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The effects of tacrolimus on the activity and expression of tissue factor in the rat ovary with ischemia–reperfusion induced injury

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ABSTRACT

In the present study, the effects of tacrolimus on the activity and expression of tissue factor (TF) were investigated in the ovarian ischemia–reperfusion induced injury in rats. Twenty-eight female rats (8–12 weeks, 300–350 g) were divided into four groups: control, ischemia–reperfusion (IR), tacrolimus treated before ischemia (TBI), and tacrolimus treated before reperfusion (TBR) groups (n = 7/per group). TF activity was measured using Quick's method, whereas TF expression was examined immunohistochemically. TF activity was significantly higher in all treated groups compared with the control group. Strong ovarian TF expression was demonstrated in the IR and TBR groups. Moreover, tacrolimus decreased TF activity in the TBI group compared with the IR group. The decreased activity of TF in the ovarian IR model may prevent IR-related inflammation during transplant procedure.

1. Introduction

Uterus and ovary transplantation is important for women who lost their fertility due to menopause or various congenital factors, and want to gain productivity and improve the quality of their life. The ovary transplant operation as a method to restore female fertility is of particular interest. The ovaries always attracted more attention than the uterus as transplantation candidates since the ovarian surgery is less demanding or risky [1]. On the other hand, the transplant procedure is related with ischemia/reperfusion (IR) injury and the surgical trauma may also lead to acute and/or chronic inflammatory reactions [2]. Prolonged oxygen deprivation in the tissue leading to hypoxia results in IR injury. IR injury is mediated by the oxygen derived free radicals, and the IR injury

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consequences include cellular ATP depletion leading to the swelling of mitochondria and the release of Cytochrome c. Cytochrome c activates the signaling cascade leading to the production of interleukin-1β (IL-1β) and the induction of apoptosis [3].

In order to prevent rejection of the transplanted organ and to maintain the patients in a good condition, the patients are co-medicated with some multiple drugs before and after organ transplantation [4]. Tacrolimus (FK506) is a calcineurin inhibitor used as an immunosuppressive agent to prevent the rejection of transplanted organs and to treat autoimmune diseases [5,6]. Tacrolimus acts similarly to cyclosprin A (CsA), exerting its effects principally *via* impairment of gene expression in target cells. Tacrolimus binds to FK506 binding protein (FKBP; immunophilin), and this complex inhibits calcineurin phosphatase. As a result, calcium-dependent events, such as interleukin-2 (IL-2) gene transcription, nitric oxide synthase activation, cell degranulation, apoptosis and T cell proliferation are inhibited [7]. On the other hand, tacrolimus has been reported to have some side effects such as hypertension and renal tubule dysfunction, including hyperkalemia, hypercalciuria and acidosis [5,6].

Tissue factor (TF) is an integral membrane protein that belongs to the γ-interferon receptor superfamily [7]. TF becomes exposed to the blood stream due to blood vessel injury and initiates the enzymatic reactions leading to blood coagulation. TF was found in circulating blood cells, and specific signaling events and various inflammatory mediators have been reported to induce its expression [9–11]. Various tissues and body fluids were demonstrated to exhibit TF activity [12–14]. Abnormal TF expression leads to intravascular thrombosis associated with various diseases, such as atherosclerosis, cancer and sepsis [15]. The role of TF in IR injury has been reported in some studies. TF inhibition has been shown to reduce renal [16], hepatic [17] and myocardial injury [18] following IR. The aim of the current study was to investigate the effects of tacrolimus on the activity and expression of TF in ovarian IR injury.

2. Materials and methods

2.1. Animals and experimental design

All animal and human protocols were approved by the Ethics Committee and the Committee on the Use of Live Animals in Teaching and Research, The University of Marmara. The rats (8–12 weeks, 300–350 g) were randomly divided into four groups ($n = 7$/group): 1/the sham group (controls, C) consisted of rats that did not receive any treatment, 2/the IR group consisted of rats exposed to 30 min of ischemia followed by 60 min of reperfusion; 3/the pre-ischemic tacrolimus group (tacrolimus before ischemia, TBI) consisted of rats that received tacrolimus *iv*. (0.3 mg/kg) 30 min before the induction of IR and 4/the post-ischemic tacrolimus group (tacrolimus before reperfusion, TBR) consisted of rats that received tacrolimus (0.3 mg/kg) immediately before reperfusion. The dosage of tacrolimus was similar to the dose reported to be protective in liver warm IR injury model [19]. The abdominal aorta in the control group was dissected under laparotomy but was not occluded. The other three groups were exposed to IR.

IR was induced by the 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 *ip*.) and xylazine (5 mg/kg *ip*.). Supplementary injections of ketamine hydrochloride were given when needed. After anesthesia, midline laparotomy was carried out on rats in supine position and under aseptic conditions. The abdominal aorta was exposed and an atraumatic microvascular clamp (bulldog clamp, Aesculap, Center Valley, PA, USA) was placed across the distal abdominal aorta above the bifurcation of iliac arteries. Then, two more vascular clamps were applied below both ovaries to prevent collateral blood supply. The abdomen was closed and the wound was covered with moist gauze to minimize heat and fluid loss. Following an ischemic period (30 min), all clamps were removed and reperfusion was allowed for 60 min [20]. All rats were sacrificed and both ovaries were carefully removed. The right ovary was transferred into a 10% neutral buffered formaldehyde for histological examination. The left ovary was kept at −20 °C until determination of TF activity.

2.2. Tissue factor activity

The ovaries were homogenized in 0.9% NaCl to obtain 10% (w/v) tissue homogenates. TF activity was determined in homogenates as was previously described [21]. The clotting reaction (37 °C) started after addition of plasma (0.1 mL) to tissue homogenate (0.1 mL) and 0.02 M $CaCl_2$ (0.1 mL). TF activity was expressed as a clotting time in seconds. Since the clotting time is inversely proportional to TF activity, the lengthening of the clotting time is a manifestation of a decreased TF activity.

2.3. Histology

Histological examinations were carried out on 4 μm slices stained with hematoxylin and eosin, and viewed under a light microscope (Carl Zeiss Axiozoom 16, Göttingen, Germany). Five sections were collected from each ovary. The whole ovary of each rat was examined in blinded fashion by the same histopathologist. Congestion, hemorrhage and edema were scored from 0 to 4 according to their severity, where 0 represented no pathological finding. The tissue damage score was evaluated for each group as follows: 1 – mild edema and congestion; 2 – moderate edema and congestion; 3 – severe edema, congestion and minimal hemorrhage and 4 – severe edema, congestion and hemorrhage.

2.4. Immunohistochemistry

Ovaries were placed in processing cassettes, dehydrated through a serial alcohol gradient, and embedded in paraffin wax blocks. Paraffin sections (4 μm) were cut and mounted on polylysine-coated glass slides. Routine deparaffinization and rehydration procedures were performed. The rabbit monoclonal antibody against TF (1:200; Abcam, Cambridge, UK) was used as primary antibody. For antigen retrieval, the slides were heated in a microwave in Tris EDTA buffer (pH 9; Abcam) and cooled to the room temperature (RT). Sections were then incubated with the primary antibody overnight at 4 °C. Next,