Effect of candesartan cilexte in Freund’s adjuvant-induced arthritis in albino rats

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Abstract

The renin-angiotensin system plays an important role in the regulation of cardiovascular, renal, and endocrine functions. Local functional renin-angiotensin systems (RAS) have been demonstrated in many organ and tissue systems.

It was found that the angiotensin II receptor blockers (ARBs) suppressed the development of severe arthritis and joint destruction in the collagen-induced arthritis model. These findings suggest that ARBs may have therapeutic potential in rheumatoid arthritis.

All animals (30 male rats) were subjected to measurements of the hind paw thickness and pressure threshold by using analgesymeter. Subsequently, Freund’s adjuvant was injected into the base of rat-tail. Sixteen days after administration of Freund’s adjuvant, animals were again subjected to the previously mentioned tests and were randomly assigned to 3 groups:

1) Control group
2) candesartan cilextil Treated group
3) celecoxib treated group

All animals were subjected to the previous tests at days 15,30 after starting treatment, then all animals were scarified, following scarification, the stomachs were removed. Gastric contents were collected for evaluation of biochemical parameters. The stomachs were opened along the greater curvature and the gastric lesions were evaluated for identifying the ulcer index.

In this work candesartan cilextil and celecoxib showed significant beneficial effect (anti-inflammatory and analgesic) compared to arthritic control non-treated group.

Introduction

The renin-angiotensin system plays an important role in the regulation of cardiovascular, renal, and endocrine functions. Local functional renin-angiotensin systems (RAS) have been demonstrated in many organ and tissue systems. Angiotensins, the effector growth factors of the RAS, are essentially cytokines and growth factors, which actively contribute to many inflammatory reactions. Recent
studies have demonstrated that angiotensin II has proinflammatory effects that may contribute to the pathogenesis of immune-mediated diseases. Moreover, Locally generated active renin and ACE could contribute to joint destruction in rheumatoid arthritis. (Cobankara et al, 2005). In vitro and in vivo studies have shown that angiotensin II blockade significantly reduces concentrations of proinflammatory mediators and oxidative stress products in numerous inflammatory models. Interruption of angiotensin II activity with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers has been beneficial for patients with inflammatory diseases. Much of this benefit occurs independent to the antihypertensive effect of angiotensin II interruption, suggesting a distinctive protective mechanism. Angiotensin II receptor blockers may represent a novel class of antiinflammatory drugs with indications far beyond cardiovascular (Dagenesis and, Jamali, 2005)

It was found that The angiotensin II receptor blockers (ARBs) , olmesartan, suppressed the development of severe arthritis and joint destruction in the collagen-induced arthritis model. These findings suggest that ARBs may have therapeutic potential in rheumatoid arthritis. (Sagawa et al, 2005)

Moreover, both the non-thiol ACE inhibitor quinapril and the angiotensin receptor blocker candesartan have significant anti-inflammatory properties, sufficient to suppress the severity of collagen-induced arthritis, either as prophylaxis or as therapy in established disease. Administration of quinapril from the time of antigen exposure may influence the priming of the immune response, as indicated by the sustained effect after quinapril withdrawal and by the reduction in bCII-specific IgG2a antibodies. The data of the present study also show that quinapril has clear effects on TNF-α production, a key cytokine in the pathology of human inflammatory arthritis. Suppression of arthritis by quinapril is also associated with reduced expression of TNF-α within the joints of mice and with diminished expression of splenocyte TNF-α production following LPS stimulation in vitro. (Dallbeth et al, 2005)

Looking to the problem from other side showing that gastroenteropathy is the most common among patients who use non-steroidal anti-inflammatory drugs for the treatment of inflammatory disorders. It is known that rheumatoid arthritic patients are more susceptible to non-steroidal anti-inflammatory -induced gastropathy than other non-steroidal anti-inflammatory users. The gastric mucosal lesions induced by indomethacin, one of conventional non-steroidal anti-inflammatory, were markedly aggravated in arthritic rats. Likewise, the healing of chronic gastric ulcers induced by thermal cauterization was significantly delayed in arthritic rats. The underlying mechanisms of these phenomena observed in arthritic rats may be attributable to the enhancement of inducible NOS/NO pathway in the former and the less expression of
various growth factors in the ulcerated mucosa, such as basic fibroblast growth factors (bFGF) or insulin-like growth factors (IGF-1) in the latter (Kato, 2001).

**Kato and Takeuchi (2002),** concluded that arthritic condition alter ulcerogenic and healing responses in stomach. In addition, cyclooxygenase-2 (COX-2) selective inhibitors, such as rofecoxib or celecoxib, proved to induce apparent gastric lesions in arthritic rats, suggesting that a caution should be paid on the use of COX-2 selective inhibitors in rheumatoid arthritic patients.

Freund’s adjuvant-induced arthritis have been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance for the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (Besson and Guilbaud, 1988). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in humans (Anderson et al., 2004).

The aim of this work is to evaluate the possible therapeutic effect of candesartan cilexetil, angiotensin receptor blocker, in Freund’s adjuvant-induced arthritis and its safety, regarding the stomach, in comparison with COX2 selective inhibitors, celecoxib.

**Materials and methods**

**Drugs**
Candesartan (Astra-Zeneca, Egypt).
Celecoxib (Pfizer co. Egypt).

**Animals**
Thirty male albino rats were used and randomly classified into 3 groups:
Control group (n=10)
Candesartan arthritis treated group (n=10)
Celecoxib arthritis treated group (n=10)

**Induction of arthritis**
An experimental model of polyarthritis was done by a single intra-dermal injection of 0.1 ml of a 10 mg/ml suspension of freeze dried mycobactrium tuberculosis (Freund’s complete adjuvant) into the base of rat tail (Dardick et al., 1986). Approximately 16 days after injection of Freund’s complete adjuvant, arthritis reach its maximum at both hind paws. The day of injection is called (-16) and the day of peak arthritis is called day (0).

**The experimental parameters**
I) Paw edema test
The reduction in the swelling of the hind paws of the adjuvant arthritic rats induced by the tested drugs was taken as a measure for the anti-inflammatory activity of the drug. The thickness of hind paw of the rats was measured by edema meter. (Winter et al., 1963)

II) Analgesymeter:
The reaction threshold to pressure is a parameter in adjuvant arthritic rats used to describe the course of adjuvant arthritis and the effect of therapy on the disease (Winter et al., 1979). The pressure was applied by the analgesymeter apparatus (Vago Basile, Italy) on the paw of the hind limb of the rat until the rat either squeaks or tries to withdraw its limb.

Animals: The study was conducted by using 30 male albino rats of local strain weighing 100-200g. The animals were allowed food and water.

Design of the work
On Day (-16), all animals (30 male rats) were subjected to measurements of the hind paw thickness and pressure threshold by using analgesymeter. Subsequently, Freund’s adjuvant was injected into the base of rat-tail in all animals. Sixteen days after administration of Freund’s adjuvant, animals were again subjected to the previously mentioned tests and were randomly assigned to 3 groups:
I) Control group that received distilled water 1 ml/kg/day orally for 30 days.
II) candesartan cilextil arthritic Treated group in a dose of 5 mg/kg/day dissolved in distilled water orally for 30 days (Dallbeth et al, 2005).
III) celecoxib arthritic treated group in a dose of 100 mg/kg/day dissolved in distilled water orally for 30 days (Kato And Takeuchi., 2002).

Assessment of Paw edema test and reaction threshold to pressure for all groups.

Fig.1)
All animals were again subjected to the previous tests at days 15,30 after starting treatment, the animals were scarified after 30 days of treatment, following scarification, the stomachs were removed. Gastric contents were collected for evaluation of biochemical parameters. The stomachs were opened along the greater curvature and the gastric lesions were evaluated for identifying the ulcer index according to (Yamamoto et al., 1984).

All photometric measurements were carried out using Shimadzu spectrophotometer UV 1201 (Japan). The collected gastric secretions were centrifuged at 3000 rpm for 10 m then the supernatant was used for determination of:
1) Volume of gastric secretion according to Shy et al., 1984.
2) Titrable acidity according to Grossman, 1963.
3) Peptic activity according to Sanyal et al, 1982.
4) Mucin concentration in gastric secretion according to Brodie and Hook 1971.

Statistical Analysis:
Results are expressed as the mean ±SE, and three different groups were compared using analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons.

Results

Anti-inflammatory and analgesic Effect of candesartan cilextil and celecoxib in Freund’s adjuvant-induced arthritis in albino rats:

Table 1 and 2 show significant (p<0.05) difference in thickness of the hind paw (mm) and hind paw pressure threshold in grams at day (-16) and days 0,15 and 30 in control and arthritic groups. Freund’s adjuvant caused statistical significant increase in hind paws thickness in all rats. Candesartan cilextil decreased the thickness of the hind paws at day 15 (16 %) and at day 30 (29.5%, P<0.05). Also, it increased hind paw pressure threshold in grams at day 15 by 55.1 % and at day 30 by 107.8 % (P<0.05) Celecoxib decreased the thickness of the hind paws at day 15 by 24% ( P<0.05) and at day 30 by 38% (P<0.05). Also, it increased hind paw pressure threshold in grams at day 15 by 95.5% (P<0.05) and at day 30 by 130.9% (P<0.05).

(Table 1) Assessment of the anti-arthritis effect of candesartan cilextil (5mg/kg/day orally) and celecoxib (100mg/kg/day orally) on thickness of right hind paw (Mean ± SE) in Freund’s adjuvant-induced arthritis in albino rats using paw-edema meter test.
### Thickness of the right hind paw (mm)

<table>
<thead>
<tr>
<th></th>
<th>Day (-16)</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control arthritic group (n=10)</strong></td>
<td>2.05 ± 0.08</td>
<td>3.86† ±0.03</td>
<td>3.82† ±0.06 (-1%)</td>
<td>3.66† ±0.05 (-5.2%)</td>
</tr>
<tr>
<td><strong>Arthritic candesartan treated group (n=10)</strong></td>
<td>2.03 ± 0.06</td>
<td>3.83† ±0.05</td>
<td>3.21†* ±0.06 (-16%)</td>
<td>2.7* ±0.13 (-29.5%)</td>
</tr>
<tr>
<td><strong>Arthritic celecoxib treated group (n=10)</strong></td>
<td>2.05 ± 0.018</td>
<td>3.72† ±0.05</td>
<td>2.87†* ±0.16 (-24%)</td>
<td>2.31* ±0.07 (-38%)</td>
</tr>
</tbody>
</table>

†=Statistically significant (p<0.05) compared to day (-16)
*=Statistically significant (p<0.05) compared to corresponding date in arthritic control group.

**NB:** % reduction is calculated compared to day 0

(Table 2) effect of candesartan cilextil (5 mg/kg/day orally) and celecoxib (100mg/kg/day orally) on hind paw pressure threshold in grams (Mean ± SE) in Freund’s adjuvant-induced arthritis in albino rats using analgesymeter.

### Hind paw pressure threshold in grams

<table>
<thead>
<tr>
<th></th>
<th>Day (-16)</th>
<th>Day (0)</th>
<th>Day 15</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control arthritic group (n=10)</strong></td>
<td>71.5 ± 1.14</td>
<td>20.11† ±1.34</td>
<td>21.1† ±1.07 (4.9)</td>
<td>24.1† ±1.13 (19.8%)</td>
</tr>
<tr>
<td><strong>Arthritic candesartan treated group (n=10)</strong></td>
<td>70.86 ± 1.92</td>
<td>20.53† ±1.06</td>
<td>31.8†* ±1.14 (55.1%)</td>
<td>42.6†* ±1.23 (107.8%)</td>
</tr>
<tr>
<td><strong>Arthritic celecoxib treated group (n=10)</strong></td>
<td>71.3 ±1.68</td>
<td>28.5† ±1.42</td>
<td>55.76†* ±1.77 (95.5%)</td>
<td>65.82*• ±1.77 (130.9%)</td>
</tr>
</tbody>
</table>

†=Statistically significant (p<0.05) compared to day (-16)
*=Statistically significant (p<0.05) compared to corresponding date in arthritic control group.

**NB:** the % of increase Hind paw pressure threshold was calculated compared to day (0)
Gastric Effects of candesartan and celecoxib in Freund’s adjuvant-induced arthritis in albino rats

Table 3 shows that Celecoxib (100 mg/kg/day) caused gastric lesion with ulcer index is 0.6 ± 0.024, which is not statistically different from control. Also, it increased significantly (P < 0.05) volume of gastric secretion (114%), titrable acidity (50.4%), peptic activity (102.2%) compared to control, but mucin concentration in gastric secretion was decreased significantly (22.8%) compared to control. On the other hand, in candesartan treated group there was insignificant difference in all parameters compared to control.

Table (3): effect of candesartan cilextil (5 mg/kg/day orally) and celecoxib (100 mg/kg/day orally) in ulcer index, volume of gastric secretion, Titrable acidity, mucin concentration and peptic activity (Mean ± SE) in Freund’s adjuvant-induced arthritis in albino rats

<table>
<thead>
<tr>
<th></th>
<th>Arthritic control group</th>
<th>Arthritic candesartan treated group</th>
<th>Arthritic celecoxib-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.6 ± 0.024</td>
</tr>
<tr>
<td>Volume of gastric secretion (ml/h)</td>
<td>0.28 ± 0.012</td>
<td>0.31 ± 0.022</td>
<td>0.6 ± 0.04†</td>
</tr>
<tr>
<td>Titrable acidity (m Eq/L)</td>
<td>13.3 ± 0.43</td>
<td>15.4 ± 0.63</td>
<td>20 ± 0.745†</td>
</tr>
<tr>
<td>Peptic activity (µM tyrosline/ml/min)</td>
<td>7.12 ± 0.159</td>
<td>8.03 ± 0.45</td>
<td>14.4±1.03†</td>
</tr>
<tr>
<td>Mucin concentration in gastric secretion (mg hexose %)</td>
<td>58.85±1.5</td>
<td>56.76±2.2</td>
<td>45.42±1.46†</td>
</tr>
</tbody>
</table>

†=Statistically significant (p<0.05) compared to arthritic control group
Ulcer index=The gastric lesions were measured, summed and scored from 1-5.

Discussion

In this work candesartan cilextil and celecoxib showed significant beneficial effect (anti-inflammatory and analgesic) compared to arthritic control non-treated group.

The proven efficacy of the angiotensin receptor blockers,candesartan cilextil is in accordance with many authors. (Price et al, 2007; Cobankara et al, 2005, Dagenes and , Jamali, 2005, Sagawa et al 2005) who investigate rennin
angiotensin interruption by either Angiotensin converting enzyme inhibitors or angiotensin receptor blockers.

Gastroenteropathy is the most common among patients who use non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of inflammatory disorders. It is known that rheumatoid arthritic (RA) patients are more susceptible to NSAID-induced gastropathy than other NSAID users. (Kato, 2001)

In this work, Celecoxib (100 mg/kg/day orally) induced gastric changes (with insignificant ulcer index compared to control arthritic group) associated with significant increase in volume of gastric secretion, titrable acidity, peptic activity and decreased mucin compared to control group. These parameters are considered aggressive factors except mucin, which is a gastroprotective factor (Huang and Hunt, 1999). The increased vascular permeability is an important factor in NSAIs induced gastric lesion (Karadi et al., 1999 and 2001).

Kato (2001) and Kato et al (2002) stated that oral administration of indomethacin caused hemorrhagic gastric lesions in both normal and arthritic rats, although the severity of lesions was significantly greater in the latter group. In contrast, neither rofecoxib nor celecoxib caused any gastric damage in normal rats even at higher dose (100 mg/kg), but both drugs provoked hemorrhagic gastric lesions in arthritic rats. The expression of COX-2 mRNA and immuno-positive cells was observed in the gastric mucosa of arthritic but not normal rats. The above authors concluded that COX-2 inhibitors produce gastric lesions in arthritic rats, similar to the nonselective COX-inhibitors.

COX-2 is up regulated in the stomach of arthritic rats, and PGs produced by COX-2 play a role in maintaining the integrity of the gastric mucosa. COX 2-inhibitors caused gastric lesion in helicobacter pylori infected animals (Takahashi et al, 2000). Also, Tachibana et al (2003) concluded that etodolac, a COX-2 selective NSAID and other NSAIDs inhibited paw swelling and caused gastric mucosal lesions in adjuvant arthritic rats in a dose-dependent manner. Also, the healing of chronic gastric ulcers was significantly impaired in rats with arthritis (Kato and Takeuchi 2002).

Dalbeth et.al.,(2005) demonstrated a role of Ang II in joint inflammation and the therapeutic benefit of ACE inhibitors in a collagen-induced mouse model of arthritis. The specificity of the anti-inflammatory effect of Ang II was confirmed using candesartan, a specific inhibitor of the AT1 receptor. However, ACE inhibitors are limited by their nonspecific effects on bradykinin and substance P,

Inhibiting ACE prevents the breakdown of bradykinin and substance P, both of which are known mediators of inflammation; thus, local inflammation is
potentially exacerbated. AT1 receptor antagonists avoid this because they selectively inhibit the action of Ang II at the receptor level. This may have an advantage, since inhibition of AT1 receptors leads to an increase in local Ang II levels (Alderman 1996)

The observations in an experimental model of arthritis are indicative for inhibiting AT1 receptors in the RA in humans, and demonstrated that generation of the proinflammatory cytokine TNFα was dose dependently inhibited by losartan (Price et. Al., 2007).

The investigations showing inhibition of collagen-induced murine arthritis using candesartan and olmesartan (Sagawa et.al., 2005), provides a strong evidence that angiotensin is likely to contribute significantly for the inflammatory process in RA (Price et. Al., 2007).

Although celecoxib increased aggressive factors and vascular permeability and decreased protective factor (mucin) but it produce insignificant ulcer index. Based on the results of the present work, candesartan has anti rheumatoid effect but with less probability of adverse effect. This finding needs to be proved clinically.

References


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تأثير الكاندزاتان في الروماتزم المخلق في فئران التجارب بواسطة فراوند

محى الدين السيد شريف, أحمد أبوالمعاطي الجزار, سامية الشيتى
قسم الفارماكولوجى, كلية طب بنها, جامعة بنها

يلعب نظام الرنين-أنجيوتنسين دوراً مهماً في تنظيم الوظائف الخاصة بالقلب والأوعية الدموية والكلى والغدد الصماء. وتشير الوظيفة المرضية لهذا النظام في عدد من الأعضاء والأنسجة.

لقد وجد أن مثبطات مستقبلات الأنجيوتنسين اتقلل حدوث التهاب المفاصل الشديد وتدمير المفاصل الناتج في موديل الألتهاب المفصلى المحدث بواسطة الكولاجين. لهذا من الممكن أن تستخدم مثبطات مستقبلات الأنجيوتنسين في علاج التهاب المفاصل.

لقد خضعت كل الحيوانات (30 فأر) إلى قياسات مقدار سمك المخلب الخلفي ومستوى الضغط عليه بواسطة استخدام جهاز احتمال الألم. ولقد تم حقن مادة فراوند في قاعدة ذيل الفأر، وبعد 16 يوم من الحقن تم تمت نفس القياسات السابقة ثم تم تقسيم الفئران عشوائياً إلى ثلاثة مجموعات 1 المجموعة الضابطة 2 المجموعة المعالجة بالكندزاتان 3 المجموعة المعالجة بالسيلوكوكسيب.

لقد تم عمل نفس القياسات السابقة لكل الفأر بعد 15, 30 يوم من بدء الغلافج، ثم تم قتل جميع الفئران وأخذ المعدة وتجميع محتوياتها لعمل الفحوص الكيميائية عليها، ثم تشغيل المغدة من ناحية المنحنى الكبير ويرجى تقييم جروح المغدة وعمل معيار للإلم في المعدة.

في هذا العمل نجد أن الكاندزاتان والسيلوكوكسيب لهم فائدة التسكين ومضاد للالتهاب ذو دلالة إحصائية مقارنة إلى المجموعة الضابطة.