Biomedicine & Pharmacotherapy 105 (2018) 645–651

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Synergistic effect of rapamycin and metformin against germ cell apoptosis and oxidative stress after testicular torsion/detorsion-induced ischemia/reperfusion in rats

Morteza Ghasemnejad-berenji[a,b,c], Mahmoud Ghazi-Khansari[b,c], Sarvin Pashapour[d], Abbas Jafari[e], Iraj Yazdani[b,c], Hojjat Ghasemnejad-berenji[f], Seyed Soheil Saeedi Saravi[g], Sonia Sadeghpour[h], Maliheh Nobakht[i], Alireza Abdollahi[j], Javad mohajer Ansari[i], Ahmad Reza Dehpour[b,c,*]

[a] Department of Pharmacology and toxicology, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran
[b] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of pediatrics, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[e] Department of Occupational Health, Faculty of Health, Urmia University of Medical Sciences, Urmia, Iran
[f] Department of Anatomy and Reproductive Biology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[g] Department of Pharmacology, Faculty of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
[h] Department of Obstetrics & Gynecology, School of Medicine, Urmia University of Medical Science, Urmia, Iran
[i] Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[j] Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Apoptosis
Metformin
Oxidative stress
Rapamycin
Testicular torsion/detorsion

ABSTRACT

The aim of this study was to investigate the effects of rapamycin (rapa) and metformin (met), combined administration on testicular torsion-detorsion (T/D) injury. A total of 108 male rats were divided randomly into six groups (n = 18), control, sham-operated, T/D, T/D + met (100 mg/kg), T/D + rapa (0.25 mg/kg) and T/D + met (100 mg/kg) + rapa (0.25 mg/kg). Except for the control and sham groups, torsion was created by rotating the right testis 720° in a clockwise direction for 1 h. Treatment groups received drug intraperitoneally, 30 min before detorsion. The right testis of 6 animals from each group was excised 4 h after detorsion for the measurement of lipid peroxidation, caspase-3, and antioxidant enzyme activities. Histopathological changes and germ cell apoptosis were determined by measuring mean of seminiferous tubules diameters (MSTD) and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick end labeling (TUNEL) test in rest of animals, 24 h after detorsion. In T/D group tissue malondialdehyde (MDA) level and caspase-3 activity increased and the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) decreased in comparison with the control group after detorsion. Met and rapa separately pre-treatment reduced MDA and caspase-3 levels, normalized antioxidant enzyme activities, reduced germ cell apoptosis and improved the MSTD in comparison with T/D group. However combined administration of met and rapa indicated a significant augmented effect as compared to the individual drug interventions on the reversal of T/D induced oxidative stress, apoptosis, and histologic changes, suggesting a synergistic response. Thus, this study shows that rapa and met combination have significant synergistic effects against oxidative stress and apoptosis and opens up further possibilities for the design of new combinatorial therapies to prevent tissue damage after ischemia-reperfusion (I/R).

1. Introduction

Testicular torsion is a urological emergency particularly seen in newborn males, children, and adolescents. This pathologic condition requires rapid diagnosis and immediate surgical intervention to prevent permanent testicular damage and loss of fertility [1]. The main pathologic event in testicular torsion detorsion is ischemia-reperfusion (I/R) injury which stimulates an intracellular cascade, including an

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran
E-mail address: mghasemnejad@alumnus.tums.ac.ir (A.R. Dehpour).

https://doi.org/10.1016/j.biopha.2018.06.012
Received 25 February 2018; Received in revised form 1 June 2018; Accepted 2 June 2018

activation of neutrophils, inflammatory cytokines and reactive oxygen species (ROS). These ROS cause lipid peroxidation in the cellular and mitochondrial membranes. Peroxidation of the lipids in membranes, changes membrane permeability or disrupts membrane integrity and thus inflict significant injury on cellular organism and induces the loss of spermatogenesis mainly through germ cell-specific apoptosis [2,3]. In addition to I/R, germ cell apoptosis is considered to be involved in various testicular pathologic conditions such as cryptorchidism, testosterone withdrawal and exposure to toxins [4]. In I/R excessive generation of ROS and leukocytes infiltration lead to germ cell specific apoptosis [5,6]. Indeed, it has been recommended that regulation of germ cell apoptosis may have a crucial role in spermatogenic dysfunctions and finding drugs capable of decreasing pathologic germ cell apoptosis could ameliorate testicular torsion as well as other causes of hypospermatogenesis [7]. Experimental studies demonstrated that metformin (met) or rapamycin (rapa), individually, have protective effects against testicular I/R injury by decreasing oxidative stress and apoptosis [8,9]. Rapa (Sirolimus), an FDA approved immunosuppressant drug for prophylaxis of allograft rejection, is a fermentation product derived from Streptomyces hygroscopius. It is a well-known specific inhibitor of the mammalian target of rapamycin (mTOR) [10]. Rapa targets several cellular functions such as proliferation, cell growth, autophagic cell death and exerts antioxidant defense through the inhibition of mTOR signaling pathway [11]. There is also evidence, showing that rapa directly protected against apoptosis following ischemia–reoxygenation injury [9,12].Met is the first-line drug for the management of type 2 diabetes and is a well-known activator of AMP-activated protein kinase (AMPK), the role of which is well-established in regulation of cellular energy balance and several diseases [13]. Met was found to protect heart, liver, brain, testis and kidney from I/R injury [8,14–16]. Studies have reported that met decreases inflammation [17], oxidative stress and apoptotic cell death in I/R injury [8]. Anti-inflammatory effect of the met relies on its ability to increase the activation of AMPK and inhibiting the activation of NF-κB [18]. Since various pathways are involved in I/R injury [19], combination therapy rather than monotherapy may be required for effective protection. Thus in the present study, for the first time we investigated the effects of combined treatment of met as an activator of AMPK and rapa as an inhibitor of mTOR on biochemical and histopathological changes in experimental testicular I/R injury in rats.

2. Material and methods

2.1. Experimental design and animal groups

The flowchart of the experimental study design is shown in Fig. 1.

One hundred and eight mature male Wistar rats weighing 220–250 g were selected. All animal experiments were performed after receiving the Tehran University of Medical Sciences Ethical Committee approval, in compliance with the principles of laboratory animal care (National Institutes of Health publication no. 85–23; revised, 1985). The rats were housed under standard conditions, on 12 h light–12 h dark cycle in a temperature-controlled room (21–22 °C) with frees access to food and water.

The rats were randomly divided into six groups (n = 18 per group):

Control group: Control rats as the baseline;

Sham group: Sham-operated rats; all the surgical steps were performed; however, T/D was not induced;

T/D group: T/D operated rats, received 2 ml injection of DMSO as a vehicle, 30 min before detorsion; (vehicle group);

T/D + met group: T/D-operated rats received a single dose of 100 mg/kg met intraperitoneally 30 min before detorsion;

T/D + rapa group: T/D-operated rats received a single dose of 0.25 mg/kg rapa intraperitoneally 30 min before detorsion;

T/D + met + rapa group: T/D-operated rats received 0.25 mg/kg rapa and 100 mg/kg met intraperitoneally 30 min before detorsion;

2.2. Experimental testicular T/D procedure

Animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and chlorpromazine (25 mg/kg). Torsion was induced by rotating right testis 720° clockwise and maintained by fixing the testis as described by Turner et al. [20]. After 1 h of torsion, the testis was counter-rotated to the natural position (detorsion) and replaced into the scrotum. The biochemical markers of oxidative stress are reported to increase just 4 h after reperfusion in testicular T/D model; thus, we removed the right testes of six animals from each study group 4 h after detorsion for assessment of oxidant/antioxidant balance. The testes of 12 other animals from each group were separated 24 h after detorsion for histopathological analysis and detection of germ cell apoptosis index, which peaks at this time point [6,20].

2.3. Biochemical assays

Four hours after detorsion, ipsilateral orchiectomy was done and samples stored in a deep freeze (−80 °C) until processing for assessment of tissue malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and caspase-3 level changes. MDA accumulation in tissues is indicative of the extent of cell membrane lipid peroxidation. MDA levels in tissue were measured spectrophotometrically as described by Ohkawa et al. We determined testes tissue SOD activity based on inhibition of NADH oxidation in the presence of chemically produced superoxide anions using the method of Paoletti and Mocali [21].CAT activity was measured spectrophotometrically according to the method of Aebi [22] in Which decomposition of H2O2 is tracked through determining the decrease in absorbance at 240 nm. GPx activities of the tissue were spectrophotometrically measured at 37 °C and 340 nm according to method of Paglia and Valentine [23,24]. For the evaluation of apoptosis caspase-3 level was measured using ELISA detection kit according to the Biotin double-antibody sandwich technology. The colorimetric change in samples at 450 nm was used to evaluate caspase-3 concentration (ng/ml) [25].

2.4. Histopathological analysis

The testicular tissues of six other rats were collected 24 h after detorsion for histopathological examinations. The tissue samples were fixed individually in Bouin's solution, embedded in paraffin wax and then were cut in 4–5 μm sections and after deparaffinization stained with hematoxylin and eosin (H&E). Each slide was evaluated by an experienced pathologist who was blinded to the experiment and the data. The slides were randomly numbered and then given to the pathologist for scoring. The tissues were examined under light microscope with 100× magnification to evaluation of testicular histological injury (Fig.2). The testicular tissue was assessed according to the four-level grading scale of Cosentino's histologic score [26]:

Grade 1: normal structure with regularly organized germ cells;

Grade 2: testicular injuries with less orderly, Loss of cohesion among regular germ cells and closely packed seminiferous tubules;

Grade 3: testicular injuries with disordered, sloughed germ cells with shrunken, pyknotic nuclei and less distinction in seminiferous tubule borders;

Grade 4: testicular injuries with intensely packed seminiferous tubules with coagulative germ cell necrosis;

Moreover, for each sample, mean seminiferous tubule diameter (MSTD) was calculated by measurement of 10 separate roundest seminiferous tubules using a light microscope-adaptable micrometer.

2.5. Detection of germ cell apoptosis

The testes of six other rats from each study group were removed