



Evaluation of the Antipyretic, Analgesic, and Anti-inflammatory Effects of Pregabalin in Chicks

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BACKGROUND: Pregabalin is an anticonvulsant medication. It's been utilized as an analgesic in animals to treat neuropathic pain that doesn't respond to NSAIDs or opiates.

Objective: This study aimed to investigate the potential antipyretic, analgesic, and anti-inflammatory effects of pregabalin in chicks as a model.

Methods: A randomized controlled study was conducted on sixty-two clinically healthy Ross chicks aged seven days. The antipyretic effect of pregabalin was determined using Baker's yeast test and anti-inflammatory effect was determined using the formalin test.

Results: Pregabalin reduced the temperature throughout the experimental period compared with that in the group treated with Baker's yeast. In the formalin test, pregabalin induced an analgesic effect against pain induced by formalin injection into the plantar region of the chick foot. This was shown by a significant increase in the onset of raising the right foot and a significant decrease in the number of the right foot raises compared with the control value. A substantial decrease in the thickness of the right foot compared to the control value was observed in the anti-inflammatory activity of pregabalin in comparison to the control group.

Conclusion: Pregabalin has an antipyretic effect which was recorded for the first time. Also it has a substantial analgesic and anti-inflammatory activity.

Keywords: Pregabalin, Pain, Fever, Baker's yeast, Formalin test, Chicks.

Introduction

Throughout the history of biology, chickens have been used as a study model. Chicks have been utilized as a model for analgesia, anesthetic, and toxicity research [1]. Pain is an unpleasant sensory and emotional experience that occurs when tissue is damaged or is at risk of being damaged [2]. "A state of raised core temperature, which is typically, but not always, part of the defensive responses of multicellular organisms (host) to the invasion of live (microorganisms) or inanimate substances recognized as pathogenic or alien by the host," according to the definition of fever [3].

Gabapentinoids are a brand-new class of anticonvulsants that offer a novel therapeutic strategy to the treatment of a variety of pain conditions. Pregabalin, a new anticonvulsant drug, has been tested in a number of clinical trials for the treatment of pain in patients with peripheral neuropathy, fibromyalgia, and irritable bowel syndrome[4].

Pregabalin has a unique mechanism of action that distinguishes it from other anxiolytic drugs. It binds to the alpha-2-delta subunit of the voltage-gated calcium channels of presynaptic neurons (VGCCs). This causes a decrease in the release of neuroexcitatory neurotransmitters and a return

to a “normal” physiologic state in “hyper-excited” neurons[5].

Pregabalin has been shown in human studies to have a clear anti-nociceptive effect when given as an acute dose before surgery. A single dose (75–150 mg) has shown significant efficacy in reducing post-operative pain in orthopedic surgery, lumbar discectomy, septoplasty, thyroidectomy, and hysterectomy [6].

The aim of our study is to reveal the antipyretic, analgesic, and anti-inflammatory effects of pregabalin in chicks as a model.

Materials and Methods

Ethical approval

The birds were handled in accordance with the animal ethics committee of the College of Veterinary Medicine. The scientific board of the department of physiology, biochemistry, and pharmacology of the college of veterinary medicine at the University of Mosul approved this study (Protocol no.1396).

Drugs

Pregabalin was a gift from Pioneer Pharmaceutical Company, Iraq. Diclofenac sodium (CLOFEN® 75mg/3ml), Gulf Pharmaceutical Industries, United Arab Emirates. To achieve the required concentrations, all drugs were diluted with normal physiological saline. The volume of administration was 5ml/kg of body weight (b.wt). Pregabalin was administered orally by gavage needle, while Diclofenac was administered intraperitoneally. The research is a part of the master’s study for the first researcher, and the doses of pregabalin and diclofenac used were determined in previous experiments.

Animals

A randomized controlled study was conducted on sixty-two clinically healthy Ross chicks aged seven days and weighing between 150 and 200gm. The animals were housed in a group inside cages provided for breeding chickens in the Mosul veterinary college’s animal house, the floor covered by sawdust, continuous lighting, and free access to feed and water.

Experimental design

Determination of the antipyretic effect of pregabalin

Thirty-five chicks were randomly separated into five groups of seven chicks per group. The treatment groups as follows:

- 1- First group (negative control): chicks were treated with physiological solution (5 ml/kg b.wt.).
- 2- Second group (positive control): chicks were treated with baker’s yeast 0.135 g/kg b.wt. i.p.[7]
- 3- Third group: chicks were treated with baker’s yeast 0.135 g/kg b.wt. i.p and diclofenac 11 mg/kg b.wt. i.p.
- 4- Fourth group: chicks were treated with baker’s yeast 0.135 g/kg b.wt. i.p and pregabalin 300 mg/kg b.wt. p.o.
- 5- Fifth group: the chicks were treated with baker’s yeast 0.135 g/kg b.wt. i.p, diclofenac 5.5 mg/kg b.wt. i.p and pregabalin 150 mg/kg b.wt. p.o.

The body temperature of all groups was recorded by inserting a digital thermometer into the cloacal orifice with a depth of 2 cm at times 0, 1, 2, 3, 4 and 5 hours.

Determination of the analgesic and anti-inflammatory effect of pregabalin

Twenty-eight chicks were randomly separated into four groups of seven chicks per group. Pain and inflammatory responses were induced in chicks by injecting 0.05 ml of 0.1% aqueous formalin into the right foot plantar [8]. As a control, a normal saline (0.05 ml) was injected into the left foot plantar. Fifteen minutes previously to formalin injection, the four chick’s groups were treated as follows:

- 1- First group (negative control): chicks were treated with a physiological solution 5 ml/kg.
- 2- Second group (positive control): chicks were treated with diclofenac 11 mg/kg i.p.
- 3- Third group: chicks were treated with pregabalin 300 mg/kg p.o.
- 4- Fourth group: chicks were treated with diclofenac 5.5 mg/kg i.p and pregabalin 150 mg/kg p.o.

The onset of lifting and number of raisings of the right foot in response to formalin administration were recorded within 3 minutes of formalin injection. Additionally, we measured foot thickness (mm) with a digital caliber (Electronics Lab, China) before and 1 hour after formalin injection to assess the anti-inflammatory effects of pregabalin and diclofenac alone or

in combination. Anti-inflammatory response was measured as following (percentage): Anti-inflammatory reaction = [alteration in control group foot thickness - alteration in thickness of foot for treatment group / alteration in control group foot thickness] $\times 100$.

Statistical analysis

The data is presented as mean \pm standard error. A one-way analysis of variance was used in the statistical analysis, which was subsequently exposed to the Least Significant Difference (LSD) test. The significance level was set at $P < 0.05$.

Results

The administration of baker's yeast with a dose of 0.135 g/kg i.p led to a rise in body temperature at the third hour and continued until the end of the experiment at the fifth hour after treatment in the group treated with baker's yeast. While we noticed the possibility of both diclofenac and pregabalin alone and together in reducing the temperature throughout the experiment period compared to the group of treated with baker's yeast (Table 1).

In the formalin test, the Pregabalin and Diclofenac induced analgesic effect against pain persuaded by injection of formalin into chick's foot planter region. This was shown through a significant

increase in the onset of raising the right foot and a significant decrease in the number of raising the right foot compared to the control value. On the other hand, there was a significant increase in the onset of raising the right foot and a decrease in the number of raising the right foot in the combination group (Pregabalin and Diclofenac) compared with the rest groups (Table 2). A substantial decrease in thickness of the right foot compared to the control value was seen in the anti-inflammatory activity of Pregabalin, Diclofenac, and combination groups. In comparison to the control group, the anti-inflammatory activity percentage was 21.6, 44.5, and 57.8 respectively (Table 2).

Discussion

There was a noticeable rise in body temperature after 3 hours of Baker's yeast administration intraperitoneally. In research trials evaluating antipyretics in animals such as rats, mice, rabbits, and birds, baker's yeast was often used as a pyrogenic agent derived from fungal sources for generating hyperpyrexia [9]. Pyrexia, tiredness, and lack of appetite are caused by baker's yeast, which is due to a rise in the concentration of inflammatory cytokines such as interleukin-6 and tumor necrosis factor- α in the blood plasma, as well as the stimulation of inflammatory transcription factors[7].

TABLE 1. Preemptive antipyretic effect of pregabalin against pyrexia induced by baker's yeast

Groups	Time (h)					
	0	1	2	3	4	5
physiological solution (negative control)	41.15 \pm 0.10 ^a	40.95 \pm 0.09 ^a	41.08 \pm 0.09 ^a	41.35 \pm 0.24 ^a	41.13 \pm 0.25 ^a	41.33 \pm 0.08 ^a
Baker's yeast 0.135 g/kg i.p (positive control)	41.15 \pm 0.19 ^a	41.35 \pm 0.15 ^a	41.60 \pm 0.18 ^a	42.31 \pm 0.13 ^b	42.28 \pm 0.17 ^b	42.08 \pm 0.17 ^b
Baker's yeast 0.135 g/kg i.p +	41.16 \pm 0.07 ^a	41.25 \pm 0.18 ^a	41.18 \pm 0.26 ^a	41.58 \pm 0.23 ^a	41.38 \pm 0.28 ^a	41.23 \pm 0.11 ^a
Diclofenac 11mg/kg i.p						
Baker's yeast 0.135 g/kg i.p +	41.65 \pm 0.33 ^a	41.30 \pm 0.36 ^a	41.16 \pm 0.32 ^a	41.90 \pm 0.22 ^a	42.00 \pm 0.15 ^a	41.58 \pm 0.30 ^a
Pregabalin 300mg/kg p.o						
Baker's yeast 0.135 g/kg IP +	40.67 \pm 0.10 ^a	40.95 \pm 0.19 ^a	41.36 \pm 0.33 ^a	41.40 \pm 0.48 ^a	41.35 \pm 0.28 ^a	41.11 \pm 0.50 ^a
Diclofenac 5.5mg/kg i.p +						
Pregabalin 150mg/kg p.o						

Values represent mean \pm SEM for 7 chicks/group.

At the 5 percent significance level, the values of each row followed by different superscript letters are significantly different.

TABLE 2. Analgesic and anti-inflammatory effects of pregabalin in the formalin test.

Groups	Onset of raising right foot(second)	Number of raising right foot(3min)	The increase in paw thickness (mm)	The anti-inflammatory activity %
physiological solution (negative control)	2.16±0.30 ^a	41.33±1.01 ^a	0.83±0.04 ^a	0
Diclofenac 11 mg/kg ip. (positive control)	4.83±0.47 ^b	29.83±1.66 ^b	0.46±0.06 ^b	44.5
Pregabalin 300mg/kg o.p	4.16±0.87 ^b	29.66±2.38 ^b	0.65±0.11 ^{ab}	21.6
Diclofenac 5.5 mg/kg ip. + Pregabalin 150mg/kg o.p	9.00±0.96 ^c	23.83±1.79 ^c	0.35±0.06 ^b	57.8

Values represent mean±SEM for 7 chicks/group.

At the 5 percent significance level, the values of each column followed by different superscript letters are significantly different.

According to our knowledge, this is the first time to record antipyretic effects of pregabalin, and this result deserves study to reveal the mechanism of antipyretic effects, and in this regard, we can present a hypothesis for this mechanism. Fever is generated by an increase in the hypothalamic set point as a result of an increased synthesis of prostaglandin E2 in the brain caused by exogenous pyrogens and pyrogenic cytokines [10]. Kilic *et al.* [11] indicated that pregabalin reduced the levels of proinflammatory cytokines TNF- α and IL-1 β in rats' serum. So the pregabalin may reverse the action of baker's yeast and prevent the occurrence of inflammation (pyrogenic cytokines) thus the temperature does not raise.

In this study, we investigated the analgesic and anti-inflammatory effects of Pregabalin on the formalin -induced paw edema. The formalin test is used as an inflammatory tonic pain model[8]. Sensory fibers respond to physicochemical stimuli that produce mediators associated with both the source of tissue damage and inflammation, and these inflammatory mediators trigger or stimulate afferent nerves, which transmit neural impulses to the spinal cord via peripheral nerves[12]. Inflammation has been linked to the release of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), as well as tumor necrosis factor-alpha (TNF- α) and cyclooxygenase-2 (COX-2)[13]. Extensive research has shown that acute Pregabalin administration reduces hyperalgesia (the hot plate test) and carrageenan- or formalin-induced peripheral inflammation [14]. Pregabalin reduced the levels of

proinflammatory cytokines TNF- α and IL-1 β in rats' serum, which increased after carrageenan injection[11].Pregabalin has been shown to have analgesic properties in the treatment of inflammatory pain by blocking neuropeptides in sensory neurons[15].

Diclofenac can reduce acute pain in a variety of ways. Long-term stimulation of C-fiber nociceptors generates glutamate release in mammals; it is presumed that this is the same in birds. Glutamate causes central sensitization through binding to N-methyl-D-aspartate receptors in the spinal cord. Through the L-arginine/NO/cGMP system, diclofenac inhibits NMDA-mediated hyperalgesia in rats[16]. By blocking the release of excitatory pronociceptive neurotransmitters, pregabalin lowers hyperalgesia and central sensitization [17]. This explains the synergistic analgesic effect of both drugs. Also diclofenac is a common drug used to treat acute inflammation [18]. It works by blocking the cyclooxygenase (COX) pathway, which prevents the formation of prostaglandin and other eicosanoids[19]. This gives a synergistic effect on the level of anti-inflammatory activity of both drugs.

Conclusion

Our results revealed for the first time that pregabalin has antipyretic effect in chicks. This result gives the drug importance in the possibility of using it from the clinical side to treat fever. It also has analgesic and anti-inflammatory effects, which adds to the drug a second clinical importance.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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تقييم التأثيرات الخافضة للحرارة والمضادة للالتهابات للبريكابالين في أفراخ الدجاج

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الخلفية العلمية: البريكابالين دواء مضاد للاختلاج. ويستخدم كمسكن للآلام في الحيوانات لعلاج آلام الأعصاب التي لا تستجيب لمضادات الالتهاب غير الستيرويدية أو الأدوية الأفيونية.

الهدف: هدفت هذه الدراسة إلى التحقق من التأثيرات المحتملة الخافضة للحرارة والمضاد للالتهابات للبريكابالين في نموذج أفراخ الدجاج.

الطريقة: تم إجراء دراسة عشوائية ذات شواهد على 63 فرخ سليم سريريا بعمر سبعة أيام. تم تحديد التأثير الخافض للحرارة للبريكابالين باستخدام اختبار خميرة الخبز وتم تحديد التأثير المضاد للالتهابات باستخدام اختبار الفورمالين.

النتائج: خفض البريكابالين درجة الحرارة طوال فترة التجربة مقارنة مع المجموعة المعالجة بخميرة الخبز. أما في اختبار الفورمالين لوحظ تأثير البريكابالين المسكن للآلم الناجم عن حقن الفورمالين في المنطقة الأخرى من قدم الفرخ. وقد ظهر ذلك من خلال زيادة ملحوظة في بداية رفع القدم اليمنى وانخفاض كبير في عدد مرات رفع القدم اليمنى مقارنة بمجموعة السيطرة، كما لوحظ انخفاض معنوي في سمك القدم اليمنى مقارنة مع مجموعة السيطرة مما يدل على التأثير المضاد للالتهابات للبريكابالين مقارنة مع مجموعة السيطرة.