ORIGINAL ARTICLE

Anti-inflammatory activity of telmisartan in rat models of experimentally-induced chronic inflammation: Comparative study with dexamethasone

Waleed K.G. Al-Hejjaja, Intesar T. Numanb, Raghdan Z. Al-Sa’adc, Saad A. Hussainb,*

a Department of Pharmacology, College of Pharmacy, University of Basrah, Iraq
b Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Iraq
c Department of Pharmacy, Babylon Directorate of Health, Babylon, Iraq

Received 22 June 2010; accepted 4 October 2010
Available online 4 November 2010

KEYWORDS
Telmisartan; PPARγ; Chronic inflammation

Abstract Recently, significant progress has been made through the application of peroxisome proliferator activated receptor-γ (PPAR-γ) agonists as anti-inflammatory drugs that are efficacious, relatively free of side effects, and can be used effectively for a long time. The present study was designed to evaluate the dose–response relationship of the anti-inflammatory activity of telmisartan in rat models of chronic inflammation. The study protocol includes four stages: First stage: 48 rats were allocated into eight groups, each containing six rats, for the study of the anti-inflammatory activity of different doses of telmisartan in rat model of formaldehyde-induced chronic inflammation. Second stage: six rats were used to study the anti-inflammatory activity of telmisartan (1.5 mg/kg) in combination with dexamethasone (0.5 mg/kg) in the same model. Third stage: 48 rats were allocated into eight groups, each containing six rats, for the study of the anti-inflammatory activity of telmisartan in rat model of cotton pellet-induced granuloma. Fourth stage: six rats were used to study the anti-inflammatory activity of telmisartan (1.5 mg/kg) when used as adjuvant with dexamethasone (0.5 mg/kg) in the same model. Telmisartan in a dose-dependent pattern (0.1, 0.2, 0.4, 0.6, 1.5, 3 mg/kg) significantly suppressed inflammation in rat models of formaldehyde-induced

* Corresponding author. Tel.: +964 7901712624.
E-mail address: saad_alzaidi@yahoo.com (S.A. Hussain).

1319-0164 © 2010 King Saud University. Production and hosting by Elsevier B.V. All rights reserved.

Peer review under responsibility of King Saud University.
doi:10.1016/j.jsps.2010.10.004
1. Introduction

Angiotensin II, the central product of the renin–angiotensin system, induces oxidative stress and inflammation by activating the angiotensin II type 1 (AT1) receptor (Welch, 2008). Telmisartan is a highly selective AT1-receptor antagonist approved for treatment of hypertension. On the other hand, telmisartan acts as a partial agonist on the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ) that has been reported to exert anti-inflammatory and anti-inflammatory effects (Benson et al., 2004). Recently, telmisartan has been reported to have additional peroxisome proliferator-activated receptor-gamma (PPAR-γ) partial agonist activity which regulates metabolic and inflammatory pathways, and improves left ventricular functions (Benson et al., 2004; Schupp et al., 2004). It has also been demonstrated to have beneficial effect on post-infarct ventricular remodeling (Geng et al., 2006). With these dual actions, telmisartan provides additional beneficial pleiotropic effects such as anti-inflammatory, antioxidative and antiproliferative effects in atherosclerosis and myocardial infarction (Imayama et al., 2006; Takaya et al., 2006). On the other hand, telmisartan does not have adverse effects like fluid retention and edema of clinically used full PPAR-γ agonists such as pioglitazone and rosiglitazone (Goebel et al., 2006). Also, candesartan and telmisartan significantly attenuated gastric mucosal lesions induced by cold-restraint stress and indomethacin in nondiabetic rats (Pavel et al., 2008; Morsy et al., 2009).

Although telmisartan has the potential to interfere with the inflammatory reaction cascade, the dose–response relationship in animal models of inflammation and comparative study with other anti-inflammatory agents is not clear enough to establish the required dose for this effect. Therefore, the present study was designed to investigate the dose–response relationship of the anti-inflammatory activity of telmisartan in animal models of chronic inflammation.

2. Experimental

2.1. General

Telmisartan powder (Boehringer Ingelheim, Germany) was dissolved in distilled water to produce a stock solution of 0.4 mg/ml, from which different doses were prepared according to the body weight of the animals. Sprague–Dawley rats weighing 180–220 g of both sexes were purchased from the National Centre for Drug Research and Quality Control, Baghdad. They were kept in the animal house of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, at 25 ± 2 °C and light:dark cycle of 12:12 h for 1 week before starting experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 12 h before the experiment, though water was allowed ad libitum. All experiments were performed according to the guidelines of laboratory animals care and the ethical guidelines for the investigations on experimental animals. In the present study, 108 rats were allocated into 18 groups (six rats each) and the study protocols were divided into four stages: First stage: 48 rats were used, and allocated into eight groups for the study of the anti-inflammatory activity of telmisartan in rat model of formaldehyde-induced chronic inflammation. Second stage: six rats were used to study the anti-inflammatory activity of the maximum effective dose of telmisartan obtained from first stage when used in combination with dexamethasone (0.5 mg/kg) (American reagent, USA) in rat model of formaldehyde-induced chronic inflammation. Third stage: 48 rats allocated into eight groups for the study of the anti-inflammatory activity of telmisartan in rat model of formaldehyde-induced granuloma. Fourth stage: six rats were used for the study of the anti-inflammatory activity of the maximum effective dose of telmisartan when used in combination with dexamethasone (0.5 mg/kg) in rat model of cotton pellet-induced granuloma.

2.2. Formalin-induced chronic inflammation

The anti-inflammatory effect of telmisartan in chronic inflammation was evaluated by formalin-induced paw edema (Chau, 1989). In this model, chronic inflammation was induced by injecting 0.1 ml of 2% formalin (Al-Julayl, Saudi Arabia) into the sub-plantar area of the right hind paw of ether anaesthetized rats. All drugs including telmisartan (0.1, 0.2, 0.4, 0.6, 1.5, and 3 mg/kg body weight), dexamethasone 1 mg/kg and distilled water 2 ml/kg were given 30 min prior to formalin injection and continued for seven consecutive days. All drugs and the vehicle were given orally once daily using oral gavage needle. In this model, the increase in paw edema was measured by vernier caliper method (Joseph et al., 2005). In vernier caliper method, the paw thickness was measured before and 6 days after induction of inflammation by using vernier caliper. The difference in paw thickness after and before induction of inflammation was calculated and presented as mean increase in paw thickness (mm). The ability of the anti-inflammatory drug to suppress paw inflammation was expressed as a percentage of inhibition of paw edema (Duffy et al., 2001).

2.3. Cotton pellet-induced granuloma

The anti-inflammatory activity of telmisartan was evaluated using cotton pellets-induced granuloma according to the method of Winter and Porter (1957). Cotton pellets weighing 10 ± 1 mg were sterilized in an autoclave for 30 min at 120 °C under 15 Ib pressure. Four pellets were implanted subcutaneously (s.c.) into the ventral region, two on either side, in
each rat under light ether anesthesia. All drugs doses and vehicle were administered as mentioned before for seven consecutive days before the day of cotton pellet implantation. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed for the determination of wet weight, and then dried in an incubator at 60°C for 18 h until a constant weight obtained (all the exudates dried); after that the dried pellets were weighed again. The exudate amount (mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined.

2.4. Statistical analysis

All results were expressed as mean ± SEM. The significance of differences between treated groups was determined using Student’s t-test and one-way analysis of variance (ANOVA). P-values < 0.05 were considered significant.

3. Results

3.1. Anti-inflammatory activity in formalin-induced chronic inflammation

In Table 1, all telmisartan doses (0.1, 0.2, 0.4, 0.6, 1.5, and 3 mg/kg body weight) significantly reduced the increases in paw thickness (in a dose-dependent pattern) compared to controls, with maximum effect produced by 1.5 mg/kg (32.2%) and 3.0 mg/kg (39.42%), respectively. Meanwhile, 1 mg/kg dexamethasone significantly inhibited the increases in paw thickness compared to controls (53.42%). Telmisartan (1.5 mg/kg body weight), when administered with dexamethasone (0.5 mg/kg body weight), resulted in 45.71% decrease in paw edema, which was significantly higher than all of the effects produced by telmisartan alone. In Fig. 1, the dose–response relationship of the anti-inflammatory activity of telmisartan in rat models of experimentally-induced chronic inflammation was found to be relatively linear within the dose ranges utilized in this study, with best linearity between 0.6 and 3 mg/kg.

3.2. Anti-inflammatory activity in cotton pellet-induced granuloma

Table 2 shows that all telmisartan doses significantly decreased the formation of inflammatory exudate (in a dose-dependent pattern) compared to controls, with the maximum effect produced by 1.5 mg/kg (34.01%) and 3 mg/kg (47.47%). Meanwhile, 1 mg/kg of dexamethasone significantly inhibited exudate formation compared to controls, and the largest effect was produced by dexamethasone (49.04%). Telmisartan (1.5 mg/kg body weight) in combination with dexamethasone (0.5 mg/kg body weight) resulted in 48.30% decrease in exudate formation, which was significantly higher than all of the effects produced by telmisartan alone. In Fig. 2, the dose–
The response relationship of the anti-inflammatory activity (in terms of attenuation of inflammatory exudate formation) of telmisartan was found to be relatively linear within the dose ranges utilized in this study. Meanwhile, telmisartan significantly decreased the formation of granuloma (in a dose-dependent pattern) compared to control, with the maximum effect produced by the dose 3.0 mg/kg (58.74%). Meanwhile, 1 mg/kg dexamethasone significantly inhibited the formation of granuloma compared to controls; the maximum effect was produced by dexamethasone (71.43%) (Table 3). Adjunct use of telmisartan (1.5 mg/kg body weight) with dexamethasone (0.5 mg/kg body weight) resulted in 68.26% decrease in the formation of granuloma, which was significantly higher than all of the effects produced by telmisartan alone. In Fig. 3, the dose–response relationship of the anti-inflammatory activity (in terms of attenuation of granuloma formation) of telmisartan was found to be relatively linear within the dose ranges utilized in the present study, with best linearity shown between 0.6 and 3 mg/kg.

4. Discussion

In the present study, formaldehyde- and cotton pellet-induced inflammatory reactions similar to that reported during arthritis, and these animal models, are standard for the evaluation of therapeutic agents with suspected anti-arthritic activity (Okoli et al., 2008). As telmisartan significantly inhibited inflammatory reactions in this model of inflammation, it can be proposed that this agent may possess anti-proliferative and anti-arthritic activities. Tissue injury induces a cascade of cellular reactions in the lesion area, accompanied with the release of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-8 and other substances, which is then followed by subsequent inflammatory reactions (Hua et al., 1996). Prostaglandins such

### Table 2
Effects of different doses of telmisartan and its combination with dexamethasone on mean weight of exudates and inhibition of exudates (%) in cotton pellet-induced chronic inflammation in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean weight of exudates (mg)</th>
<th>% inhibition of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>108.5 ± 0.84</td>
<td>–</td>
</tr>
<tr>
<td>Telmisartan 0.1 mg/kg</td>
<td>100.4 ± 0.31**</td>
<td>7.47</td>
</tr>
<tr>
<td>Telmisartan 0.2 mg/kg</td>
<td>91.0 ± 0.09**</td>
<td>16.13</td>
</tr>
<tr>
<td>Telmisartan 0.4 mg/kg</td>
<td>89.8 ± 0.12**</td>
<td>17.24</td>
</tr>
<tr>
<td>Telmisartan 0.6 mg/kg</td>
<td>74.9 ± 0.20**</td>
<td>30.97</td>
</tr>
<tr>
<td>Telmisartan 1.5 mg/kg</td>
<td>71.6 ± 0.05**</td>
<td>34.01</td>
</tr>
<tr>
<td>Telmisartan 3 mg/kg</td>
<td>57.0 ± 0.26*</td>
<td>47.47</td>
</tr>
<tr>
<td>Telmisartan 1.5 mg/kg + 0.5 mg/kg dexamethasone</td>
<td>56.1 ± 0.15**</td>
<td>48.30</td>
</tr>
<tr>
<td>Dexamethasone 1 mg/kg</td>
<td>55.3 ± 0.12**</td>
<td>49.04</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error; *n = 6 animals in each group; *significantly different compared to control (*P < 0.05); values with non-identical superscripts (a, b, c, d, e, f, g) were considered significantly different (*P < 0.05).

### Table 3
Effects of different doses of telmisartan and its combination with dexamethasone on mean weight of dry granuloma and inhibition of granuloma (%) in formaldehyde-induced chronic inflammation in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Mean dry weight of granuloma (mg)</th>
<th>Granuloma inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>31.5 ± 0.16</td>
<td>–</td>
</tr>
<tr>
<td>Telmisartan 0.1 mg/kg</td>
<td>21.8 ± 0.17**</td>
<td>30.8</td>
</tr>
<tr>
<td>Telmisartan 0.2 mg/kg</td>
<td>19.1 ± 0.02**</td>
<td>39.37</td>
</tr>
<tr>
<td>Telmisartan 0.4 mg/kg</td>
<td>18.2 ± 0.03**</td>
<td>42.23</td>
</tr>
<tr>
<td>Telmisartan 0.6 mg/kg</td>
<td>17.1 ± 0.02**</td>
<td>45.72</td>
</tr>
<tr>
<td>Telmisartan 1.5 mg/kg</td>
<td>14.7 ± 0.04**</td>
<td>53.34</td>
</tr>
<tr>
<td>Telmisartan 3 mg/kg</td>
<td>13.0 ± 0.03**</td>
<td>58.74</td>
</tr>
<tr>
<td>Telmisartan 1.5 mg/kg + 0.5 mg/kg dexamethasone</td>
<td>10.00 ± 0.15**</td>
<td>68.26</td>
</tr>
<tr>
<td>Dexamethasone 1 mg/kg</td>
<td>9.0 ± 0.12**</td>
<td>71.43</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error; *n = 6 animals in each group; *significantly different compared to control (*P < 0.05); values with non-identical superscripts (a, b, c, d, e) were considered significantly different (*P < 0.05).
Figure 3  Dose–response relationship of the effect of telmisartan on granuloma inhibition (%) in cotton pellet-induced granuloma in rats.

as PGE₁ and PGE₂, which are produced at elevated levels in inflamed tissues like rheumatoid synovium, increase local blood flow and potentiate the effects of mediators such as bradykinin that induce vasopermeability (Yoshida et al., 2006). Meanwhile, many studies suggested that activation of PPAR-γ with many classes of ligands (including telmisartan) may decrease production of the hypertrophic prostanooids PGE₂, with consequent amelioration or modulation of some component of inflammation (Norihiro et al., 2006). Telmisartan, the selective AT₁ receptor antagonist, was proved effective in reducing the generation of reactive oxygen species and pro-inflammatory mediators. The antioxidant and anti-inflammatory effects of telmisartan are related to its ability to prevent the activation of nuclear factor-κB signaling pathway which promotes the transcription of NADPH oxidase, tumor necrosis factor-α and inducible nitric oxide synthase genes (Takaya et al., 2006; Morishima et al., 2009). This is in agreement with the present results which revealed that telmisartan treatment significantly prevented the formation of inflammatory edema due to challenge with formalin, and suppressed both exudate and granulation tissue formation as a result of subcutaneous cotton pellet implantation. Other mechanisms independent of AT₁ receptor blockade are responsible for the antioxidant and anti-inflammatory activities of telmisartan. The drug acts as a partial agonist at the PPAR-γ (Benson et al., 2004). Activation of this receptor induces catalase gene expression and inhibits nuclear factor-κB, thus combating oxidative stress and down regulating most of the pro-inflammatory responses (Blessing et al., 2008). In addition, the anti-inflammatory effect of telmisartan observed in the present study can be attributed to its PPAR-γ agonist activity. The fact that expression of PPAR-γ was modulated during the course of many inflammatory disorders represents the solid base for the use of highly effective PPAR-γ ligands for the aim of attenuation and/or modulation of the course of inflammation. Altered expression of PPAR-γ was observed in several other inflammatory disorders. For instance, PPAR-γ expression was shown to be reduced in many pathological conditions of inflammatory bases including atherosclerotic tissues (Klotz et al., 2005), in peripheral blood mononuclear cells from patients with multiple sclerosis (Kitamura et al., 1999) and in alveolar macrophages from patients with allergic asthma (Benayoun et al., 2001). Meanwhile, ligand activation of PPAR-γ down regulates the transcription of genes encoding inflammatory molecules, inflammatory cytokines, growth factors, proteolytic enzymes, adhesion molecules, and chemotactic factors (Koji et al., 2007; Qingping et al., 2009). Such finding gives a hope for conducting trials to evaluate the anti-inflammatory activity of potent PPAR-γ activators, including telmisartan and other ARBs, when the safety concern of these agents was completely resolved. Therefore, a possible involvement of PPAR-γ in the attenuation of inflammatory response afforded by telmisartan in animal models of chronic inflammation utilized in the present study cannot be excluded. However, this needs to be confirmed by further investigations.

5. Conclusion

In conclusion, telmisartan, in a dose-dependent pattern, was effective in attenuating formaldehyde-induced paw edema and cotton-pellet-induced granuloma in rat models of chronic inflammation, and therefore it could be investigated as a potential treatment for chronic inflammatory conditions in humans.

Acknowledgment

The authors gratefully thank the College of Pharmacy, University of Baghdad, for supporting the present work.

References


