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Upregulation of peroxisome proliferator activated receptor alpha by fenofibrate in induced testicular ischemia reperfusion

Marwa M.M. Refaie

Department of Pharmacology, Faculty of Medicine, Minia University, 61511, Minia, Egypt

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ABSTRACT

Torsion of the spermatic cord is a common urological emergency among infants and adolescents that can lead to testicular necrosis and infertility. We investigated the effect of fenofibrate (FEN) on induced testicular ischemia reperfusion (testicular (I-R). FEN (100, 300 mg/kg/day) was administered orally in presence or absence of testicular (I-R). We measured testicular weight changes and serum testosterone level. In addition; Testicular tissue reduced glutathione (GSH), malondialdehyde (MDA), total nitrites (NO_x) and superoxide dismutase (SOD) activity were measured. Moreover; tumor necrosis factor alpha (TNF-α), nuclear factor kappa B (NF-κB) immunoexpressions and histopathology were evaluated. Testicular (I-R) induced group showed significant decrease in serum testosterone level and testicular weight with increase in testicular tissue MDA and NO_x levels. Testicular (I-R) induced group showed the histopathological changes of marked testicular damage according to Johnsen's score. In addition, there was significant reduction in GSH and SOD testicular tissue levels but significant increase in TNF-α and NF-κB immunoexpressions. FEN was able to markedly improve testicular (I-R) induced changes through its action on peroxisome proliferator activated receptor alpha (PPARα), anti-oxidant, anti-inflammatory and anti-apoptotic effects.

1. Introduction

Testicular Torsion is considered as urological emergency which requires early diagnosis and surgical intervention to prevent testicular damage. Management of testicular torsion may be accompanied by further damage to the testes. Reperfusion of ischemic tissue leads to sequence of events that injure the tissue [1–3]. These injuries can be more severe than injuries induced by ischemia [4]. Ischemia-reperfusion of testis stimulates an intracellular signaling cascade in the endothelial cells that leads to neutrophil recruitment, reactive oxygen species (ROS) formation, inflammation, release of cytokines, such as interleukin-1b, tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB). Recruitment of neutrophils and macrophages causes testicular atrophy, disruption of spermatogenesis and germ cell apoptosis [4]. ROS can cause tissue damage or death through cell membrane lipid peroxidation, protein denaturation and DNA impairment [5].

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors. The group includes three isotypes encoded by different genes: PPAR α, β, γ, δ. These receptors are known for their involvement in fatty acid, lipoprotein metabolism, glucose homeostasis, cellular proliferation and differentiation and the immune response [6]. Accordingly, PPARs are important targets in treatment of metabolic disorders such as type 2 diabetes mellitus and inflammatory diseases. Different studies identified other functions of PPARs in blood pressure regulation, neuroinflammation, neuroprotection and inflammatory pain reduction [7].

The fibrate family contains several compounds that are all peroxisome proliferator activated receptor alpha (PPARα) agonists. PPARα is one of the three subtypes of the nuclear receptor PPAR [8]. PPARα activation leads to the expression of target genes such as Cu/Zn SOD or glutathione peroxidases, glutathione reductase and glutathione S-transferase which increase the redox state. Moreover, PPARα activation represses NF-κB and activator protein-1 signaling pathways thereby down regulates the inflammatory response and oxidative stress [9].

1.1. Purpose of the study

In current study we tried to evaluate the role of upregulation of PPARα receptors in induced testicular (I-R) model by using a potent PPARα agonist, anti-inflammatory, antioxidant and anti-apoptotic drug

E-mail addresses: marwamonier@yahoo.com, marwamonier@mu.edu.eg.

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Table 1
Effect of FEN on testicular weights and serum testosterone in testicular (I-R) induced rats.

Group	Testicular weight/g	Serum testosterone (nmol/ml)
Control	1.80 ± 0.04	2.3 ± 0.08
FEN1	1.9 ± 0.02	2.1 ± 0.06
FEN2	1.9 ± 0.02	2.4 ± 0.14
testicular (I-R)	1.2 ± 0.04[a]	0.2 ± 0.02[a]
testicular (I-R) + FEN1	1.6 ± 0.06[a,b]	1.9 ± 0.06[a,b]
testicular (I-R) + FEN2	1.8 ± 0.02[b]	2.0 ± 0.05[b]

Values are representation of 6 observations in each group as means ± S.E.M. Results are considered significantly different when $P < .05$.

[a] Significant difference compared to control sham operated group.

[b] Significant difference compared to testicular ischemia reperfusion induced group. FEN1 is fenofibrate given group (100 mg/kg), FEN2 is fenofibrate given group (300 mg/kg). Testicular (I-R) is testicular ischemia reperfusion induced group and testicular (I-R) +FEN1 is the testicular ischemia reperfusion and fenofibrate given group (100mg/kg). Testicular (I-R) + FEN2 is the testicular ischemia reperfusion and fenofibrate given group (300 mg/kg).

Table 2
Effect of FEN on testicular tissue GSH and SOD levels in testicular (I-R) induced rats.

Group	GSH (μmol/g tissue)	SOD (unit/mg tissue)
Control	81.7 ± 3.1	3.3 ± 0.15
FEN1	82.97 ± 3.2	3.2 ± 0.11
FEN2	84.7 ± 4.3	3.4 ± 0.16
testicular (I-R)	18.2 ± 4.1[a]	2.4 ± 0.13[a]
testicular (I-R) + FEN1	55.2 ± 1.7[a,b]	2.9 ± 0.02[a,b]
testicular (I-R) + FEN2	80.6 ± 2.6[b]	3.2 ± 0.04[b]

Values are representation of 6 observations in each group as means ± S.E.M. Results are considered significantly different when $P < .05$.

[a] Significant difference compared to control sham operated group.

[b] Significant difference compared to testicular ischemia reperfusion induced group. FEN1 is fenofibrate given group (100 mg/kg), FEN2 is fenofibrate given group (300 mg/kg). Testicular (I-R) is testicular ischemia reperfusion induced group and testicular (I-R) +FEN1 is the testicular ischemia reperfusion and fenofibrate given group (100 mg/kg). Testicular (I-R) + FEN2 is the testicular ischemia reperfusion and fenofibrate given group (300 mg/kg).

Table 3
Effect of FEN on MDA and NOX levels in testicular (I-R) induced rats.

Group	MDA (nmol/g tissue)	NO_X (nmol/g tissue)
Control	123.2 ± .1.5	138.2 ± 1.9
FEN1	125.5 ± .1.5	135.3 ± 1.9
FEN2	123.8 ± 1.3	138.2 ± 1.8
testicular (I-R)	171.8 ± 1.9[a]	187.3 ± 4.9[a]
testicular (I-R) + FEN1	135.7 ± 1.4[a,b]	166.5 ± 4.9[a,b]
testicular (I-R) + FEN2	124.7 ± 1.4[b]	142.2 ± 2.9[b]

Values are representation of 6 observations in each group as means ± S.E.M. Results are considered significantly different when $P < .05$.

[a] Significant difference compared to control sham operated group.

[b] Significant difference compared to testicular ischemia reperfusion induced group. FEN1 is fenofibrate given group (100 mg/kg), FEN2 is fenofibrate given group (300 mg/kg). Testicular (I-R) is testicular ischemia reperfusion induced group and testicular (I-R) +FEN1 is the testicular ischemia reperfusion and fenofibrate given group (100 mg/kg). Testicular (I-R) + FEN2 is the testicular ischemia reperfusion and fenofibrate given group (300 mg/kg).

fenofibrate (FEN) on induced testicular ischemia reperfusion (testicular (I-R) in rats.

2. Materials and methods

2.1. Chemicals

FEN powder was from Mina Pharm Company. Testosterone ELISA kit (Cayman Chemicals., USA). Polyclonal TNF-α antibody, polyclonal NF-κB antibody and Immunostaining Detection Kit were from Lab Vision Laboratories, USA.

2.2. Animals and experimental design

Adult male Wistar albino rats weighing 250–300 g were from the animal house, Giza, Egypt. Animals were left in standard housing conditions in cages, 3 rats/cage, and were left to acclimatize. Rats were given laboratory chow and tap water. This work was in the Pharmacology Departement, Faculty of Medicine, Minia University, Egypt and the animal experimental protocol was approved by the faculty board in accordance with European (EU) directive 2010/63/EU.

Rats were randomly divided into 6 groups (n = 6 each group)

Group I was sham group. It was subjected to all operative procedures, except vessels occlusion. This group was given vehicle (1% carboxymethylcellulose) once orally [10].

Group II was treated with FEN (100 mg/kg) once orally [11].

Group III was treated with FEN (300 mg/kg) once orally [12].

Group IV was testicular (I-R) induced group [4].

Group V was treated with FEN (100 mg/kg) once orally [11] + testicular (I-R) [4].

Group VI was treated with FEN (300 mg/kg) once orally [12] + testicular (I-R) [4].

Explanation of the operative procedures in each group:

All rats were weighed then groups I, IV, V, VI were anesthetized using ip injection of 20% Urethane hydrochloride. After anesthesia, the rats were kept in a supine position and underwent antisepsis of the scrotal region with 2% iodine alcohol. Surgery was performed through a left scrotal incision. Unilateral testicular torsion was created in groups IV, V, VI by twisting the left testis 1080° in a clockwise direction and fixed within hemiscrotum with a 3/0 silk suture. The incision was then closed using 2/0 silk suture in groups I, IV, V, VI. Testicular (I-R) induced rats were subjected to 1hour of ischemia and 3h of reperfusion. Groups V, VI were received single dose of FEN (100, 300 mg/kg) respectively 30 min before reperfusion of the testes orally. Rats were sacrificed immediately after 3 h.

2.3. Evaluation of serum testosterone

Measurement of serum testosterone was measured according to testosterone ELISA kit (Cayman Chemicals., USA). This measurement is based on the competition between testosterone and a testosterone acetylcholine esterase conjugate (testosterone tracer).This antiserum testosterone complex binds to mouse monoclonal anti-rabbit IgG that has previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent is added. The reaction gives color measured at 412 nm.

2.4. Evaluation of testicular homogenate

All of the testicular tissues were washed with saline solution then the testicles were weighed on Mettler Toledo scale, Swizer Land. Ipsilateral testes were kept at −80 °C. Testes were homogenized (Glas-Col homogenizer, USA) and homogenate was prepared in ice cold Tris HCl buffer solution for biochemical analysis. The homogenate was centrifuged at 4000 rpm for 15 min at 4 °C in cooling centrifuge and the supernatant was kept at −80 °C till used.

2.5. Evaluation of testicular reduced glutathione (GSH) and superoxide dismutase (SOD) levels

Evaluation of testicular antioxidant defense mechanisms was done by assessment of testicular tissue GSH and SOD enzyme levels.

GSH measurement depends on that the sulfhydryl groups of GSH react with5, 5-dithio-bis-2-nitrobenzoic acid (Ellman's reagent). That gives a yellow colored 5-thio-2-nitrobenzoic acid. The color density was