The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: a biochemical and histopathologic evaluation

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Objective: To evaluate the effects of the immunosuppressant tacrolimus as an antioxidant and analyze the histopathologic changes in rat uteri exposed to experimental ischemia-reperfusion (I/R) injury.
Design: Experimental study.
Setting: Experimental surgery laboratory in a university.
Animal(s): Twenty-eight female rats exposed to experimentally induced uterine I/R injury.
Intervention(s): Group I: control group; group II: uterine I/R injury–induced group; group III: pre-ischemia tacrolimus group; group IV: post-ischemia tacrolimus group.
Main Outcome Measure(s): Uterine tissue malondialdehyde (MDA) level as a marker of lipid peroxidation and glutathione (GSH) level and superoxide dismutase (SOD) and catalase (CAT) activities as markers of tissue antioxidant capacity; histopathologic examination of all uterine rat tissue.
Result(s): Following aortic I/R injury, MDA levels were significantly increased whereas GSH levels and CAT and SOD activities were found to be decreased compared with control animals. MDA levels were found to recover prominently after the administration of tacrolimus in both groups III and IV. Administration of tacrolimus improved uterine GSH levels and CAT activity in the tacrolimus-treated groups.
Conclusion(s): Our results indicate that tacrolimus reduces oxidative damage in rat uteri exposed to I/R injury induced by distal abdominal aortic occlusion. Histologic evaluation reveals that tacrolimus attenuates the inflammatory response and protects the tissue damage induced by I/R injury. (Fertil Steril® 2014;101: 1176–82. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ischemia-reperfusion injury, tacrolimus, uterus, rat, transplantation

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Uterine transplantation (UT) has been gaining popularity around the world in the past few years. Patients with uncorrectable uterine-factor infertility are considered to be ideal candidates for UT and include those with the congenital absence of the uterus (Mayer-Rokitansky-Küster-Hauser syndrome) and patients hysterectomized owing to benign conditions, malignant tumors, or massive blood loss after delivery. Furthermore, patients with nonfunctional uterine cavities are also candidates for UT (1). Currently, options for patients with uterine-factor infertility are gestational surrogacy or adoption. However, surrogacy is legal in only a few countries, owing to concerns about

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ethical, social, and legal issues (2). In light of current studies, UT may become the first treatment option for women with uterine-factor infertility in these and other countries. The first attempt of UT in humans was carried out in Saudi Arabia in 2000 (3). Approximately 3 months after the transplantation, the uterus had to be removed owing to uterine prolapse and necrosis. It was thought that this attempt of human uterine transplantation had been made without sufficient animal research. Since that first attempt, many studies have been conducted in animals. Successful transplantation and pregnancy has been achieved in mice, sheep, and rats (4–6). Based on data accumulated from animal studies, the second attempt of UT in humans was conducted from a cadaver donor in Turkey in 2011. Although it was technically successful, it could not produce an uneventful pregnancy with birth of a healthy baby (7). Following this, a Swedish group performed 2 procedures, which were the world's first living-donor UTs from mothers to their daughters (8).

Ischemia-reperfusion (I/R) injury has detrimental effects on transplanted organs (9). In general, I/R injury is mediated by several mechanisms, including the release of reactive oxygen species (ROS), activation of leukocytes, endothelial system dysfunctions and activation of complement pathways (10, 11). ROS are one of the most important components of I/R injury causing apoptotic and necrotic cell death via lipid peroxidation in a variety of organs (12). Lipid peroxidation is a catalytic mechanism leading to oxidative destruction of cellular membranes. Therefore, lipid peroxidation has been suggested to be closely related to I/R injury–induced tissue damage, and malondialdehyde (MDA) is a good indicator of the rate of lipid peroxidation (13). Endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), protect cells from the detrimental effects of ROS. The levels of antioxidant enzymes have been used to indicate the magnitude of oxidative stress that occurs during I/R injury (14).

Tacrolimus, a calcineurin inhibitor, is a crucial immunosuppressant used after kidney, liver, and pancreas transplantations (15–17). It has diverse actions that result in the reduction of I/R injury. Several investigators have documented decreased ROS production in association with a reduction in I/R injury when tacrolimus is administered before ischemia (18, 19). Endothelial damage triggers interactions among platelets, leukocytes, and endothelial cells, thus leading to the increased secretion of cytokines and infiltration of mononuclear cells. Tacrolimus attenuates this cascade by inhibiting the secretion of ROS and reducing the expression of adhesion molecules including P-selectin and intercellular adhesion molecule 1 (18, 20).

The aim of the present study was to examine the effects of tacrolimus on uterine I/R injury induced by distal abdominal aortic occlusion. For this purpose, the rat uterine tissue levels of MDA, GSH, SOD, and CAT were measured. Furthermore, the rat uterine specimens were histologically evaluated.

MATERIALS AND METHODS

Animals

A total of 28 female Wistar albino rats (3–4 months old) weighing 230–270 g obtained from the Marmara University Experimental Animal Research Laboratory were enrolled in the study. They were given free access to water and standard laboratory rodent pellet food. The rats were maintained in the laboratory under controlled environmental conditions (room temperature 22 ± 1°C) and 12-hour:12-hour light-dark cycles. All rats were used in compliance with the national guidelines for the use and care of laboratory animals. The protocols were designed according to the Committee of Ethics on Animal Experimentation and approved by the Institutional Review Board of Marmara University (report no. 83.2013.mar.).

Chemicals

Tacrolimus (Prograf, 5 mg/mL), ketamine hydrochloride (Ketalar, 50 mg/mL), and xylazine (Rompun, 20 mg/mL) were purchased from Astellas, Pfizer, and Bayer, respectively.

Experimental Groups and Surgical Technique

The rats were randomized into four groups of seven rats each. All rats included in the study were synchronized in the diestrus phase of estrous cycle, which was judged as described by Marcondes et al. (21). Group I (the control group) consisted of rats that did not receive any treatment, group II (the uterine I/R injury group) of rats exposed to 0.5 hour of ischemia and 1 hour of reperfusion, group III (the pre-ischemia tacrolimus group) of rats that received intravenous tacrolimus (0.3 mg/kg) 0.5 hour before the induction of I/R, and group IV (the post-ischemia tacrolimus group) of rats that received tacrolimus (0.3 mg/kg) immediately before reperfusion. In groups III and IV, the given dosage of tacrolimus (0.3 mg/kg) was decided according to that reported to be protective in liver warm ischemia reperfusion injury model (22).

The abdominal aorta in the control group was dissected under laparotomy but was not occluded. The other three groups were exposed to ischemia and reperfusion by occlusion of the distal abdominal aorta and collateral occlusion of the ovarian arterial blood supply below the level of the ovaries.

The rats were anesthetized with a combination of ketamine hydrochloride (60 mg/kg intraperitoneally) and xylazine (5 mg/kg intraperitoneally), and supplementary injections of ketamine hydrochloride were applied as needed. When the anesthesia was accomplished, the rats were placed in a supine position and a midline laparotomy was carried out under aseptic conditions. The abdominal aorta was exposed by gently deflecting the loops of intestine to the left side with moist gauze swabs. An atraumatic microvascular clamp (Bulldog clamp; Aesculap) was placed across the distal abdominal aorta just above the bifurcation of iliac arteries, and two more vascular clamps were applied below both ovaries to prevent collateral blood supply. After this, the abdomen was closed and the wound was covered with moist gauze to minimize heat and fluid loss. After a 30-minute ischemia period, all clamps were removed and reperfusion was allowed for 60 minutes. All rats were then killed under anesthesia and both uterine horns were carefully removed. The left uterine horns were reserved for biochemical assays and stored at −80°C until analysis. The right uterine horns