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Nephroprotective effect of PPAR Agonists on Thioacetamide-induced Nephrotoxicity in Rats

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Introduction

Since their isolation by Issemann and Green in 1990, peroxisome proliferator activated receptor (PPAR) became an area of wide and endless interest [1]. To date, three isoforms of PPARs have been identified, namely PPARα, PPARβ/δ, and PPARγ. Importantly, PPARs have been increasingly recognized as key players in the function of many organs including kidney [2]. At the same time, renal diseases became a new epidemic of the twentieth and twenty-first centuries. At present, it is a global problem, mainly because a variety of risk factors is being involved in its etiology and pathophysiology. Although emerging evidence support PPARs may serve as therapeutic targets for treating the nephrotoxicity, there remains a lack of definitive data on their effect on renal functions [3]. There is a controversy about effect of PPAR agonists on renal functions [4,5]. fibrates are PPARα agonists used in treating dyslipidemia, which interplays with renal diseases. Dyslipidemia is a consequence of kidney disease [6] and a large body of clinical and experimental studies support that altered lipid metabolism may contribute to the pathogenesis and progression of kidney disease [7]. telmisartan, an angiotensin type 1 receptor blocker (ARB), is used in treatment of hypertension. In addition, telmisartan has a partial agonistic effect on PPARγ and showed nephroprotective effect in ischemia/reperfusion injury [8]. Worldwide, hypertension is a common cause of end-stage renal disease, which is the last stage of chronic kidney disease [9,10]. the present study aimed to evaluate the effect of PPARα and PPARγ agonists on a model of nephrotoxicity induced in rats by thioacetamide (TA). Furthermore, comparing a possible nephroprotective effect of PPARα agonists versus PPARγ agonists.

Material and Methods

Drugs and chemicals

Bezafibrate, losartan and telmisartan powder were generously supplied by Egyptian International Pharmaceuticals and Medical Union Pharmaceuticals (Egypt, Cairo) respectively. TA and pyrogallol were purchased from Sigma-Aldrich (USA, Missouri). All other chemicals of analytical grade and were obtained from commercial sources.

Animals

The present study was conducted on adult male wistar rats weighing 205-280 g. Rats were obtained from the animal house, El-Giza, Egypt. Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for one week prior to inclusion in the experiments. All experimental designs were conducted according to the ethical standards approved by the faculty board committee of faculty of medicine, Minia University, Egypt.

Experimental Design

Induction of nephrotoxicity: Nephrotoxicity was induced by intra-peritoneal TA at dose of 50 mg/kg, dissolved in saline 2 ml/kg twice weekly (Monday and Thursday) for 6 weeks. The dose of TA as well as the duration of the study was selected on the light of our pilot experiment and with previous studies [11,12]. All treatments were administered from the first day of TA-intoxication.

Grouping: The animals were randomly divided into 8 experimental groups of 6 animals each. Duration of the study was 6 weeks. (1) Normal control: rats received oral carboxy methyl cellulose (CMC) (p.o.) daily and saline (i.p.) twice weekly; (2) losartan-treated: rats were administered losartan (10 mg/kg, p.o.) [13] suspended in CMC daily and saline (i.p.) was also given twice weekly. (3) Telmisartan-treated: rats were administered telmisartan (10 mg/kg, p.o.) [14] suspended in CMC daily and saline (i.p.) was also given twice weekly. (4) (2) bezafibrate-treated: rats were administered bezafibrate (50 mg/kg, p.o.) [15] suspended in CMC daily and saline (i.p.) was also given twice weekly. (5) TA-treated: TA (50 mg/kg in saline, i.p.) twice weekly and CMC was given daily; (6) TA+Losartan: TA and losartan (10 mg/kg orally) suspended in

CMC; (7) TA+Telmisartan: TA and telmisartan (10 mg/kg orally) suspended CMC. (8) TA+Bezafibrate: TA and bezafibrate (50 mg/kg, p.o.) suspended in CMC.

Sample Collection and Storage

All animals were sacrificed 48 h after the last TA administration. Blood samples were collected and centrifuged at 3000 g for 10 min to obtain clear sera. Kidneys were excised from each rat and then washed by cold saline and divided into parts which were snap frozen in liquid nitrogen, stored at -80 °C, and subsequently homogenized in cold potassium phosphate buffer (pH 7.4) for various biochemical analyses.

Biochemical Analysis

Evaluation of kidney functions: Creatinine and urea levels were determined using commercial kits from Spectrum Diagnostics (Cairo, Egypt).

Renal oxidative stress parameters: Superoxide dismutase (SOD) activity was measured by method of Marklund and Marklund [16] with a slight modification. This method is based on inhibition of the autoxidation of pyrogallol by SOD. The percentage of inhibition for the samples was calculated by the aid of running a control with no sample under the same conditions. SOD enzyme activity was expressed as U/mg protein, where one unit was defined as the amount of the enzyme that inhibited the rate of pyrogallol autoxidation by 50%. Malondialdehyde (MDA), a measure of lipid peroxidation, was evaluated by a method that depends on the reaction between MDA with thiobarbituric and the color developed was measured spectrophotometrically at 535 nm against a blank. Standard curve by 1,1,3,3-tetramethoxypropane was prepared. From this curve, the MDA concentration was expressed as nmol/g tissue then multiplied in the tissue dilution factor [17].

Determination of renal nitric oxide content: The stable oxidation end products of nitric oxide (NO), nitrite (NO_2^-) and nitrate (NO_3^-) were measured after the reduction of nitrate to nitrite by copperized cadmium granules. Quantitation of NO_2^- was based on the Griess reaction and the absorbance of developed color was measured at 545nm against a blank. Concentration of NOx in samples was determined from a standard curve of $NaNO_3$ (0–100 nmol/ml) [18].

Real-time Reverse Transcription Polymerase Chain Reaction for the Relative Quantification of PPARα and PPARγ

Total RNA was extracted from homogenized kidney specimen using ribozol RNA extraction reagent (Amresco, Solon, USA) following the manufacturer's instructions. cDNAs were synthesized using SensiFAST TM cDNA synthesis Kit (Bioline). cDNA was synthesized at 42 °C for 15 min then at 85°C for 5 min followed by immediate cooling on ice. Real-time polymerase chain reaction (RT-PCR) was performed using 10µL of SYBER Green QPCR Mix (SensiFAST™ SYBER ® Lo-ROX Kit, Bioline). The SYBER green data were analyzed with a relative quantification to GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) as reference gene The sets of primers used were as follows: PPARα sense, 5′- ACGATGCTGTCCTCCTTGATG -3′, and antisense, 5′- GCGTCTGACTCGGTCTTCTTG-3′ PPARγ sense primer; 5'- ATTCTGGCCCACCAACTTCGG -3 ' and antisense 5'- TGGAAGCCTGATGCTTTATCCCCA -3' GAPDH sense primers: 5'- GTCGGTGTGAACGGATTTG -3 ' and antisense 5'- CTTGCCGTGGGTAGAGTCAT -3 '. The relative expression level of each gene was calculated using the formula 2(-ΔΔCt). They were scaled relative to controls. Thus, results for all experimental samples were graphed as relative expression compared with the control [19].

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by the Bonferroni's post analysis test to analyze the results for statistically significant difference. p values less than 0.05 were considered significant. Graph Pad Prism was used for statistical calculations (version 6 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). The density of PCR product was measured using Scion Image J software (Scio Cooperation, Fredrick, Maryland).

Results

Effect of Telmisartan and Bezafibrate on Serum Urea and Creatinine

Administration of losartan, telmisartan and bezafibrate alone did not produce any significant change in serum urea and creatinine, as compared to control group. At the same time, a significant increase in serum urea and creatinine noticed in TA group, as compared to control group. As compared to TA group, significant reduction in serum urea and creatinine were noticed in groups TA+Losartan, TA+Telmisartan and TA+Bezafibrate but significant reduction occurred in TA+Telmisartan group as compared to TA+Losartan group (Figure 1).

Effect of Telmisartan and Bezafibrate on Renal Malondialdehyde

Similarly, losartan, telmisartan and bezafibrate administration did not produce any significant change in renal MDA, as compared to control group. In TA group, significant increase in renal MDA occurred, as compared to control group. Although significant reduction in renal MDA was noticed in groups TA+Losartan, TA+Telmisartan and TA+Bezafibrate, there was a significant reduction occurred in TA+Telmisartan group as compared to TA+Losartan group (Figure 2).

Effect of Telmisartan and Bezafibrate on Renal Superoxide Dismutase and Nitric Oxide

As compared to control group, administration of losartan, telmisartan and bezafibrate alone did not produce any significant change in renal SOD and NO. Administration of TA

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caused significant decrease in renal SOD and NO, as compared to control group. In groups TA+Losartan, TA+Telmisartan and TA+Bezafibrate significant increase in renal SOD and NO were noticed, however, significant incresae occurred in TA+Telmisartan group as compared to TA+Losartan group (Figure 2).

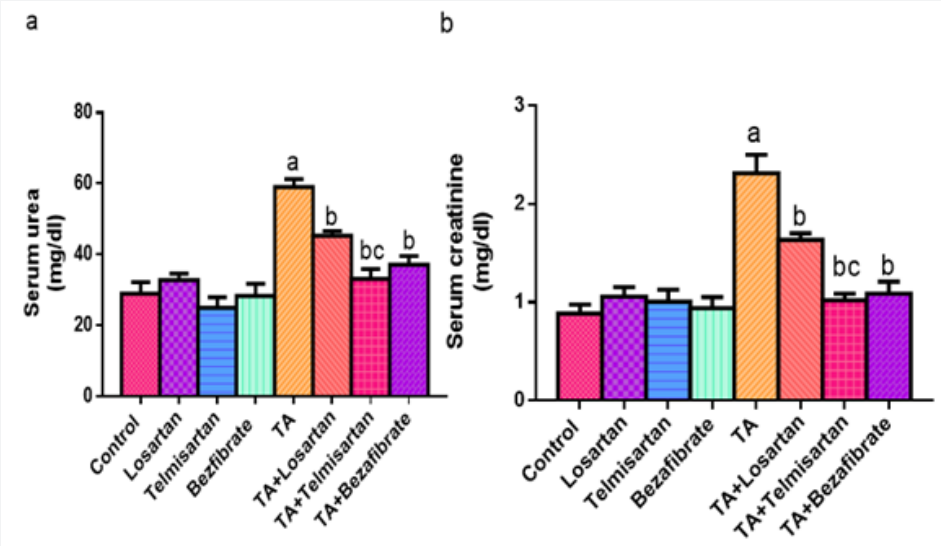

Figure 1: Effect of losartan, telmisartan and bezafibrate on serum urea

a: and creatinine.

B: TA; thioacetamide-treated group.

[a]significance from control group, [b]significance from TA group and [c]significance from TA+losartan group. Significance at $P < 0.05$.

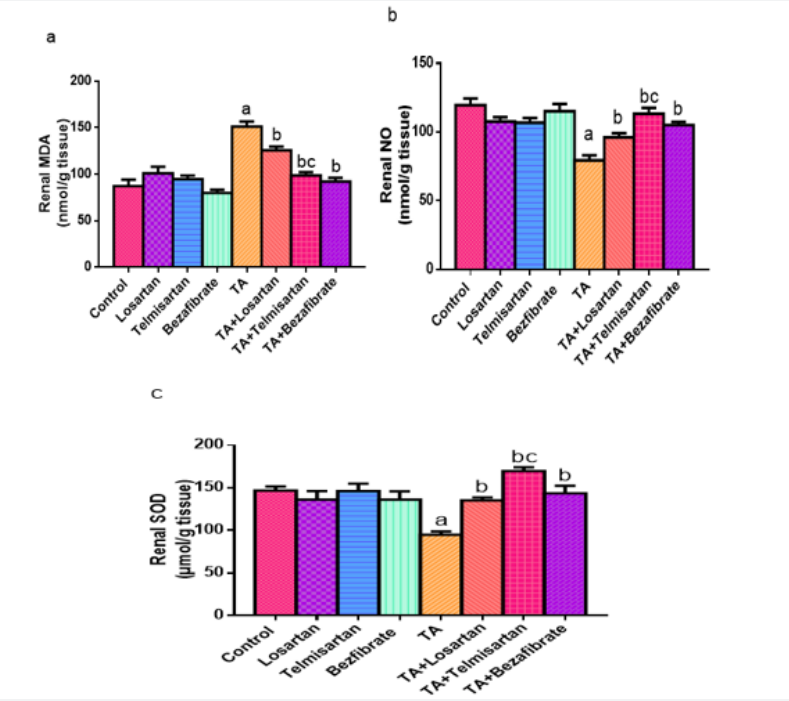

Figure 2: Effect of losartan, telmisartan and bezafibrate on renal MDA.

a: NO.

b: and SOD.

c: TA; thioacetamide-treated group

[a]significance from control group, [b]significance from TA group and [c]significance from TA+losartan group. Significance at $P < 0.05$.

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Effect of Telmisartan and Bezafibrate on Renal Expression of PPAR-Γ and PPAR-A

Administration of TA caused significant decrease in renal PPAR-γ expression, as compared to control group. In groups TA+Telmisartan significant increase in renal PPAR-γ expression were noticed, as compared to TA group, meanwhile, no increase occurred in TA+Losartan and TA+bezafibrate groups. On the other hand, significant decrease in renal PPAR-α expression was noticed in TA group, as compared to control group. At the same time, significant increase in renal PPAR-α expression were noticed in TA+Bezafibrate group but no increase occurred in TA+Telmisartan and TA+losartan groups (Figure 3).

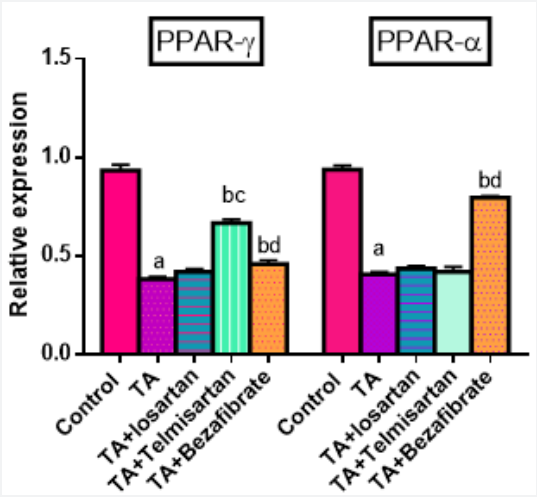

Figure 3: Effect of losartan, telmisartan and bezafibrate on expression of PPAR-γ and PPAR-α in renal tissue.

[a]significance from control group, [b]significance from TA group, [c]significance from TA+losartan group and [d]significance from TA+telmisartan group. Significance at $P < 0.05$.

TA: Thioacetamide-treated group.

Discussion

Understanding the role of PPAR activation in nephrotoxicity remains a matter of great interest. In the current study, different agonists of PPAR were used to explore the role of PPAR in protection against TA-induced nephrotoxicity. Severe renal damage can be caused by some environmental and industrial toxicants by induction of highly reactive free radicals generation. One of the most extensively studied chemicals and industrial toxicants is TA which is known to induce injury to the terminal portion of the proximal renal tubule. After administration of TA it undergoes an extensive metabolism forming sulfoxide and sulfone which circulate through various organs in body before finally being transformed into acetate and excreted into urine within 24 hours [20].The present investigation revealed that administration of TA for 6 weeks resulted in functional disturbances manifested by a significant increase in serum urea and creatinine levels together with an increase in MDA and reduction in both NO and SOD. As a measure of renal function status, blood urea and creatinine are often regarded as reliable markers of renal damage. Current results agree with previous researches, which reported the nephrotoxic effect of TA and its induction of oxidative stress as seen by increased lipid peroxidation and alteration of antioxidant status [20-22].

Bezafibrate administration was protective against TA-induced nephrotoxicity evidenced by significant decrease in serum urea, creatinine and renal MDA together with increase in both NO and SOD. This effect is accompanied by induction of PPAR-α expression in renal tissue which was reduced with TA treatment. Similar results were obtained referring to the nephroprotective effect of bezafibrate in other models of nephrotoxicity. Those investigators also found the increased expression was associated with reduction in oxidative stress parameters and such reduction was parallel to the nephroprotective effect of fibrates. Also, fibrates ameliorates the apoptotic cell death of renal cells [23,24]. Our study here corroborates the observation that inhibition of PPAR-α expression is linked to nephrotoxic effect of TA, while its induction is linked to nephroprotective effect of bezafibrate.

Telmisartan has dual mechanism of action, an ARB and a PPAR-γ agonist. To clarify the effect of PPAR-γ on this model of nephrotoxicity, we used a selective ARB; losartan. Telmisartan antagonized the development of nephrotoxicity with TA administration. It reduced urea, creatinine and MDA, which was increased with TA. At the same time, it increased NO and SOD in renal tissue that was reduced with TA administration. This protective action was gathered with increased in PPAR-γ expression that was dramatically reduced with TA administration. Our findings agree with above-mentioned studies, which reported the nephroprotective of telmisartan and stated its antioxidant, anti-apoptotic and anti-inflammatory actions in various models of nephrotoxicity [25,26]. At the same time, the protective effect of losartan was significantly lower

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than the effect of telmisartan indicating the role of PPAR-γ agonistic activity of telmisartan in such nephroprotective effect. In conclusion, our study showed that TA administration caused deterioration in renal function, which was ameliorated by PPAR-α agonist; Bezafibrate and PPAR-γ agonist; telmisartan. The proposed mechanism is antagonizing oxidative stress induced by TA. These findings support the use of fibrates and telmisartan to ameliorate nephrotoxic effect of TA.

Authors' Contribution

Remon R. Rofaeil carried out study design, performing experiment, biochemical study, analysis, interpretation of data and writing the manuscript. Ahlam K. Abdellah and Nagwa M. Zenhom performed and wrote gene expression part.

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