Remote organ injury induced by myocardial ischemia and reperfusion on reproductive organs, and protective effect of melatonin in male rats

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Objective: Myocardial ischemia and reperfusion (MI-R) leads to remote organ injury associated with oxidative stress. Melatonin is a well-known antioxidant and free-radical scavenger. This study was conducted to examine whether MI-R causes damage in the testes and sperm quality, and to investigate the possible protective effect of exogenous melatonin on these parameters in an in vivo rat model.
Design: Experimental study.
Setting: Experimental Research Center, Fırat University Medical School, Elazığ, Turkey.
Patient(s): Eight-week-old male Wistar rats (n = 18).
Intervention(s): To produce MI-R, a branch of the descending left coronary artery was occluded for 30 minutes, followed by 120-minute reperfusion. Melatonin (10 mg/kg) or vehicle was given 10 minutes before ischemia via the jugular vein.
Main Outcome Measure(s): Reproductive organ weights and epididymal sperm concentration, sperm motility, abnormal sperm rate, and testicular-tissue malondialdehyde (MDA) and glutathione (GSH) levels were examined after reperfusion.
Result(s): MI-R significantly decreased epididymal sperm motility, and increased the testes-tissue level of MDA, compared to the control group. Administration of melatonin reversed the harmful effects of MI-R significantly. However, MI-R did not change sperm concentration, GSH levels, and reproductive organ weights.
Conclusion(s): These findings indicate that MI-R leads to damage of testis tissue and sperm motility, and melatonin protects against MI-R-induced reproductive-organ injury. These results may also encourage the use of antioxidants to reduce remote organ injury in the testis after MI-R. (Fertil Steril® 2007;88:188–92. ©2007 by American Society for Reproductive Medicine.)

Key Words: Melatonin, myocardial ischemia and reperfusion, oxidative stress, sperm characteristics, remote organ

Cardiovascular disease is a leading cause of death worldwide, and remains one of the major killers in modern society. It can be initiated by multiple factors, including ischemia-and-reperfusion (I-R) injury (1). Myocardial ischemia and reperfusion (MI-R) represents a clinically relevant problem associated with thrombolysis, angioplasty, and coronary bypass surgery (2). Several studies showed that MI-R leads to remote organ injury (3, 4), and that oxygen-based reactants may play a central role in this injury (5). Myocardial ischemia and reperfusion cause severe damage, as indicated by free radicals and loss of membrane phospholipids (6, 7).

Reactive oxygen species (ROS) are free radicals such as the hydroxyl radical ($OH^{\cdot}$) and the superoxide anion ($O_2 \cdot^-$), or molecules such as hydrogen peroxide (H_2O_2). The production of ROS is a normal physiological event in various organs, including the testis. However, the overproduction of ROS stimulates DNA fragmentation and can be detrimental to sperm function, associated with peroxidative damage to the mitochondria and plasma membrane. In addition, spermatozoa are especially susceptible to peroxidative damage because of a high concentration of polyunsaturated fatty acids and low antioxidant capacity (8, 9).

Melatonin has been gaining acceptance as potent radical scavenger and antioxidant (10, 11). It was shown to inhibit MI-R-induced oxidative changes and reduce I-R-induced arrhythmias, mortality, and infarct size (11–13). On the other hand, the efficacy of melatonin on the oxidative changes in testis tissue and sperm characteristic due to MI-R has not been well-documented.

In this study, we aimed to investigate the effect of MI-R on remote organ injury in testis tissue, and the possible protective effects of the exogenous administration of melatonin after MI-R in an in vivo rat model. We evaluated

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morphological changes in reproductive organs and sperm characteristics such as epididymal sperm concentration, motility, and abnormal sperm rate, in addition to levels of malondialdehyde (MDA), a stable metabolite of the free radical-mediated lipid peroxidation cascade which is widely used as a marker of oxidative stress, and glutathione (GSH), an important endogenous antioxidant whose levels are influenced by oxidative stress.

MATERIALS AND METHODS

Experimental Groups

Eighteen healthy, adult, male Wistar rats (weighing 250–300 g, 8 weeks old) were kept under standard laboratory conditions (12 hours light:12 hours dark, and 21°C ± 2°C). Experiments were performed between 9 AM–5 PM. This study was performed in accordance with the guidelines for animal research from the National Institutes of Health (Bethesda, Maryland), and was approved by our Local Committee on Animal Research.

The rats were divided into three groups of six rats each. The control group received isotonic saline; the MI-R group received melatonin vehicle; and the MI-R-plus-melatonin group received a single dose of melatonin. Melatonin (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol and further diluted in saline to give a final concentration of 1%, and at a dose of 10 mg/kg was administered by IV injection 10 minutes before ischemia.

Ischemia and Reperfusion Procedure

Rats were anesthetized with intraperitoneal urethane (1.2–1.4 g/kg, Fluka Chemical, Buchs, Switzerland). The jugular vein and trachea were cannulated for drug administration and artificial respiration, respectively. Blood pressure and electrocardiography were monitored during I-R; the results are not described here because these parameters were used only to verify MI-R. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, about 2 mm to the left of the sternum.

Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 mL/100 g body weight at a heart rate of 60 beats/minute to maintain normal partial pressures of CO_2 (pCO_2) and O_2 (pO_2), and pH parameters. After the pericardium was incised, the heart was exteriorized by gentle pressure on the right side of the rib cage. A 6/0 silk suture attached to a 10-mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest, and the animal was allowed to recover for 20 minutes. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery was then occluded by applying tension to the ligature, and reperfusion was achieved by releasing the tension. The artery was occluded for 30 minutes, and then reperfused for 120 minutes (13). The jugular vein was cannulated for drug administration.

As soon as reperfusion was completed, the animal was euthanized by cervical dislocation. The testes, epididymides, seminal vesicles, and prostate were removed and cleared of adhering connective tissue. Measurement of testis weight, length, and thickness was performed, and epididymal, seminal-vesicle, and prostatic weight were evaluated along with epididymal sperm concentration, sperm motility, and sperm morphology. Testis samples were stored at −20°C until biochemical assays.

Epididymal Sperm Concentration, Motility, and Abnormal Sperm Rate

Spermatozoa in the right epididymis were counted by a modified method of Yokoi et al. (14). Briefly, the epididymis was minced with anatomical scissors in 5 mL of physiological saline, placed in a rocker for 10 minutes, and allowed to incubate at room temperature for 2 minutes. After incubation, the supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 25 mg eosin per 100 mL of distilled water. Total sperm number was determined by use of a hemocytometer. Approximately 10 μL of the diluted sperm suspension were transferred to each counting chamber of the hemocytometer, and allowed to stand for 5 minutes. The cells that settled during this time were counted with the use of a light microscope at ×200 magnification.

Progressive motility was evaluated by an method described by Sönmez et al. (15). The fluid obtained from the left cauda epididymis with a pipette was diluted to 0.5 mL with Tris buffer solution. A slide was placed in a light microscope with a heated stage, an aliquot of this solution was placed on the slide, and the percentage of motility was evaluated visually at a magnification of ×400. Motility estimates were performed in three different fields from each sample. The mean of the three estimates was used as the final motility score. Samples for evaluation of motility were kept at 35°C. Slides were prepared with Indian ink for determination of the percentage of morphologically abnormal spermatozoa. In total, 300 sperm cells were counted on each slide under a light microscope at ×400 magnification (16).

Biochemical Assays

Testes tissue was homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride, to obtain 1:10 (w/v) whole homogenate. Concentrations of MDA, as an index of lipid peroxidation (LPO), were measured in the homogenate. Then homogenates were centrifuged at 18,000 × g (4°C) for 30 minutes to determine reduced GSH levels. Concentrations of MDA were assayed according to a modified method of Ohkawa et al. (17), based on their reaction to thiobarbituric acid, and were expressed as nmol/mL. Tissue GSH concentrations were measured by a kinetic assay by means of a dithionitrobenzoic acid-recycling method described by Ellman (18), and were expressed as μmol/mL.