EFFECT OF PENTOXIFYLLINE ON ADJUVANT-INDUCED ARTHRITIS IN ALBINO RATS

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ABSTRACT
Rheumatoid arthritis (RA) is a common inflammatory autoimmune disorder. Non-steroidal anti-inflammatory drugs (NSAIDs) have become an integral part of RA therapy. Adverse effects of these drugs are widely expanding. Data implicate the cytokine tumor necrosis factor-alpha (TNF-α) in the pathophysiology of RA as well as involved in the indomethacin induced gastrointestinal damage. Pentoxifylline (PTX), a methylxanthine derivative is documented to possess anti-inflammatory and anti-TNF-α properties.

The present study was conducted to investigate the effect of PTX on edema, serum malondialdehyde (MDA) and TNF-α in experimentally induced collagen II adjuvant arthritis in albino-rats. Forty-two male albino rats weighing 200-250 grams were used throughout the study. The animals were divided into seven equal groups. Group (1): Non-arthritis control rats received daily 0.5 ml intragastric isotonic saline for 6 weeks. Group (2): Arthritic control rats received daily 0.5 ml isotonic saline intragastrically for 6 weeks. Group (3): Arthritic rats treated intragastrically with indomethacin (1.3 mg/kg/day) for 6 weeks. Group (4): Arthritic rats treated with daily intragastric PTX (50 mg/kg) for 6 weeks. Group (5): Arthritic rats treated with PTX (100 mg/kg/day), intragastrically for 6 weeks. Group (6): Arthritic rats treated with PTX (50 mg/kg/day) given 30 minutes before administration of indomethacin.

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of peripheral vascular disease because of its potent hemorrheological properties (6) subsequently, PTX was found to have anti-inflammatory properties mediated via inhibition of phosphodiesterases (7).

In vitro, PTX inhibits monocyte production of TNF-α (8) that thought to play a central role in the pathogenesis of many diseases like RA. Modest clinical effects have also been observed in RA (5,34). Beneficial effects of PTX have been reported in idiopathic dilated cardiomyopathy (9), childhood type I diabetes (10), and systemic vasculitis (11). Furthermore, PTX inhibits lipopolysaccharide induced production of TNF-α by monocytes and T-cells as well as interleukin-2 induced (IL-2) adherence to leukocytes (12,13). Substantial evidence indicates that inflammatory cytokines subserve a crucial role in joint destruction and disease propagation in RA (14). Among these cytokines, TNF-α has been considered as the pivotal factor to induce and sustain tissue damage by activating the inflammatory mediator cascade, stimulating the mechanism of angiogenesis and up-regulating the vascular endothelial adhesiveness (15). TNF-α is found in elevated level in sera of RA patients (15). Thus this study was carried out to examine whether treatment with PTX ameliorates edema and inflammation in rats with CIA. Furthermore this study aimed to test the hypothesis that PTX inhibits pro-inflammatory cytokine (TNF-α) production in CIA in rats.

MATERIALS AND METHODS

Drugs used:

- Pentoxifylline (PTX) powder is supplied by Sigma Co, dissolved in sterile isotonic saline. Control rats received an equivalent volume of the vehicle.
- Indomethacin, Sigma Co, dissolved in sterile isotonic saline.

Statistical Analysis:

One-way analysis of variance (ANOVA) was done to compare between the studied groups followed by Student’s "T" test according to Phipkins (17) to compare statistically the significant changes between control group and test group. P values ≤ 0.5 were considered to be statistically significant.

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base of each rat. A boaster dose was given after 3 weeks. After 45 days the systemic arthritis was manifest in both hind paws. Inflammatory and anti-inflammatory effects (pain tolerance and edema development and suppression) were assessed by using the analgesimeter and paw edema tests respectively (21,22). At the end of the experiment, all rats were sacrificed by knife. Trunk blood of each rat was collected and centrifuged at 1000 r.p.m for 15 min. the unhaemalyzed serum samples were separated carefully and stored at -70°C until used for assay. Serum CRP was measured by using latex particles of agglutination (23,24). Lipid peroxidation was assessed spectrophotometrically by measuring serum MDA using thiobarbituric acid method (25). In addition to measurement of serum tumor necrosis factor-α according to Carti et al (26).

RESULTS

Induction of collagen-II adjuvant arthritis produced a significant decrease in analgesometric pressure tolerance, significant increase in paw edema thickness as well as significant increase in serum CRP, MDA and TNF-α (Tab.1).

Daily administration of indomethacin (1.3 mg/kg) for 6 weeks produced significant increase in analgesometric pressure tolerated by the arthritic rats, significant decrease in serum CRP and MDA but significant increase in serum TNF-α (tab. 2&3).

Intragastric administration of PTX in a dose of 50 or 100 mg/kg/day produced significant improvement of arthritic rats. This improvement was indicated by significant decrease in serum CRP and MDA as well as increase in analgesometric pressure tolerated by the rats. Also of PTX in either doses produced significant decrease in TNF-α as compared to the arthritic control (Tab.2 &3). Moreover, administration of PTX in either daily dose 50 mg/kg or 100 mg/kg 30 minutes before administration of indomethacin (1.3 mg/kg/day, intra-gastrically for 6 weeks) induced significant increase in analgesometric pressure tolerated by the arthritic rats and significant decrease in paw edema thickness as well as significant decrease in serum TNF-α as compared to administration of indomethacin alone (tab. 2 &3).
Table (2): Effect of intragastric administration of indomethacin and pentoxifylline (PTX) for 6 weeks on analgesic metric pressure, paw edema thickness and serum C-reactive protein (CRP) in arthritic rats. (Mean ±SE).

<table>
<thead>
<tr>
<th>Group</th>
<th>Analgesic pressure (grams)</th>
<th>Paw edema thickness (cm)</th>
<th>Serum CRP (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>Arthritic control</td>
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<tr>
<td>n = 6</td>
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<tr>
<td></td>
<td>Right paw (R)</td>
<td>Left paw (L)</td>
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<tr>
<td></td>
<td>128 ± 9.5</td>
<td>120 ± 10.1</td>
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<tr>
<td></td>
<td>3.1 ± 0.12</td>
<td>3.3 ± 0.13</td>
<td>105 ± 6.7</td>
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<tr>
<td>Indomethacin treated (1.3 mg/kg/day)</td>
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<tr>
<td></td>
<td>350 ± 12.3*</td>
<td>348 ± 11.6*</td>
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<tr>
<td></td>
<td>2.0 ± 0.2*</td>
<td>2.1 ± 0.17*</td>
<td>No agglutination (&lt; 6 mg/L) *</td>
</tr>
<tr>
<td>PTX-treated (50 mg/kg/day)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>209 ± 10.1*</td>
<td>203 ± 8.9*</td>
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<tr>
<td></td>
<td>2.5 ± 0.3*</td>
<td>2.3 ± 0.1*</td>
<td>No agglutination*</td>
</tr>
<tr>
<td>PTX-treated (100 mg/kg/day)</td>
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</tr>
<tr>
<td></td>
<td>215 ± 12.3*</td>
<td>212 ± 10.5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.3 ± 0.2*</td>
<td>2.6 ± 0.1*</td>
<td>No agglutination*</td>
</tr>
<tr>
<td>Indomethacin (1.3 mg/kg/day) + PTX (50 mg/kg/day)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>352 ± 3.4*</td>
<td>345 ± 11.5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.1*</td>
<td>2.2 ± 0.1*</td>
<td>No agglutination*</td>
</tr>
<tr>
<td>Indomethacin (1.3 mg/kg/day) + PTX (100 mg/kg/day)</td>
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<tr>
<td></td>
<td>345 ± 4.1*</td>
<td>358 ± 6.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.3 ± 0.2*</td>
<td>2.1 ± 0.14*</td>
<td>No agglutination*</td>
</tr>
</tbody>
</table>

SE = standard error
* = significant difference between arthritic treated versus arthritic control groups (p ≤ 0.05).
* = significant difference between PTX or PTX+ indomethacin treated versus indomethacin
DISCUSSION

Collagen induced arthritis (CIA) is an experimental model of autoimmune disease \(^{(27)}\). It can be induced in mice \(^{(27)}\), rats \(^{(28)}\), and monkeys \(^{(29)}\) by immunization with type II collagen (C11). Many features of CIA resemble those of RA in human \(^{(30)}\).

In the present study CIA developed within 45 days after ID injection of C11 in CFA as evidenced by significant decrease in pain threshold and increase the mean hind paw edema thickness accompanied by increase in serum CRP, MDA and TNF-\(\alpha\). These results are in accord with those observed by Rodriguez et al \(^{(31)}\) and Yamaki et al \(^{(32)}\).

Intragastric administration of indomethacin in a dose of 1.3mg/kg/day for 6 weeks to rats with CIA produced a potent anti-inflammatory activity as assessed by decrease paw edema thickness, increased analgesicmetric pressure tolerance, and serum CRP. These findings are in agreement with Yamaki et al \(^{(32)}\). Furthermore, administration of indomethacin to CIA rats resulted in significant increase in TNF-\(\alpha\). This finding is supported by the study of Reuter and Wallace and by the study of Souza et al \(^{(19,33)}\). Also, they suggested that PTX prevented acute gastric mucosal damage and neutrophil migration induced by indomethacin and reduced indomethacin induced release of TNF-\(\alpha\). TNF-\(\alpha\) acting via tumor necrosis factor alpha receptors-1 (TNF-\(\alpha\). R1) is involved in indomethacin induced gastric damage and granulocyte infiltration. In present study administration of indomethacin to arthritic rats induced a significant decrease in serum MDA. This findings is consistent with Twoney and Dale \(^{(16)}\) as they have been documented that NSAIDs can either attenuate or depress the neutrophil respiratory burst that occurs when cells become activated by specific stimuli. The respiratory burst generates the superoxide anion which give rise to tissue damaging oxygen metabolites.

In the present study, administration of PTX to the arthritic rats in a dose of 50 mg/kg/day or 100 mg/kg/day, intragastrically for 6 weeks resulted in significant improvement as indicated by decreased serum CRP, MDA as well as increase in an anal-
day, 30 minutes before administration of indomethacin produced significant increase in analgesmetric pressure tolerated by the arthritic rats and a significant decrease in paw edema thickness, as well as of significant decrease in serum TNF-α, as compared to administration of indomethacin alone. These results could be explained on the light of study of Couturier et al (44). Those authors reported that the intestinal damage produced by indomethacin could be significantly attenuated by pretreatment with specific type IV phosphodiesterase (PDE) inhibitor (RO-20-1724) by decreasing TNF-α. On the other hand Reuter and Wallace (19), reported that PDE inhibitor (PTX) had its protective effect against NSAID enteropathy through a mechanism independent of TNF-α synthesis inhibition. So the present study support the concept that antagonizing the action of TNF-α is successful in treating inflammatory disorders (RA) and for optimal effect in treating RA, PTX may have to be used as adjuvant therapy.

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المتخصص العربي
تأثير عقار البنتوكسيفليين على التهاب الفاصل الروماتيزمي المحدث معملياً في الفشان البيضاء
(دور معامل تحلل الأورام - ألفا)

كروان محمد عبد الرحمن
قسم الفارماكولوجيا الإكلينيكية - كلية الطب - جامعة المنصورة

أجري هذا البحث لدراسة مدى تأثير عقار البنتوكسيفليين على معامل تحلل الأورام - ألفا والشقوق الحرة في الفشان المصاب بمرض الالتهاب الفاصل. استخدم في أجراء هذا البحث عدد 24 فائراً أحياناً وقسمت إلى 7 مجموعات متساوية كالتالي:

المجموعة الأولى: مجموعة ضابطة عادية لم يحدث بها التهاب مفصلي روماتيزمي وأعطيت يومياً محلول ملح متوسط بقدر 1/2ملم عن طريق الفم وذلك لدة 6 أسابيع متتالية.

المجموعة الثانية: أحدث بها التهاب مفصلي وأعطيت محلول الملح كما سبق (مجموعة ضابطة للالتهاب المفصلي).

المجموعة الثالثة: أحدث بها التهاب مفصلي وعولجت بدواء الالتدوميثيدين بجرعة تعادل 1,3 مجم/كجم يومياً عن طريق الفم لمدة 6 أسابيع متتالية.

المجموعة الرابعة: مصابة بالتهاب مفصلي وأعطيت دواء البنتوكسيفليين بجرعة مقدراها 50 مجم/كجم يومياً وتفسئ المدة السابقة.

المجموعة الخامسة: تكونت من فشان مصابة بالتهاب المفصلي وعولجت بدواء البنتوكسيفليين بجرعة 100 مجم/كجم يومياً عن طريق الفم ولدته 6 أسابيع.

المجموعة السادسة: فشان مصابة بالتهاب المفصلي وعولجت بدواء البنتوكسيفليين بجرعة 50 مجم/كجم يومياً ثم أعطيت بعد 30 دقيقة دواء الالتدوميثيدين بجرعة 1,3 مجم/كجم يومياً ولدته 6 أسابيع متتالية.

المجموعة السابعة: مثل المجموعة السابقة ولكن تختلف عنها في جرعة البنتوكسيفليين حيث أعطى

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