Original article

Telmisartan attenuates fatty-acid-induced oxidative stress and NAD(P)H oxidase activity in pancreatic β-cells

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Abstract

Aim. – Angiotensin II receptor blockers (ARB) have been shown to lower insulin resistance in obese diabetic animal models and to reduce the risk of new-onset diabetes in hypertensive patients. In the present study, we studied whether telmisartan, an ARB with partial peroxisome proliferator-activated receptor-γ (PPARγ) activity, can exert a direct effect against fatty-acid-induced oxidative stress in pancreatic β-cells.

Methods. – The effect of telmisartan on lipotoxicity was evaluated using mouse insulin-secreting clonal MIN6 and isolated mouse pancreatic islet cells. Reactive oxygen species, protein kinase-C (PKC) activity and NAD(P)H oxidase activity were examined to clarify the underlying mechanisms.

Result. – Telmisartan decreased the accumulation of palmitate-induced reactive oxygen species in MIN6 cells by 25% and in mouse islet cells by 55%. Telmisartan also decreased palmitate-induced PKC activity by 36% and NAD(P)H oxidase activity by 32% in MIN6 cells.

Conclusion. – These findings indicate that telmisartan attenuated fatty-acid-induced oxidative stress and NAD(P)H oxidase activity in pancreatic β-cells. Our observations pave the way to the possible use of ARB as a means of protecting β-cell survival and preserving insulin secretion capacity in patients with diabetes mellitus.

Keywords: Angiotensin II receptor blockers; Pancreatic β-cells; Oxidative stress; PKC; NAD(P)H oxidase activity

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Résumé

Le telmisartan diminue dans les cellules β pancréatiques le stress oxydant et l’activité de la NAD(P)H oxydase induits par les acides gras.

Objectif. – Une diminution de l’insulinorésistance a été mise en évidence dans des modèles animaux diabétiques obèses et une réduction du risque de diabète a été montrée chez les patients hypertendus avec des inhibiteurs des récepteurs de l’angiotensine II (ARB). Dans la présente étude, nous avons cherché si le telmisartan, ARB qui possède une activité peroxisome proliferator-activated receptor gamma (PPARγ) partielle, pouvait exercer un effet direct vis-à-vis du stress oxydant induit par les acides gras dans la cellule β pancréatique.

Méthodes. – L’effet du telmisartan sur la lipotoxicité a été évalué en utilisant une lignée de cellules de souris MIN6 insulinosécrétrices et des îlots pancréatiques de souris isolés. Les espèces réactives de l’oxygène, l’activité de la protéine kinase C (PKC) et l’activité de la NAD (P) H oxydase ont été examinées pour préciser les mécanismes.

Résultats. – Une réduction de 25 % de l’accumulation des espèces réactives de l’oxygène induites par le palmitate a été observée dans les cellules MIN6, et une réduction de 55 % dans les cellules d’îlots de souris. Le telmisartan a également diminué de 36 % dans les cellules MIN6 l’activité de la PKC et de 32 % celle de la NAD (P) H oxydase induites par le palmitate.

Conclusion. – Ces résultats suggèrent que le telmisartan pourrait diminuer dans les cellules β pancréatiques le stress oxydant, ainsi que l’activité de la NAD (P) H oxydase induits par les acides gras. La perspective offerte est une utilisation des ARB comme moyen susceptible d’améliorer la survie des cellules β du diabétique et de préserver l’insulinosécrétion résiduelle des diabétiques de type 2.

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Mots clés : Inhibiteurs des récepteurs de l’angiotensine II ; Cellules β pancréatiques ; Lipotoxicité ; Stress oxydant ; PKC ; NAD (P) H oxydase

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1. Introduction

The activity of the local renin-angiotensin system (RAS) is an important determinant of structure and function in a variety of organs [1]. Angiotensin II (Ang II) has several functions, such as stimulation and inhibition of cell proliferation, induction of apoptosis, generation of reactive oxygen species, and proinflammatory and profibrogenic actions [2]. Recently, an intrinsic RAS was also identified in the pancreatic islets [3]. As Ang II activates NAD(P)H oxidase, the increased RAS in β-cells could aggravate oxidative stress-induced β-cell dysfunction and apoptosis [4]. On the basis of these data, Ang II receptor blockers (ARB), a type of antihypertensive drugs, have received a great deal of attention as a therapeutic tool for obesity-related metabolic disorders.

Vitale et al. [5] showed that telmisartan, an ARB with partial peroxisome proliferator-activated receptor-γ (PPARγ) activity, could improve insulin sensitivity and reduce the incidence of type 2 diabetes in patients with hypertension. In rats fed a high-fat, high-carbohydrate diet, orally administered telmisartan was able to reduce weight gain and significantly increase levels of plasma glucose, insulin and triglycerides [6].

Uncoupling protein-2 (UCP-2), an inner mitochondrial membrane protein, dissipates the proton electrochemical gradient by uncoupling fuel oxidation from adenosine triphosphate (ATP) accumulation in certain experimental systems [7,8]. Free fatty acids serve as weak uncouplers of mitochondrial respiration. The expression of UCP-2 was induced by chronic exposure to free fatty acids in INS-1 insulinoma cell lines, resulting in impaired β-cell function via depletion of cellular ATP [9]. Excess energy metabolism, as seen with lipid exposure, induces multiple adaptations that lead to cell damage, such as ceramide formation [10] and apoptosis in islets [11,12]. Reactive oxygen species are thought to be key players in β-cell apoptosis [12].

A recent report demonstrated that high glucose levels stimulate reactive oxygen species accumulation in the pancreatic β-cell line MIN6 through protein kinase-C (PKC)-dependent activation of NAD(P)H oxidase, which is known to induce cellular oxidative stress by the activation of the AT1 receptor [13]. The results suggest that NAD(P)H oxidase may be involved in increasing oxidative stress in β cells.

In the present study, we observed a direct effect of telmisartan against β-cell lipotoxicity, using MIN6 and mouse islet cells, and have attempted to clarify the mechanism(s) underlying the effect.

2. Materials and methods

2.1. Materials

Dulbecco’s Modified Eagle’s Medium (DMEM) and palmitate (sodium salt) were purchased from Sigma (St Louis, MO, USA), and telmisartan was kindly donated by Yamanouchi Pharmacology (Tokyo, Japan). Penicillin G, streptomycin, amphotericin B, fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI) medium 1640 were obtained from GibCO (Auckland, New Zealand), and TRIZol from Life Technologies (Oslo, Norway). Protein assay reagent was from Bio-Rad (Hemel Hempstead, UK). All procedures were approved by the University of Miyazaki Institutional Animal Care and Use Committee.

2.2. Animals

Six-month-old female C57BL/6J mice were purchased from Charles River Laboratories (Yokohama, Japan) and housed at 22 °C under a 12:12 light:dark cycle, with free access to tap water and standard chow pellets (CLEA Japan, Tokyo, Japan).

2.3. Isolation and culture of islets

A modified version of the collagenase digestion method described by Lacy and Kostianovsky [14] was used. The mice were anaesthetized with intraperitoneal pentobarbital (6 mg/100 g body weight). Collagenase was injected into the common bile duct at a concentration of 1 mg/mL in 1.5 mL Hanks’ solution. The pancreas was removed from each mouse, placed in ice-cold Hanks’ solution and minced with scissors. The mixture was shaken in a 37 °C water bath for 15 min. The supernatant of the mixture was removed, and the remaining pellet re-suspended in Hanks’ solution several times to remove exocrine tissues. Islets were hand-picked using stereomicroscopy, then cultured in RPMI 1640 medium – supplemented with 10% heat-inactivated FBS, 10,000 units/mL penicillin G, 10,000 μg/mL streptomycin, 25 μg/mL amphotericin B and 8 mM glucose – under an atmosphere of 5% CO2/95% air maintained at 37 °C.

2.4. Culture of MIN6 cells

MIN6 cells were cultured in a medium (DMEM supplemented with 10% heat-inactivated FBS, 10,000 units/mL penicillin G, 10,000 μg/mL streptomycin and 25 μg/mL amphotericin B) containing 25 mM glucose under an atmosphere of 5% CO2/95% air maintained at 37 °C [15]. After 60–80% confluence, the MIN6 cells were further cultured with or without 10 μM telmisartan and/or 0.5 mM palmitate at different glucose concentrations (5.6 mM or 25 mM) for 48 h. The media used for further cultures of the proliferated MIN6 cells contained 0.1% dimethyl sulphoxide and/or 0.17% ethanol.

2.5. Reactive oxygen species measurement by confocal microscopy

The MIN6 cells were plated (2 × 10⁵ cells/well) onto EZView culture plates (24-well LB, Asahi Techno Glass, Osaka, Japan) and cultured for 48 h. They were then exposed to DMEM containing either 5.6 mM or 25 mM glucose (low or high glucose, respectively) in the presence or absence of 10 μM telmisartan and/or 0.5 mM palmitate for 48 h.

Islets were also transferred to EZView culture plates, covered with a medium containing 8 mM glucose, and cultured in
an atmosphere of 5% CO₂/95% air maintained at 37 °C. After 48 h, the islets were exposed to a medium containing either 5.6 mM or 25 mM glucose (low or high glucose, respectively) in the presence or absence of 10 μM telmisartan and/or 0.5 mM palmitate.

The MIN6 cells and islets were then stained with dihydrorhodamine (DHR)-123 (Molecular Probes, OR, USA) for 30 min and washed twice with 1 × PBS. Oxidation of the non-fluorescent DHR-123 by the reactive oxygen species H₂O₂, superoxide anion or peroxynitrite yields cationic fluorescent Rh-123, which is then sequestered by active mitochondria [16]. Rh-123 reflects mainly mitochondrial reactive oxygen species, but could be influenced by reactive oxygen species passing through the cell membrane to the mitochondria. Fluorescence of Rh-123 was monitored, using a Leica TCS-SP2 AOBs laser scanning confocal microscope (Leica Microsystems, Mannheim, Germany), with excitation from the 543-nm line of an argon–krypton laser. The accumulation of reactive oxygen species was quantified using a histogram menu.

2.6. PKC enzyme assay

PKC activity was measured with a PKC enzyme assay system (Stressgen Bioreagents Corp., Victoria, BC, Canada). MIN6 cells were washed with PBS and treated with a lysis buffer (20 mM MOPS, 50 mM β-glycerolphosphate, 50 mM sodium fluoride, 1 mM sodium vanadate, 5 mM EGTA, 2 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM benzamidine, 1 mM phenylmethylsulphonyl fluoride (PMSF), and 10 μg/mL leupeptin and aprotinin) for 10 min on ice. The cell lysate was centrifuged (13,000 × g, 15 min), and the suspension collected. This constituted the cytosolic fraction, while protein concentrations were determined by biophotometer. The substrate, which is readily phosphorylated by PKC, was precoated on the wells of the plates. The cytosolic fraction was added to the wells, followed by the addition of ATP to initiate the reaction. After the kinase reaction was terminated, a phospho-specific substrate antibody was added to the wells to bind specifically to the phosphorylated peptide substrate. The phospho-specific antibody was then bound by a peroxidase-conjugated secondary antibody. After washings, a substrate solution – acted upon by the bound enzyme to produce a colour – was added and the resulting absorbances measured at 450 nm.

2.7. NAD(P)H oxidase activity assay

Cells were scraped into 1 mL PBS and homogenized on ice at low speed, then centrifuged at 3000 rpm for 10 min to remove unbroken cells and debris [17]. Supernatants (20 μL) were added to glass scintillation vials containing 250 μM lucigenin. The chemiluminescence observed over the subsequent 1 min in response to the addition of 100 μM NADPH was recorded. In preliminary experiments, homogenates alone – without the addition of NADPH – gave only minimal signals. Also, NADPH did not evoke lucigenin chemiluminescence in the absence of the homogenate.

2.8. Statistical analysis

Data are presented as means ± standard error of mean (SEM). Statistical analyses were performed using parametric ANOVA with Fisher’s protected least significant difference test or Student’s unpaired t test. Differences were considered significant at P < 0.05. All analyses were carried out using StatView 4.1 software (Abacus Concepts Inc., Berkeley, CA; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Effect of telmisartan on reactive oxygen species accumulation

Intracellular reactive oxygen species accumulation was monitored by DHR-123 and visualized by laser scanning confocal microscopy (Figs. 1 and 2A), and fluorescence intensity was measured by histogram quantification menus (Figs. 1 and 2B). High-glucose, compared with low-glucose, incubation significantly enhanced reactive oxygen species accumulation in MIN6
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Fig. 2. Effect of telmisartan on reactive oxygen species accumulation in mouse islet cells. A. Typical fluorescent images: a: islets cultured with low glucose; b: islets cultured with high glucose; c: islets cultured with high glucose and 10 μM telmisartan; d: islets cultured with high glucose and 0.5 mM palmitate; and e: islets cultured with high glucose, 0.5 mM palmitate and 10 μM telmisartan. B. Reactive oxygen species accumulation was quantified using histogram menus. Data are expressed as means ± SEM for five separate experiments. *P < 0.01 vs islets cultured with low glucose; **P < 0.01 vs islets cultured with high glucose; #P < 0.01 vs islets cultured with high glucose and palmitate.

3.2. Effect of telmisartan on PKC activity

High glucose vs low glucose incubation enhanced PKC activity in MIN6 cells by 2.8 times (Fig. 3). Also, compared with high glucose alone, the addition of palmitate significantly increased PKC activity by 1.7 times. Telmisartan decreased high-glucose- and palmitate-induced PKC activity by 36%.

3.3. Effect of telmisartan on NAD(P)H oxidase activity

Compared with low glucose incubation, high glucose incubation enhanced NAD(P)H oxidase activity in MIN6 cells and mouse islet cells. The addition of palmitate to the high glucose dose led to an extremely enhanced reactive oxygen species accumulation in MIN6 and mouse islet cells. In contrast, telmisartan suppressed the high-glucose- and palmitate-induced reactive oxygen species accumulation in MIN6 cells by 25% and in mouse islet cells by 55%. Although telmisartan only partly reduced the high-glucose- and palmitate-induced oxidative stress in MIN6 cells, in mouse islet cells, it reduced oxidative stress close to levels without palmitate.

3.4. Discussion

It has been reported that chronically elevated fatty acid levels can impair pancreatic β-cell function [18,19], and that fatty-acid-induced oxidative stress in pancreatic β-cells can be “rescued” by the oral Ang II type 1 (AT1) receptor blocker irbesartan in Zucker diabetic fatty (ZDF) rats [20]. In the present study, we investigated the direct effect of telmisartan against

Fig. 3. Effect of telmisartan on palmitate-induced PKC activation. Cells were cultured in the presence or absence of 10 μM telmisartan and/or 0.5 mM palmitate for 48 h. Data are expressed as means ± SEM for five separate experiments. *P < 0.05 vs cells cultured with low glucose; #P < 0.05 vs cells cultured with high glucose.

by 3.1 times (Fig. 4), and the addition of palmitate significantly increased NAD(P)H oxidase activity compared with high glucose alone. Telmisartan decreased the high-glucose- and palmitate-induced NAD(P)H oxidase activity by 32%.

Fig. 4. Effect of telmisartan on palmitate-induced NAD(P)H activation. Cells were cultured in the presence or absence of 10 μM telmisartan and/or 0.5 mM palmitate for 48 h. Data are expressed as means ± SEM for five separate experiments. *P < 0.01 vs cells cultured with high glucose; #P < 0.01 vs cells cultured with high glucose and palmitate.
fatty-acid-induced oxidative stress in pancreatic β-cells using MIN6 and mouse islet cells.

Unger et al. [21] reported that the accumulation of intracellular triglycerides might play a pivotal role in β-cell lipopoptosis, while Cnop et al. [22] reported that exposure of rat β cells to free fatty acids (oleic or palmitic acid) resulted in concentration-dependent cell death. In their study, the addition of troglitazone (10 μM), a full agonist of PPARγ, failed to increase palmitate-induced apoptosis, although it slightly increased reactive oxygen species within 48 h. The present study showed that telmisartan, a partial agonist of PPARγ, suppressed intracellular triglyceride accumulation in MIN6 cells (data not shown). Troglitazone also lowered intracellular triglyceride content [23], but the mechanism(s) for this are not yet known. One possible explanation is that the thiazolidinedione compound induces the expression of two enzymes involved in fatty acid oxidation—acyl-CoA oxidase and carnitine palmitoyltransferase I [10,23]. Another possibility is that acetyl-CoA carboxylase (ACC) activity is suppressed by the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) through PPARγ signalling, as shown in mouse skeletal muscle [24]. However, further studies are required to clarify these hypothetical mechanisms.

One mechanism underlying the protective effect of ARB on β-cells is reduction of oxidative stress. Lau et al. [25] recently identified a local RAS in the pancreatic islet. Tikellis et al. [20] reported that treatment with irbesartan attenuated the increased oxidative stress in islets of ZDF rats and that the blockade of RAS could be mediated through inhibition of NAD(P)H oxidase expression. Other sources of reactive oxygen species generation include increased formation of advanced glycosylation end-products, enhanced polyol pathway, increased xanthine oxidase activity and increased release of superoxides from mitochondria [26]. However, the precise mechanisms responsible for the increased oxidative stress in β-cells remain to be elucidated. Nakayama et al. [4] were the first to show that the increased expression of the NAD(P)H oxidase components gp91phox and p22phox correlated with increased oxidative stress in islet cells in animal models of type 2 diabetes, and that the inhibitory effect of the AT1 ARB valsartan on the expression of these components can be seen in the islets of db/db mice, together with inhibition of oxidative stress and preservation of insulin contents. Their study demonstrated that the beneficial effects of ARB treatment was partly due to the prevention of loss of the β-cell mass through reduction of oxidative stress. Another study found that candesartan treatment decreased the expression of gp91phox and p22phox, the membranous components of NAD(P)H oxidase [27]. In the present study, telmisartan decreased palmitate-induced reactive oxygen species accumulation in MIN6 cells by 25% and in mouse islet cells by 55%. The induction of oxidative stress could be contributory to the increased PKC and NAD(P)H oxidase activity. Activation of the PKC–NAD(P)H oxidase pathway by diacylglycerol production during the process of triglyceride synthesis could be one of the mechanisms of oxidative stress seen in lipotoxic β-cells. However, it remains unclear as to whether or not ARB in themselves serve as scavengers to prevent oxidative stress.

Li et al. [28] reported that UCP-2 played a role in β-cell antioxidant defenses, and exposure to antioxidants (vitamin E with selenite) was found to lower UCP-2 mRNA expression. Chu et al. [29] suggested that the ARB losartan inhibited oxidative stress via down-regulation of NAD(P)H oxidase and suppressed UCP-2 expression in vivo. In our study, telmisartan decreased palmitate-induced reactive oxygen species accumulation and up-regulation of UCP-2 mRNA expression (data not shown). The toxicity of long-term elevated fatty acids makes it plausible that UCP-2 may play a protective role for β-cells by mitigating the consequences of excess free-fatty-acid-induced reactive oxygen species accumulation. Thus, to avoid lipotoxicity through reactive oxygen species in the presence of chronic exposure to elevated free fatty acids, β-cells could become subject to partial mitochondrial uncoupling that could, in turn, result in impaired insulin secretion.

5. Conclusion

Telmisartan, an ARB with partial PPARγ activity, suppressed fatty-acid-induced oxidative stress and the activation of NAD(P)H oxidase in pancreatic β cells. Our observations pave the way to the possible use of telmisartan as a means of protecting β-cell survival and preserving insulin secretion capacity in patients with diabetes mellitus.

References


