Evaluation of the Anti-Inflammatory Effect of Pioglitazone in Experimental Models of Inflammation in Rats

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Abstract

The antidiabetic thiozolidinediones (TZDs) a class of peroxisome proliferators-activated receptor (PPAR) ligands has recently been the focus of much interest for their possible role in regulation of inflammatory response. The present study was designed to evaluate the anti-inflammatory activity of pioglitazone in experimental models of inflammation in rats. The present study was conducted to evaluate the anti inflammatory effect of TZDs (pioglitazone 3mg/Kg) on acute, sub acute and chronic model of inflammation by using egg-albumin and formalin–induced paw edema in 72 rats, relative to reference drugs Dexamethasone 5mg/Kg and Piroxicam 5mg/Kg. In each inflammation model, 24 rats were allocated into four subgroups, each containing six rats representing control, two standards, and test groups. All treatments were given (I.P) 30 minutes before induction of inflammation and the increase in paw edema was measured at certain time intervals by using vernier caliper. Pioglitazone produced nonsignificant reduction (P>0.05) of egg albumin-induced acute inflammation of the rat hind paw, while significantly produced time-related reduction of formalin-induced sub-acute and chronic inflammation of the rat hind paw. In conclusion, pioglitazone possesses anti-inflammatory activity in the animal models of sub-acute and chronic inflammations.

Key Words: Pioglitazone, PPAR-γ, anti-inflammatory activity

الخلاصة

أخذ الأهتمام يزداد في الآونة الأخيرة بدراسة الفعالية المحتملة المضادة للألتهاب لمشتقات الثايوزوليدينديون والمستخدمة حاليا بشكل فعال في معالجة داء السكري. تم تصميم الدراسة الحالية لتقييم الفعالية المضادة للألتهاب لمادة بيوكليتازون في النماذج التجريبية للألتهابات في الجرذان، حيث تمت دراسة التأثير المضاد للألتهاب لجرعة مقدارها ٣ملغم/كغم من مادة بيوكليتازون في حالات الألتهاب الحادة المستحدثة بواسطة زلال البيض، وتحت الحادة والمزمنة المستحدثة بواسطة الفورمالين في أقدام الجرذان وقياس مستوى تكون الوذمة المتكونة قبل وبعد استخدام المركب قيد الدراسة، ومقارنة مثل هذا التأثير مع ذلك الذي تسببه المركبات القياسية المضادة للألتهاب مثل البيروكسيكام والديكساميثازون. تم استخدام ٢٤ جرذا لكل نموذج من نماذج الألتهاب وتم تقسيمها الى أربعة مجموعات تمثل كل من مجموعة السيطرة، مجموعتا المركبات القياسية المضادة للألتهاب ومجموعة البيوكليتازون، حيث تم اعطاء جميع المركبات عن طريق الزرق في البريتون قبل استحداث الألتهاب بثلاثين دقيقة ومن ثم قياس حجم الوذمة المتكونة بواسطة الورنية في فترات زمنية محددة. أظهرت النتائج مقدرة البيوكليتازون على الحد من تكون الوذمة وبفارق معنوي يعتمد على الفترة الزمنية بعد الأعطاء في حالات الألتهاب تحت الحادة والمزمنة، أما في حالات الألتهاب الحادة فلم يظهر مثل هذا التأثير. يمكن الأستنتاج بأن للبيوكليتازون فعالية مضادة للألتهاب في النماذج التجريبية للألتهابات تحت الحادة والمزمنة المستحدثة في الجرذان.

Introduction

Inflammation is a complex biological set of interactions between soluble factors and cells that can arise in any tissue due to disturbed homeostasis in response to traumatic, infectious, post-ischemic, toxic or autoimmune injuries [(1)]. The inflammatory process is often viewed as being comprised of three closely linked phases: initiation, propagation and resolution. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [(2)]; however, if the targeted destruction and assisted repair are not properly phased, inflammation can lead to persistent tissue damage contributing to the pathogenesis of common chronic inflammatory diseases such as atherosclerosis, arthritis, inflammatory bowel disease and multiple sclerosis [(1,3,4)]. The current anti-inflammatory therapies designed to limit or interrupt the synthesis or action of mediators that drive the host's response to injury i.e. limit the initiation and propagation phases [(2)]. However, it is increasingly recognized that therapies aimed at enhancing the resolution phase will be important in limiting the damage associated with inflammation-based disease [(5)]. Recently, the modulatory role of peroxisome proliferator-activated receptors (PPARs) has been proposed in the inflammatory response of different tissues and organs [(6)].

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Three different isoforms of this receptor have been recognized; PPARα, PPARδ and PPARγ [7], the later is predominantly detected in adipose tissue, intestine and macrophages. PPARγ activators, such as pioglitazone, are a new class of oral antidiabetic drugs that ameliorate insulin resistance with an improvement in glucose control [8,9]. Next to their anti diabetic properties, these drugs were shown to exert variety of anti-inflammatory and vasoprotective effects in diabetic and non diabetic subjects [10,11,12]. These recent findings provide opportunities for the potential therapeutical application of these drugs in chronic inflammatory diseases with fewer side effects than traditional anti-inflammatory drugs.The present study was carried out to evaluate the anti-inflammatory effect of pioglitazone relative to commonly used anti-inflammatory drugs piroxicam and dexamethasone.

Materials and Methods

The present study was carried out on 72 Sprague-Dawley rats of both sexes weighing 180-250 gm, selected from the animal house of the College of Pharmacy, University of Baghdad. The animals were maintained on normal temperature, humidity and light/dark cycle. They fed standard rat pellet diet and had free access to water until the night of the day of investigation. The animals were allocated into three main groups each of 24 animals for evaluation the anti-inflammatory effect of Pioglitazone on acute, sub acute and chronic inflammation models. For egg albumin-induced acute inflammation, after an overnight fasting 24 animals were allocated into four subgroups (each of six rats), the control group was treated with dimethylsulfoxide 2 ml/Kg (vehicle), the two standard groups were treated with piroxicam 5mg/Kg, and dexamethasone 5mg/Kg respectively, while the test group was treated with pioglitazone 3mg/kg. All drugs were administered intraperitoneally**.** Thirty minutes after drug treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin [13,14] into the dorsal surface of the right hind paw. The increase in paw edema as a result of inflammation was measured using vernier caliper method [15], where thickness was measured by vernier before and 1hr, 2hr, 3hr and 4hr after induction of inflammation. The difference in paw thickness after and before induction of inflammation was calculated and determined as mean increase in paw thickness (mm). The ability of anti-inflammatory drug to suppress paw inflammation was expressed as percentage of inhibition of paw edema [16].In formalin-induced sub acute inflammation, the test group was treated with pioglitazone 3mg/Kg and the two standard groups were treated with piroxicam 5mg/Kg and dexamethasone 5mg/Kg, while the control group was treated with dimethylsulfoxide 2ml/Kg. All drugs were administered intraperitoneally 30 minutes before induction of inflammation and the paw thickness was measured by vernier caliper[15] immediately prior to drug administration (at zero time) and then at 1.5hr, 24hr, and 48hr after formaldehyde injection. Mean increase in paw thickness and the percentage of inhibition then calculated as mentioned previously. Chronic inflammation was induced by injection of 0.1ml of 2% formalin into sub planter area of the right hind paw of rat [17]**.** All treatments were administered 30 minutes prior to formalin injection and continued for seven consecutive days**.** The increase in paw thickness was measured by vernier caliper method [15] before and six days after induction of inflammation**.** The mean increase in paw thickness and the percentage of inhibition was calculated as in previous models. All data were expressed as mean ±SEM. Comparisons between groups were performed by ANOVA and Student's t-test to evaluate the statistical differences. The *P* value < 0.05 was considered significant.

Results

The anti-inflammatory effect Pioglitazone on acute inflammatory model was illustrated in table 1 and figure 1. Treatment with dexamethazone and piroxicam significantly reduced egg albumin-induced paw edema ($P<0.05$) compared to control group after 1hr, 2hr, 3hr and 4hr after induction of inflammation, while treatment with Pioglitazone results in non-significant reduction ($P>0.05$) in paw thickness compared to control group all over the period of investigation.

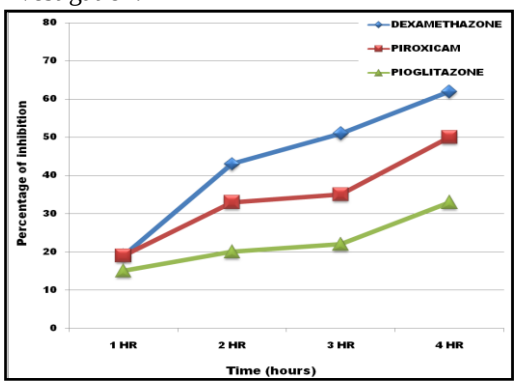

Figure 1. Effect of Pioglitazone on egg albumin-induced acute inflammation in rats.

Table 1. Effect of Pioglitazone on egg albumin-induced acute inflammation in rats.

Treatment Groups	Mean increase in paw thickness (mm)				% of inhibition			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Dimethyl sulfoxid	3.07 ± 0.24	2.47 ± 0.20	1.95 ± 0.18	1.52 ± 0.16	–	–	–	–
Dexamethazone	2.47 ±0.11$^{*\,a}$	1.40 ±0.11$^{*\,a}$	0.95 ±0.04$^{*\,a}$	0.58 ± 0.03$^{*\,a}$	19	43	51	62
Piroxicam	2.48 ±0.05$^{*\,a}$	1.65 ±0.09$^{*\,a}$	1.27 ±0.05$^{*\,b}$	0.76 ± 0.14$^{*\,a}$	19	33	35	50
Pioglitazone	2.62 ±0.22$^{*\,a}$	1.98 ±0.15$^{*\,a}$	1.53 ±0.17$^{*\,a}$	1.02 ±0.19$^{*\,a}$	15	20	22	33

Data were expressed as mean ± SEM; number of animals = 6 in each group; * P< 0.05 with respect to control group; values with non-identical superscripts (a, b) are considered significantly different (P<0.05).

The suppressive effect of pioglitazone in formalin-induce sub-acute inflammation was shown in table 2 and figure 2; all drug treatments significantly reduced the paw edema during the whole time of assessment compared to control group at 1.5 hr, 24 hr and 48 hr (P<0.05). Pioglitazone (3 mg/Kg) showed significant reduction in paw thickness (P<0.05) compared to piroxicam and dexamethazone over all the time of assessment. Table 3 demonstrated the effect of Pioglitazone on formalin-induced chronic inflammation; all treatments significantly reduced the paw edema induced by formalin (P<0.05) compared with control group. Both Pioglitazone and piroxicam produced comparable effect on formalin-induced chronic inflammation and no significant differences were detected between them; while their effect was significantly different compared to that produced by dexamethazone (P<0.05), which produced the greatest effect.

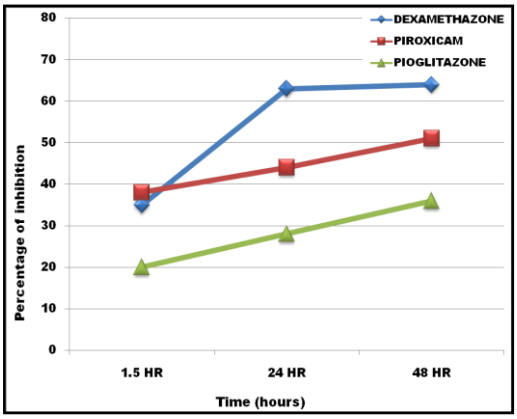

Figure 2. Effect of Pioglitazone on formalin-induced sub-acute inflammation in rats.

Table 2. Effect of pioglitazone on formalin-induced sub-acute inflammation in rats

Treatment Groups	Mean increase in paw thickness (mm)			% of inhibition		
	1.5 h	24 h	48 h	1.5 h	24 h	48 h
Dimethyl sulfoxide	3.12 ±0.17	2.87 ±0.19	2.68 ±0.20	_	_	_
Dexamethasone	2.03 ±0.15*^{a}	1.07 ±0.09*^{a}	0.97 ±0.10*^{a}	35	63	64
Piroxicam	1.93 ±0.05*^{a}	1.62 ±0.06*^{b}	1.32 ± 0.05*^{b}	38	44	51
Pioglitazone	2.50 ±0.06*^{b}	2.08 ±0.11*^{c}	1.72 ±0.07*^{c}	20	28	36

Data were expressed as mean ± SEM; number of animals = 6 in each group; * P<0.05 with respect to control group; values with non-identical superscripts (a, b, c) are considered significantly different (P<0.05).

Discussion

Egg albumin-induced paw edema in rats an *in vivo* model of inflammation [18], which has long accepted as a useful tool to study and evaluate drugs with anti inflammatory activity in acute inflammation [19,20]**.** The degree of swelling in paws injected with egg albumin was maximal 1hr after injection and then decreased with time**.** In the present study, the effect of Pioglitazone was evaluated on egg albumin- induced edema as a model for acute inflammation, where the data (table 1; figure 1) revealed no significant reduction in paw edema compared to control group; this could be explained by the fact PPARγ ligands regulate gene expression [21,22], which consequently produce their expected effects after a characteristic lag time that may extend to several hours. So, the improvement of the induced pathological state not occur immediately, but require enough time which represent that required for the synthesis of new signaling protein [23]. In sub-acute and chronic models of inflammation, injection of formaldehyde in the hind paw of rats produced pain and peripheral tissue inflammation [24], which is biphasic and includes a phase of inflammatory response, where histamine, 5-HT, PGs and bradykinin are involved [25]. In the model of sub-acute inflammation, Pioglitazone (3mg/Kg) produced significant reduction (P<0.05) in paw thickness along the period of investigation compared to control group, and the level of inhibition is found to be less than that produced by standard anti-inflammatory drugs (piroxicam and dexamethazone) as shown in table 2 and figure 2; this effect may be attributed to repression of synthesis of many inflammatory mediators. Many studies have demonstrated that PPARγ agonists inhibit the production of several inflammatory cytokines [26,27], including those that plays an important role in the nociceptive and inflammatory responses induced by formaldehyde, moreover, Inhibition of the production of ecosanoids and NO has also been demonstrated after treatment with PPARs agonists [28,29, 30]. In chronic inflammation, this represents a continuous inflammatory state that could be driven by the development of an immune response to an endogenous antigen [31]. The effect of Pioglitazone on formalin-induced paw edema, as a chronic inflammatory model, was assessed by vernier caliper method. Pioglitazone significantly reduced paw thickness (P<0.05) and the level of inhibition was found to be higher than that of piroxicam but less than dexamethazone inhibitory effect as shown in table 3. This result may provide an indication about the possible usefulness of Pioglitazone in the management of chronic inflammation of many diseases. Recently, Pioglitazone was tested in different chronic inflammatory diseases including neurological, cardiovascular and gastrointestinal diseases**.** It significantly accelerates ulcer healing in experimental animals due to hyperemia at ulcer margin and the anti inflammatory action including suppression of pro inflammatory cytokine, down regulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) at the level of mRNA and protein synthesis [32]**.** Also pioglitazone has been observed to ameliorate pancreatic damage associated with Cerulin-induced pancreatitis (CIP) by inhibiting the production and release of IL-1β [33]**.** It effectively provides neuroprotection against LPS insult in dopaminergic neurons through the inhibition of JNK-NF-kB pathways as well as suppression of COX-2 activity and decreased PGE_2 production [34]**.**In conclusion, pioglitazone showed reproducible anti-inflammatory activity in sub-acute and chronic models of inflammation in rats within the therapeutic dose utilized to increase sensitivity to insulin, which is comparable to that produced by piroxicam and less than that produced by dexamethasone.

Table 3. Effect of Pioglitazone on formalin-induced chronic inflammation in rats.

Treatment Groups	Mean increase in paw thickness (mm) after 6 days	% of inhibition
Dimethyl sulfoxide	3.30 ± 0.15	–
Dexamethazone	1.18 ± 0.14*[a]	64
Piroxicam	2.08 ± 0.13*[b]	37
Pioglitazone	1.85 ± 0.09*[b]	44

Data were expressed as mean ± SEM; number of animals = 6 in each group;
P < 0.05 with respect to control group; values with non-identical subscription (a, b) are considered significantly different (P<0.05).

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