg/kg IP</td>
<td>2.35±0.6</td>
<td>195.68±9.8</td>
<td>6.02±1.1</td>
<td>216.22±1.3</td>
</tr>
</tbody>
</table>

Value are presented as the mean ± SE of 4 rabbits/group

DISCUSSION

Many reports have been published an analgesic combination such as Ketoprofen with Acetaminophen, Tramadol with Acetaminophen (19 and 20), it had manifested these combinations of drugs acting on different receptors may produce super or sub additive interaction in antinociceptive effects (17). In the present study had been used electrical stimulator for induce pain then detect the analgesic pain tolerance analgesic induction of and to evaluate analgesic effect of Medetomidine or Tramadol alone or as a combination quantitively and qualitatively in rabbits. This device was used firstly by Al-Mashhadany to evaluate the analgesia by Medetomidine in goats (33) also used to evaluate the analgesic effects of xylazine in chicks (15), In this study used electrical stimulator to evaluate the analgesic action of some analgesic drugs such as Medetomidine and Tramadol and to detect the best onset and duration of analgesia, also to detect the best analgesic dose. The results were indicate the Medetomidine at 100, 200 micro g/kg B.W. (I.M) and Tramadol at 0.5, 1, 2 mg/kg B.W. (I.P) in rabbit to relief of pain sensation as dose dependent response to electrical stimulation by electrical stimulant via voltage increasing (increase of pain threshold) compared with its voltage before injection and control. The best analgesic doses of Medetomidine and Tramadol at 200 micro g/kg B.W.(I.M) and 2 mg/kg B.W. (I.P), respectively, induced rapid significantly analgesia compared with other doses in each of them and persist more than 90 minutes of injection, these results of Medetomidine
were agreement with previous studies in goats (33) chicks (15) and Tramadol results were agreement with previous studies in chicks (34), rats (35 and 36) and mice (37). The analgesic effects of Medetomidine due to activation of pre-synaptic alpha 2- adrenoceptor in the peripheral and central nervous system leading to decrease of catecholamine release and turnover (13 and 38) but analgesic effects of Tramadol result from dual action, first; it has affinity to binding with μ-opioid receptor in the CNS and the second; it had inhibited effect of pre-synaptic reuptake of nor epinephrine and serotonin (20).

The clinical signs in this study were manifested by ataxia, hair erecting, urination, defecation, lacrimation, closed eye depress and decumbency, they were agreement with other studies in analgesic effects of Medetomidine in goats (33), chicks (15), camel (11), cats (3), horses (10) and in analgesic effects of Tramadol in chicks (34and 15), dogs (2) and in mice (39).

In the present study evaluating the analgesic effects of drug interaction (synergism) between sedative doses of Medetomidine at 50 micro g / kg B.W. (I.M) with Tramadol 0.5 mg / kg B.W. (I.P) leading to excellent analgesic effect as 100 % of treated rabbits compared with each of them given alone (not have analgesic effects) and the causing of this synergism revert to may be due to Tramadol has weak opioid agonist with antinociceptive effects through its action on Mu-receptor or by inhibiting the neural reuptake of both noradrenalin and serotonin (26). Many studies demonstrated the Tramadol has analgesic effect through acting on descending noradrenergic pathways and this play a role in analgesic properties of the non-opioid, stimulating this pathway produces antinociceptive effect from the activation of the spinal alpha 2- adrenergic receptor by noradrenergic neuron (39). Previous study suggested that the coexpression of the synergistic receptor pair alpha 2- adrenoceptor and Mu-receptor on primary afferent nociceptive fibers may representing on substrate for analgesic synergy, perhaps as a result of interacting between neural G protein coupled receptor (40). Alpha 2- adrenergic receptor coupled to sensitive inhibitory G-- protein that causing inhibition of adenylyl cyclase which result in decrease cAMP formation, is an important consequence of alpha 2- adrenoceptor activation (41), and the drug interaction in sedative dose was not manifested any significantly differences in onset and duration of sedation in animals when given as a single or combined dose, and not appeared any CNS side effects as well as, not induced any significant difference in glucose, glutathione, AST and ALT levels in rabbits.
تأثيرات الجرعة تحت المسكنة (المصدر) للميديتوميدين والترامادول معا لأحداث التسكين في الأرانب

محمود بشير محمود
فرع الأدوية، كلية الطب البيطري، جامعة دوّلك، دوّلك، العراق

الخلاصة

كان الهدف من الدراسة الحالية هو تحديد أفضل جرعة مسكنة للالم (وقت بدء التسكين ومدته) لبعض المسكنات كالديتوميدين والترامادول في الأرانب عند معاملتها بالجرع المسدر (حتى المسكنة) لهذه المسكنات عند إعطاءها معا بشكل مركبات واستخدام جهاز المحفز الكهربائي. وكانت أفضل جرعة مسكنة للالم لعقار الميديتوميدين لوحده هي 200 مابيروغرام/كم من وزن الجسم بالعسلة والأدوية من للتراشادول لوحده هي 2 ملغ/كم من وزن الجسم بالقلب ، حيث أدت الى زيادة معنوية في الفئات المسببة للالم عند الأوقات (15،30،45،60،75،90) دقيقة من الحنف مع مجموعة السيطرة وال المجامع الأخرى لكل عقار. في حين أن اعداد عقار الميديتوميدين والترامادول معاً بجرعة مسدرة (حتى المسكنة) عند 50 مابيروغرام/كم بالعسلة و 0.5 ملغ/كم بالقلب على التوالي إلى إحداث تسكين ممتاز من الألم ونسبة 100% مقارنة مع المجامع المعاملة بكل عقار لوحده ( عند الجرع تحت المسدرة نفسها) . تم تظهر هذه تأثيرات جانبية ضارة على الحيوانات بالإضافة الى عدم حصول تغيرات معنوية في مستوى كل من الكولسترول والكولسترول في الأجسام المصابة بجزء المسدرة معا. لقد أظهرت الدراسة بأن المزج (الداخل) بين الميديتوميدين والترامادول في الجرع الواطئة هو تأري وبعد هذا المزج مثالياً مناسبة لأحداث تسكين آمن وجيد من الألم بالإضافة الى ارتفاع الانتصاب الهيكلية في الأرانب.

REFERENCES


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