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Original Research Article

Fluoxetine Potentiate the Analgesic Effect of Diclofenac in Male Mice

Selman Mohammed Selman
College of Medicine, University of Babylon, Hilla, IRAQ

E-mail:selman_mohammed@yahoo.com

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Abstract

Fluoxetine is a selective serotonin re-uptake inhibitors (SSRI) drug, which is the most widely used in the treatment of depression. The aims of this study were to evaluate the analgesic effects of fluoxetine, and the analgesic effect of combination of fluoxetine and diclofenac in model of thermal pain in male mice.
Mice were divided into four groups, six in each. Group 1 received normal saline. Group 2received diclofenac. Group 3 received fluoxetine. Group 4 received fluoxetine before the administration of diclofenac. Evaluation of the analgesic action of the drug was achieved by the tail flick test.
Tail-flick latency significantly increased ($P < 0.05$) in the group 2 in the 2 time intervals as compared to control group. In the group 3, there were no significant changes ($P > 0.05$) in the tail-flick latency after 30 min as compared to corresponding time of control group, while it significantly increased ($P < 0.05$) after 60 min as compared to corresponding time of control group**.** In the group 4caused significant increase ($P < 0.05$) in the time of tail-flick latency after 60 min as compared to corresponding time of diclofenac group and fluoxetine group. In this study, combination of low dose diclofenac with fluoxetine caused a significant prolongation of tail-flick latency proposing additive antinociceptive effect. Thus, combination of diclofenac with fluoxetine hypothetically reduce the dose requirement and adverse effects for each drug.. Further clinical studies are needed to prove these effects.

Key Words: diclofenac, fluoxetine, tail-flick test, mice**.**

الفلوكسيتين يقوي تاثير الدايكلوفيناك كمسكن للالم في ذكور الفئران

الخلاصة

الفلوكسيتين هو دواء مانع انتقائي لامتصاص السيروتونينو يستعمل على نطاق واسع في علاج مرض الكابة. اهداف البحث هذا هو لتقييم عمل الفلوكسيتين لمسكن للالم وتاثير مزج الفلوكسيتين مع الدايكلوفيناك كمسكن للالم في الفئران.

تم تقسيم الفئران الى أربعة مجموعات و في كل مجموعة ٦ فئران. المجموعة الأولى أعطيت محلول ملحي. المجموعة الثانية أعطيت الدايكلوفيناك. المجموعة الثالثة أعطيت الفلوكسيتن. المجموعة الرابعة اعطيت الفلوكسيتين قبل إعطاء الدايكلوفيناك. تم قياس تاثير الدواء كمسكن للالم عن طريق اختبار اهتزاز الذيل.

ان وقت استجابة اهتزاز الذيل ازداد زيادة معنوية ($P < 0.05$) في المجموعة الثانية في كلتا الفترتين الزمنيتين مقارنة مع المجموعة الأولى. في المجموعة الثالثة لا توجد زيادة معنوية ($P > 0.05$) بعد ٣٠ دقيقة في وقت استجابة اهتزاز الذيل مقارنة بالوقت الموازي للمجموعة الأولى، لكنه ازداد زيادة معنوية ($P < 0.05$) بعد ٦٠ دقيقة مقارنة مع الوقت الموازي له في المجموعة الأولى. في المجموعة الرابعة، فان مزج الدايكلوفيناك مع الفلوكسيتين سبب زيادة معنوية ($P < 0.05$) في وقت استجابة اهتزاز الذيل بعد ٦٠ دقيقة مقارنة مع الوقت الموازي له في مجموعة الدايكلوفيناك و مجموعة الفلوكسيتين. ففي هذه الدراسة، ان مزج جرعة قليلة من الديكلوفيناك مع الفلوكسيتين سبب زيادة معنوية بوقت استجابة اهتزاز الذيل و هذا يعطي تاثيرا مضافا لتسكين الألم. لذا، فان مزج الدايكلوفيناك مع الفلوكسيتين يقلل، نظريا، الجرعة المطلوبة و الاثار الجانبية لكل دواء. ان دراسات سريرية إضافية مطلوبة لبرهان هذه التأثيرات.

الكلمات المفتاحية: دايكلوفيناك، فلوكسيتين، اختبار هز الذيل، الفئران.

Introduction

Pain which associated with tissue damage and inflammation possibly results from stimulation of the fibers of the pain and enhanced pain sensitivity (hyperalgesia), which is a part of increased excitability of the neurons in spinal cord(central sensitization) [1]. Cytokines such as IL-1, IL-8 and TNFα have an important role in producing the pain of inflammation. These mediators release prostaglandins and perhaps other agents such as substance P that produce hyperalgesia. Intramuscular or sub-cutaneous injections of PGF2 or PGE2 cause severe local pain. Also, prosta-glandins can produce vascular pain and headache when infused intravenously. Prostaglandins sensitize pain receptors to chemical and mechanical stimulation due to the ability of prostaglandins to reducing of the threshold of the polymodalno-ciceptors of fibers type C [2].

NSAIDs interfere with the prostaglandins biosynthesis by inhibition fatty acid cyclooxygenase-1 (COX-1) which convers arachidonic acid into the PGG2, and PGH2 [3]. Diclofenac is an inhibitor of COX-1 and its potency greater than that of several agents of NSAIDs such as naproxen, or indomethacin [4]. The drug is rapidly absorbed after oral dose, and the peak plasma concentrations are reached after 2 to 3 hours. Administration of diclofenac with food reduce the rate of absorption [5]. Only 50% of the drug is available systematically because it face first-pass effect. It is bound to plasma protein extensively (99%) [6,7]. The duration of the therapeutic effect of the drug is longer than the half-life due to the accumulation of the drug in the synovial fluid after intramuscular or oral administration. Metabolism of diclofenac occurs in the liver by a cytochrome P450 isozyme after sulfation and glucuronidation, and the metabolites are excreted in bile and urine [8,9].

Fluoxetineis a selective serotonin re-uptake inhibitors (SSRI) drug, which is the most widely used in the treatment of depression [10]. It is also used to treat another type of anxiety disorder called obsessive-compulsive disorder. It has less anticholinergic side effects than those of tricyclic antidepressants (TCAs), [11] in addition to that, it causes no 'cheese reactions' which occurs in monoamines inhibitors (MAOIs) [12]. Fluoxetine is well absorbed after oral administration, and the half-life is 24-96 hours. The drug needs 2-3 weeks to elicit its therapeutic effects [13].

Many studies proved that antidepressant drugs have analgesic effect specially SSRIs [14] fluoxetine is able to reverse or prevent hyperalgesia in animal model of pain [15]. Pain of diabetic neuropathy, migraine [16] and perhaps rheumatoid arthritis response to antidepressants [17]. The aims of this study were to evaluate the analgesic effects of fluoxetine, and the analgesic effect of combination of fluoxetine and diclofenac in model of thermal pain in mice.

Materials and Methods

Animals:

Twenty fourmale adult mice(25- 30 g) were enrolled in this experiment. The animals were retainedin the Animal House of College of Medicine/University of Babylon, 12 hours light-dark cycles and the temperature was controlled on 25 °C. Tap water with standard diet *ad libitum* were provided. After 2 weeks for accommodation, the micewere divided randomly into four groups, each group had six mice.

Group 1 (control group): The animals received normal saline equal to the volume of the diclofenac which administered intra-peritineally.

Group 2: In this group, each mouse received diclofenac, 3 mg/kg, intra-peritoneally.

Group 3: In this group, each mouse received 10 mg/kg of fluoxetine, orally.

Group 4: In this group, each mouse received 10 mg/kg of fluoxetine , orally thirty min before the administration of diclofenac, 3 mg/kg, intraperitoneally.

Evaluation of antinociceptive effect of the drugs

Evaluation of antinociceptive action of drugs was achieved by the tail flick test [18]. Each mouse was restrained by suitable container which fixed on the water bath allowing the last 2 cm of the tail immersed in the heated water (50 ± 0.2 °C). Measurement of the time to tail twistingor flicking i.e. the reaction considered as the tail-flick latency.

Each mouse was tested at time intervals 0 min, 30 min, and 60 min after the drug administrations. In addition to that, each mouse was tested three trials in each interval separated by 3-5 min. For each mouse, the mean of 3 measurements was considered as the time of tail-flick latency. In each trial, the cut-off time of 3 min to prevent tissue injury.

Drugs

Fluoxetine capsule, 20 mg (Actavis, Irland) 20 was dissolved in 10 ml of isotonic saline (0.9% NaCl) and the final product was 2 mg/ml. Diclofenac sodium ampule 75/2ml (Olfen-75, Acino, Switzerland) was used in this experiment.

Statistical analysis

Results of this study were expressed as mean ± standard error mean (SEM) and one-way ANOVA was used for comparison between means. Value was considered as a statistically significant when the P value< 0.05.Statistical analysis was done by using SPSS (version 18).

Results

1- Control group

In the control group, there were no significant differences in the time of tail-flick latency after 30 min as compared to the tail-flick latency after 60 min ($P > 0.05$). (Tables 1 and 2)

2- Effects of diclofenac

Tail-flick latency significantly increased ($P<0.05$) in the group 2 (received diclofenac 3mg/kg) in the 2 time intervals as compared to control group. Diclofenac produced significant differences in the time of the tail-flick latency after 30 min as compared to the tail flick latency at 0 min but there were no significant changes in the time of the tail-flick latency ($P > 0.05$) after 60 min as compared to the tail-flick latency after 30 min. The time of the tail-flick latency significantly increased after 30 min as compared to corresponding time of fluoxetine ($P<0.05$), while there were no significant differences in the time of tail-flick latency after 60 min as compared to corresponding time of fluoxetine ($P>0.05$) (Tables 1 and 2).

3- Effects of fluoxetine

In the group 3 (received fluoxetine, 10 mg/kg), there were no significant changes in the time of tail-flick latency after 30 min as compared to corresponding time of control group ($P>0.05$), while it significantly increased ($P<0.05$) after 60 min as compared to corresponding time of control group. Fluoxetine produced no significant changes in the time of the tail-flick latency after 30 min as compared to the time of the tail-flick latency at 0 min but it significantly increased after 60 min as compared to tail-flick latency after 30 min ($P < 0.05$). (Tables 1 and 2)

4- Effects of combination of dilclofenac and fluoxetine

In the group 4 (received diclofenac, 3mg/kg and fluoxetine, 10 mg/kg), the time of the tail-flick latency significantly increased after 30 min as compared to the corresponding times of control group and fluoxetine group ($P<0.05$), while there were no significant changes ($P > 0.05$) as compared to the corresponding time of diclofenac group. Diclofenac and fluoxetine caused significant changes in the time of the tail-flick latency after 30 min as compared to the tail-flick latency in 0 min. In addition, it significantly increased after 60 min as compared to tail-flick latency after 30 min ($P < 0.05$). Combination of diclofenac and fluoxetine caused significant increase ($P < 0.05$) in the time of the tail-flick latency after 60 min as compared to corresponding time of diclofenac group and fluoxetine group. (Tables-1&2).

Table 1: Effects of diclofenac (3mg/kg), fluoxetine (10mg/kg), and combination of fluoxetine (10mg/kg) and diclofenac (3mg/kg) on the tail-flick latency. (n = 6 in each group)

Drug (mg/kg)	Tail-flick latency in sec (mean ± SEM)	
post-drug time (min)		
0	30	60
Control (saline)	4.1 ± 264714.5 ± 42817	4.3333 ± .33333
Diclofenac	5.4 ± 48316 15.3333 ± 1.14504	181667 ± 144722
Fluoxetine	4.5 ± 83186 5.0 ± 3.5159	14.8333 ± 2.02347
Diclofenac+fluoxetine	5.3 ± 31016	16.6667 ± 1.17379 33.1667 ± 2.60021*

* The mean difference is significant (P< 0.05).

Table 2: A comparison of the mean differences of the tail-flick latency according post-drug time (30 min and 60 min) for each group. G 1 received NS (control group). G 2 (diclofenac, 3mg/kg). G 3(fluoxetine,10mg/kg). G 4(combination of fluoxetine, 10mg/kg and diclofenac, 3mg/kg) (n = 6 in each group)

	G 1 30	**G 1 60**	**G 2 30**	**G 2 60**	**G 3 30**	**G 3 60**	**G 4 30**	**G 4 60**
G 1 30	X	0.16667	-10.8333*	-13.66667*	- 0.50	-10.33333*	-12.16667*	-28.6667*
G 1 60	-0.16667	X	-11.0000*	-13.83333*	-0.66667	-10.50000*	-12.33333*	-28.83333*
G 2 30	10.8333*	11.0000*	X	-2.83333	10.33333*	0.50	-1.33333	-17.83333*
G 2 60	13.66667*	13.83333*	2.83333	X	13.16667*	3.33333	1.50	-15.00
G 3 30	0.50	0.66667	-10.33333*	-13.16667*	X	-9.83333*	-11.66667*	-28.16667*
G 3 60	10.33333*	10.50000*	-0.50	-3.33333	9.83333*	X	-1.83333	-18.33333*
G 4 30	12.16667*	12.33333*	1.33333	-1.50	11.66667*	1.83333	X	-16.50000*
G 4 60	28.6667*	28.83333*	17.83333*	15.00	28.16667*	18.33333*	16.50000*	X

* The mean difference is significant (P<0.05).

Discussion

This study showed that the antinociceptive action of low dose Diclofenac (3mg/kg) against thermal pain in mice was obvious after intraperitoneal injection, expressed as prolongation in the tail-flick latency after 30 min, then this effect increased after 60 min. Diclofenacis classified as a NSAID which can inhibit the actions of both types of cyclooxygenase enzymes (cyclooxygenase -1 and cyclooxygenase-2). These agents are useful in the management of inflammatory disorders and pain [19]. Non-steroidal anti-inflammatory drugs are widely used because these drugs relieve inflammation and pain and the patients return to usual functions [20].

Data of this study revealed that after 60 min of oral dose of 10 mg/kg, fluoxetine caused anti-nociceptive effect. This result is in agreement with the several clinical and experimental researches have reported the analgesic activity of antideprassants [21,22]. The major effect of anti-depressants is inhibition of re-uptake of

monoamines [23]. Several reports have linked the actions of fluoxetine to the serotonergic pathway [24-26].

Many brain areas supplies periaque-ductal grey area and supposed it is a gateway in control the pain. Periaque-ductal grey area stimulates the nucleus raphemagnusin the spinal cord, that connected by synapses with dorsal horn interneurons.

At these synaptic connections, the main neurotransmitter is serotonin and the tract from nucleus raphe magnusto the substantiagelatinosa which is located in the dorsal horn [27]. Stimulation of this path inhibits nociceptive pathway. At the dorsal horn of spinal cord, 5-HT3 receptors mediate the analgesic action [28]. SSRIs inhibit the serotonin reuptake leading to increase the amount of serotonin in the synaptic cleft, extension of this effect on the serotonergic pathway in the spinal cord may explain the antinociceptive action of SSRI drugs.

Recently, many researchers reported that naloxone can antagonized fluoxetine-induced antinociceptive action [16,29]. Therefore it likely that fluoxetine exhibits its actions via opioid pathways including the μ receptors.

In this study, combination of low dose diclofenac with fluoxetine caused a significant prolongation of tail-flick latencyproposing additive antinociceptive effect. Therefore, fluoxetine significantly potentiate the analgesic action of diclofenac in pain model when given together. Many agents which are used for treatment other than pain, maybe have antinociceptive effects in specific situations, these agents called Co-analgesics [30]. This combination enhances the analgesic effect of the agent without increasing the dose of the analgesic agent. Thus, it can moderatethe occurrence of unwanted side effects [31].

It is a great value in treatment of the pain, when a combination of two types of agents at doses less than therapeutic doses [32]. Thus, combination of diclofenac with fluoxetine hypothetically reduce the dose requirementand adverse effects for each drug. Further clinical studies are needed to prove these effects.

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