Nephroprotective effect of peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ agonists on thioacetamide-induced nephrotoxicity in rats

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Aim
This study, for the first time, evaluates the effect of PPARα/γ agonists on thioacetamide (TA)-induced nephrotoxicity in rats.
Methods
Male wistar rats were treated with TA (50 mg/kg, twice weekly for 6 weeks) to induce nephrotoxicity.
Results
Our results showed that bezafibrate and telmisartan caused significant decrease in urea, creatinine and renal malondialdehyde (MDA) which were elevated with TA administration. At the same time, both drugs caused significant increase in renal nitric oxide (NO) and renal superoxide dismutase (SOD) which were reduced with TA. Meanwhile, TA caused significant reduction in PPARα and PPARγ expression, telmisartan increased only PPARγ expression and bezafibrate increased only PPARα expression.
Conclusion
Our findings suggests that bezafibrate and telmisartan are protective against TA-induced nephrotoxicity possibly through their antioxidant activity.

Keywords:
fibrates, telmisartan, PPAR

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Introduction

Since their isolation by Issemann and Green [1], peroxisome proliferator-activated receptor (PPAR) became an area of wide and endless interest. To date, three isoforms of PPARs have been identified, namely PPAR-α, PPAR-β/δ, and PPAR-γ. Importantly, PPARs have been increasingly recognized as key players in the function of many organs including the kidneys [2].

At the same time, renal diseases became a new epidemic of the 20th and 21st 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 that PPARs may serve as therapeutic targets for treating nephrotoxicity, there remains a lack of definitive data on their effect on renal functions [3]. There is a controversy about the effect of PPAR agonists on renal functions [4,5].

Fibrates are PPAR-α 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 diseases [7].

Telmisartan, an angiotensin type 1 receptor blocker (ARB), is used in the treatment of hypertension. In addition, telmisartan has a partial agonistic effect on PPAR-γ 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 PPAR-α and PPAR-γ agonists on a model of nephrotoxicity induced in rats by thioacetamide (TA), furthermore, comparing a possible nephroprotective effect of PPAR-α agonists versus PPAR-γ agonists.

Material and methods

Drugs and chemicals

Bezafibrate, losartan, and telmisartan powder were generously supplied by the Egyptian International Pharmaceuticals and Medical Union Pharmaceuticals (Egypt, Cairo), respectively. TA and pyrogallol were purchased from Sigma-Aldrich (St Louis, Missouri,

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USA). All other chemicals were of analytical grade and were obtained from commercial sources.

Animals

The present study was conducted on adult male Wistar rats weighing 205–280 g.

The rats were obtained from the animal house, El-Giza, Egypt. They were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for 1 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 intraperitoneal TA at a 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 eight experimental groups of six animals each. The duration of study was 6 weeks. (a) Normal control: the rats received oral carboxy methyl cellulose (CMC) (postoperatively) daily and saline (intraperitoneal) twice weekly; (b) losartan-treated: the rats were administered losartan (10 mg/kg, postoperatively) [13] suspended in CMC daily and saline (intraperitoneal) was also given twice weekly; (c) telmisartan-treated: the rats were administered telmisartan (10 mg/kg, postoperative) [14] suspended in CMC daily and saline (intraperitoneal) was also given twice weekly; (d) (2) bezafibrate-treated: the rats were administered bezafibrate (50 mg/kg, postoperatively) [15] suspended in CMC daily and saline (intraperitoneal) was also given twice weekly; (e) TA-treated: TA (50 mg/kg in saline, intraperitoneal) twice weekly and CMC was given daily; (f) TA+losartan: TA and losartan (10 mg/kg orally) suspended in CMC; (g) TA+telmisartan: TA and telmisartan (10 mgt/kg orally) suspended CMC. (h) TA+bezafibrate: TA and bezafibrate (50 mg/kg, postoperative) suspended in CMC.

Sample collection and storage

All animals were killed 48 h after the last TA administration. Blood samples were collected and centrifuged at 3000*g* for 10 min to obtain clear sera. The kidneys were excised from each rat and then washed with cold saline and were 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 the method of Marklund and Marklund [16] with a slight modification. This method is based on the 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 and 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 545 nm 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 peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ

Total RNA was extracted from homogenized kidney specimens using the ribozol RNA extraction reagent (Amresco, Solon, Cleveland, Ohio, USA) following the manufacturer's instructions. cDNAs were synthesized using the SensiFAST TM cDNA

synthesis kit (Bioline, London, UK). cDNA was synthesized at 42°C for 15 min and then at 85°C for 5 min followed by immediate cooling on ice. Real-time PCR was performed using 10 µl 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 the reference gene. The sets of primers used were as follows: PPAR-α sense, 5′-ACGATGCTGTCCTCCTTGATG-3′, and antisense, 5′-GCGTCTGACTCGGTCT TCTTG-3′ PPAR-γ sense primer; 5′-ATTCT GGCCCACCAACTTCGG-3′ and antisense 5′-TGGAAGCCTGATGCTTTATCCCCA-3′ GAPDH sense primers: 5′-GTCGGTGTGAAC GGATTTG-3′ and antisense 5′-CTTGCCGTGG GTAGAGTCAT-3′. The relative expression level of each gene was calculated using the formula $2^{(-\Delta\Delta Ct)}$. They were scaled relative to controls. Thus, results for all experimental samples were graphed as a relative expression compared with the control [19].

Statistical analysis

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

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 with the control group. At the same time, a significant increase in serum urea and creatinine was noticed in the TA group, as compared with the control group. As compared with the TA group, significant reductions 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 with the TA+losartan group (Fig. 1).

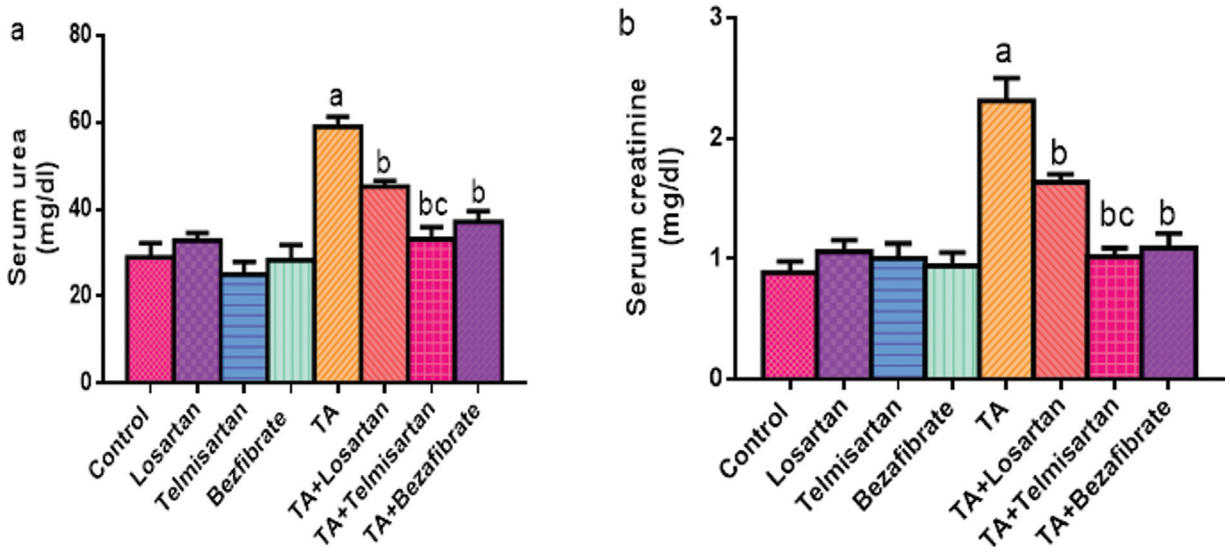
Effect of telmisartan and bezafibrate on renal malondialdehyde

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

Effect of telmisartan and bezafibrate on renal superoxide dismutase and nitric oxide

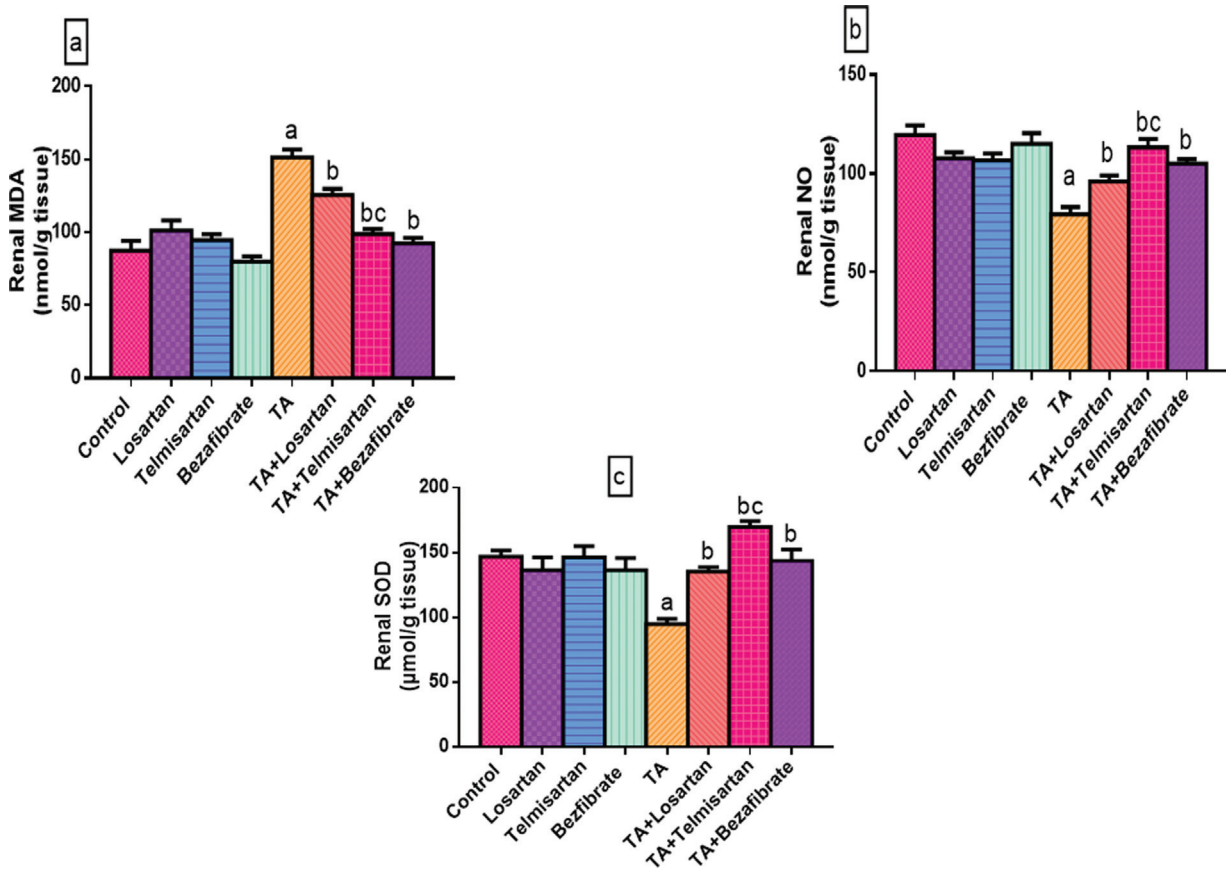
As compared with the control group, administration of losartan, telmisartan, and bezafibrate alone did not produce any significant change in renal SOD and NO. Administration of TA caused a significant

Figure 1



Effect of losartan, telmisartan, and bezafibrate on serum urea (a) and creatinine (b). TA, thioacetamide-treated group. a, Significance from the control group; b, significance from the TA group; and c, significance from the TA+losartan group. Significance at a *P* value of less than 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 the control group; b, significance from the TA group; and c, significance from the TA+losartan group. Significance at a *P* value of less than 0.05. MAD, malondialdehyde; SOD, superoxide dismutase.

decrease in renal SOD and NO, as compared with the control group. In groups TA+losartan, TA+telmisartan, and TA+bezafibrate significant increases in renal SOD and NO were noticed; however, a significant increase occurred in the TA+telmisartan group as compared with the TA+losartan group (Fig. 2b and c).

Effect of telmisartan and bezafibrate on renal expression of peroxisome proliferator-activated receptor-γ and peroxisome proliferator-activated receptor-α

Administration of TA caused a significant decrease in renal PPAR-γ expression, as compared witht the control group. In groups TA+telmisartan significant increases in renal PPAR-γ expression were noticed, as compared with the TA group; meanwhile, no increase occurred in TA+losartan and TA+bezafibrate groups. On the other hand, a significant decrease in renal PPAR-α expression was noticed in the TA group, as compared with the control group. At the same time, significant increases in renal PPAR-α expression were noticed in the TA+bezafibrate group but no increase occurred in TA+telmisartan and TA+losartan groups (Fig. 3).

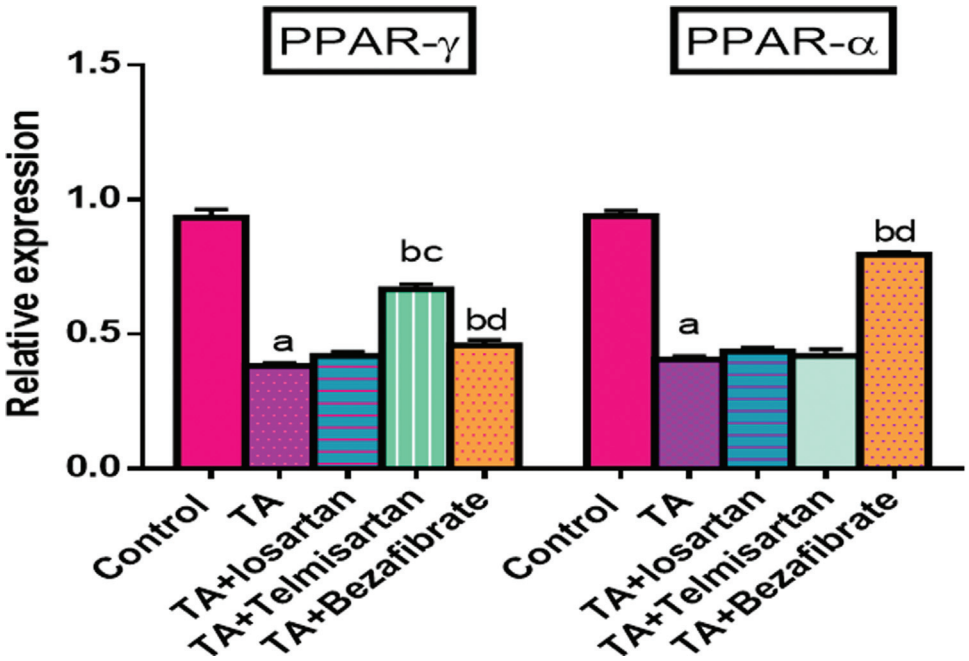
Discussion

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 the induction of highly reactive free radical 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 the body before finally being transformed into acetate and excreted in the urine within 24 h [20].

The present investigation showed 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

Figure 3



Effect of losartan, telmisartan, and bezafibrate on the expression of peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ in the renal tissue. TA, thioacetamide-treated group. a, Significance from the control group; b, significance from the TA group; c, significance from the TA+losartan group; and d, significance from the TA+telmisartan group. Significance at a *P* value of less than 0.05.

function status, blood urea and creatinine are often regarded as reliable markers of renal damage. Current results are in agreement 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 a significant decrease in serum urea, creatinine, and renal MDA together with an increase in both NO and SOD. This effect is accompanied by the 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 that the increased expression was associated with a reduction in oxidative stress parameters and such reduction was parallel to the nephroprotective effect of fibrates. Also, fibrates ameliorate 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 the renal tissue that was reduced with TA administration. This protective action was gathered with increased PPAR-γ expression that was dramatically reduced with TA administration. Our findings are in agreement with the 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 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 the PPAR-α agonist, bezafibrate and the 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 the nephrotoxic effect of TA.

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

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Conflicts of interest
There are no conflicts of interest.

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