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## Effects of Gingko Glycosid (EGb 761) on Some Vascular Endothelial Functions and Renal Histology in Rabbits With Acute Cyclosporine Nephrotoxicity

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**Abstract :** In recent years, Endothelin-1 (ET-1), Platelet Activating Factor (PAF) and thromboxane have been considered responsible for the pathogenesis of acute cyclosporine nephrotoxicity (ACN). The aim of this study was to investigate the effects of the PAF antagonist gingko glycosid (EGb 761) on both renal histology and ET-1 and Nitric Oxide (NO) levels in experimental ACN.

This study was carried out on 30 albino rabbits, divided into three groups. They were: group A (n=10) the control, group B (n=10), which was given intravenous cyclosporine (15mg/kg/day) and group C (n=10), which was given oral gingko glycosid (2.5mg/kg/day) in addition to cyclosporine. Plasma ET-1 and NO levels and renal histology were investigated in all of the groups. Data analysis was performed by Kruskal-Wallis one-way Anova and Mann Whitney-U tests.

Plasma ET-1 levels in groups B and C were higher than in the controls ( $p<0.0002$ ,  $p<0.0002$ , respectively). Plasma NO levels in groups B and C were lower than in the controls ( $p<0.0002$ ,  $p<0.0047$ , respectively). However, group C had high NO levels and low ET-1 levels compared to group B ( $p<0.004$ ,  $p<0.0002$ , respectively). Renal histological findings of ACN were observed in group B, and to a lesser extent in group C.

Consequently, it was established that the PAF antagonist Gingko glycosid Egb 761 partially improved both the renal histology and plasma ET-1 and NO levels in experimental ACN.

**Key Words:** Acute nephrotoxicity, Cyclosporine, Gingko glycosid.

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### Introduction

The immunosuppressive agent cyclosporine improves graft survival in renal transplantation (1). Cyclosporine-induced acute nephrotoxicity is reversible and is characterized by a decrease in the glomerular filtration rate secondary to vasoconstriction, occurring generally on the afferent arteriole (2,3). The mechanism of this vasoconstriction is not yet entirely clear but has been linked more recently to the release of endothelin-1 (ET-1), platelet activating factor (PAF) and thromboxane (3,4).

The aim of the present study was to investigate the effects of the PAF antagonist gingko glycosid (EGb 761) on both renal histology and plasma ET-1 and nitric oxide (NO) in rabbits with acute cyclosporine nephrotoxicity (ACN).

### Materials and Methods

The present study was performed using 30 albino rabbits (weight 2250-3000g, age 13-15 months). Animals were fed a laboratory diet ad libitum and tap water.

#### Drugs, Reagent and Chemicals

Cyclosporine was obtained from Sandoz (Sandimmun, 50mg/mL amp., Basel, Switzerland). The PAF antagonist Gingko glycosid (EGb 761) was obtained from Abdi Ibrahim (Tebokan forte gutts, 9.6mg/mL/20gutts by Dr. Willmar Schwabe License, Istanbul, Turkey). Aprotinin was obtained from Bayer (Trasyolol, 50mL, 500.000KIU, Istanbul, Turkey). A radioimmunoassay kit (Code RPA 535) for endothelin-1 was purchased from Amersham (Arlington Heights, Ill., USA). To determine nitric oxide (NO), 35% sulphosalysilic acid, Griess reagent (1g/L sulphanilamide, 25g/L phosphoric acid, 0.1g/L N-1 naphthylethylenediamine), 50µmol/L nicotinamide adenin dinukleotid phosphate (NADPH), 5mmol/L flavin adenin

dinucleotide, 10mg/L lactic dehydrogenase, 10mmol/L sodium pyruvate and 200U/L nitrate reductase were used. The nitrate reductase was purchased from Boehringer Mannheim (Germany), while the others were obtained from Sigma (USA).

**Administration of drugs**

The animals were divided into three groups. There were 10 animals in each group. The first group (group A) was selected as the control. Cyclosporine was given to the second group (group B). Cyclosporine and Gingko glycosid Egb 761 were given to the third group (group C).

A catheter was introduced into the right jugular vein and was extracted through a tunnel under the interscapular area. 15 mg/kg/day of cyclosporine was infused intravenously through this catheter for five days in both group B and group C.

2.5 mg/kg body weight/day of the Gingko glycosid Egb 761 was given orally together with food and water. It was started 3 days before the cyclosporine and continued throughout the experiment.

**Procedures**

Venous blood samples from all the rabbits were put into silicone tubes, containing 7.5mM EDTA for the plasma ET-1 and NO determinations. The samples were taken after the administration of drugs in groups B and C. Aprotinin (500KIU/mL blood) was added to the plasma for measuring ET-1.

The rabbits were anesthetized intraperitoneally with Ketamine (100mg/kg). Kidneys were isolated from the surrounding tissues through a median abdominal incision in both the control group and the experimental groups, and were then harvested. Then, the animals were killed with high dose sodium thiopental (2 g) given intravenously. For histological evaluation, the harvested kidneys were fixed in 10% formalin for at least 3 days. A longitudinal piece was embedded in paraffin. Sections 3 μm thick were cut and stained with hematoxylin-eosin (H&E), periodic acid schiff (PAS) and Masson’s trichrome. Pathology and histology specialists, who had no information about the study, examined the slides using a light photomicroscope (Olympus BX-50, Japan).

Plasma endothelin-1 levels (pg/mL) were determined using the method of Deray et al. (5). It is very difficult to determine NO due to its radical nature and very short half life (6). The plasma NO level is most often measured by

the determined stable end products of NO, nitrite and nitrate (7). In the present study, plasma nitric oxide levels (nmol/mL) were measured using the method of Moshage et al (8). According to this method, total nitrite and nitrate concentrations in plasma are determined using a Griess assay. In addition, deproteinized plasma and background controls for each sample are used in the Griess assay to avoid obtaining artifactually high nitrite and nitrate concentrations in plasma. Although, in some published studies, samples were deproteinized, it is not clear whether the controls were included (9,10).

**Statistical analysis**

The data were analyzed using Kruskal-Wallis one-way Anova and Mann Whitney-U tests in the Statistical Package for Social Sciences (SPSS). Statistical significance was taken to be P<0.05. All data are expressed as mean (X) ± standard deviation from the mean (SD).

**Results**

The plasma ET-1 (range:18-90pg/mL) and NO levels (range:5-20nmol/mL) of all the rabbits are summarized in Table 1.

Table 1. Plasma ET-1 and NO levels (mean±SD) and ranges in groups

	ET-1(pg/mL)	NO(nmol/mL)
Group A	22±2.9 (18-27)	16.0±2.4 (12-20)
Group B	77±8.0 (68-90) <sup>#</sup>	8.0±1.9 (5-11) <sup>#</sup>
Group C	39±5.3 (30-47) <sup>#,&amp;</sup>	12.7±1.8 (10-16) <sup>ψ,⊗</sup>

<sup>#</sup>p<0.0002 when compared with group A;

<sup>&</sup>p<0.0002 when compared with group B;

<sup>ψ</sup>p<0.004 when compared with group A;

<sup>⊗</sup>p<0.004 when compared with group B.

The plasma ET-1 and NO levels of the animals in groups A, B and C were significantly different (p<0.00001, p<0.00001, respectively). Compared to those of the controls, ET-1 levels were high in group B and group C. Although the ET-1 level of group C had decreased compared to group B, its level was still higher than that in the controls. NO levels were lower in group B and group C than in the controls. The NO level of group C was higher than that of group B, but its level was still

low. Thus, Gingko glycosid partially improved the levels of plasma ET-1 and NO in ACN.

#### Histological data

In the rabbits given cyclosporine, the tubules were dilated, minimal interstitial edemas and a small number of lymphocytes in the peritubular area were present with isometric cytoplasmic vacuoles in some of the proximal tubular epithelial cells (Figure 1A), prominently thickening in the walls of arterioles associated with muscle layers (Figure 1B) and obliteration with thrombi in some arterioles (Figure 1C).

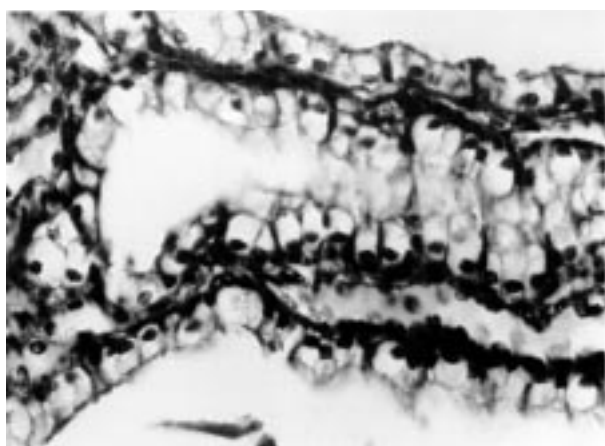


Figure 1A. Isometric cytoplasmic vacuoles in renal proximal tubular cells (H&Ex400) in rabbits with acute cyclosporine nephrotoxicity.

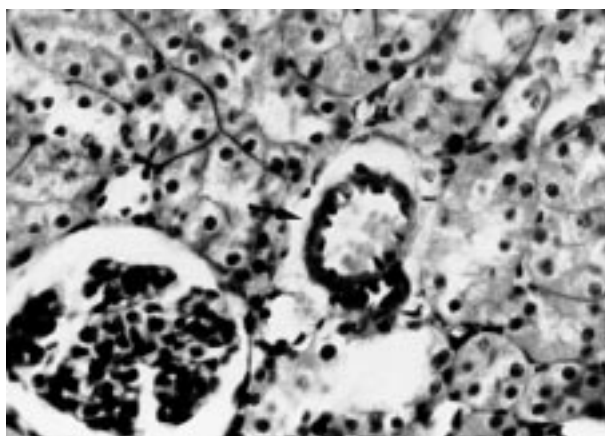


Figure 1B. The prominent thickening in the arteriole wall associated with muscle layer (arrow) (H&Ex200).

In the rabbits given ginkgo glycosid (EGb 761) together with cyclosporine, no thrombosis in the

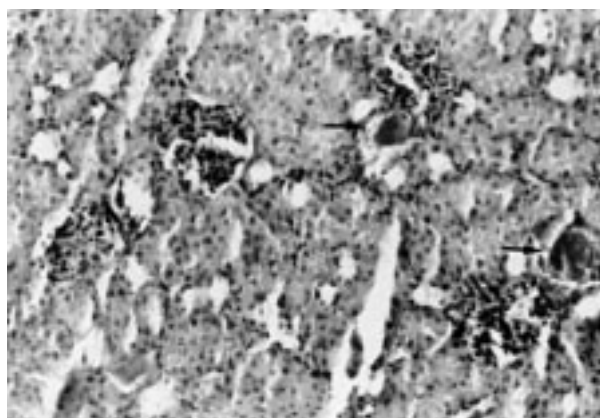


Figure 1C. Thrombosis in the arterioles (arrow) (H&Ex100).

arterioles was observed and there were less interstitial edemas and vacuoles in the proximal tubular epithelial cells (Figure 2).

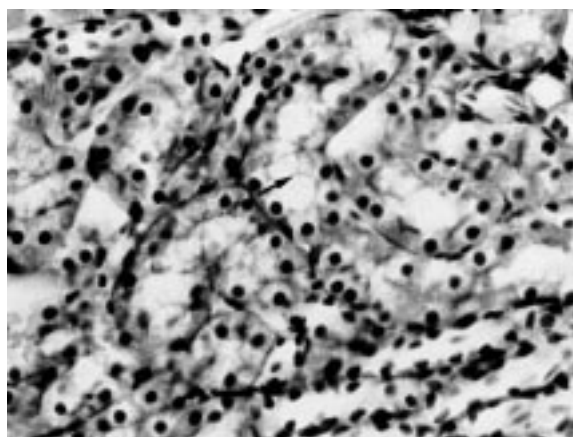


Figure 2. The effects of Ginkgo glycosid Egb 761 on the renal histology of rabbits with acute cyclosporine nephrotoxicity. The reduction at vacuolization in the proximal tubular cells are indicated (arrow) (H&Ex200).

#### Discussion

Cyclosporine directly damages vascular endothelial cells in culture and in vivo (11, 12). The endothelial cell damage leads to the release of the prostaglandins, NO and ET-1. It has been found that plasma ET-1 levels are high in animals given cyclosporine (13). ET-1 mediates the acute renal vasoconstriction induced by cyclosporine. In addition, pharmacologic blockade of the ET-1 receptor has been found to be beneficial in treating ACN (14). It has been reported that cyclosporine-induced nephropathy

improves through chronic dietary supplementation of the NO substrate L-arginine (15, 16). Therefore, this renal disease is presented as an example of NO deficiency states (17). In the present study, high ET-1 and low NO levels were found in the plasma. These results confirm that there is vascular endothelial damage in ACN.

Upon histologic examination of ACN, classical data include epithelial cell vacuolization associated with vascular changes consisting of endothelial cell swelling, intimal thickening, hyalinosis and luminal occlusion (18). Similar findings were obtained in the present study.

Endothelin-1 stimulates Platelet Activating Factor (PAF) synthesis in endothelial cells (19). The kidney has the greatest amount of PAF of all the organs (20). However, PAF is synthesized by platelets, polymorphonuclear neutrophils, monocytes, and endothelial cells (21, 22). PAF has a biphasic effect on afferent arterioles, dilating them that at low concentrations while constricting them at higher concentrations (23). It has been found that PAF is a direct mediator of ACN (24).

PAF receptor antagonist reduces cyclosporine nephrotoxicity by improving whole kidney clearances and glomerular morphology (25). In the present study, when

the PAF receptor antagonist ginkgo glycoside EGb 761 was given orally to rabbits associated with cyclosporine, plasma ET-1 levels decreased and NO levels increased. In addition, there were fewer histologically abnormal findings than in rabbits given only cyclosporine. Thus, it may be suggested that the cyclosporine-induced impairment of renal function and histology is minimized by a PAF antagonist. These results are in accordance with the findings of other studies (24, 26). Furthermore, PAF antagonist improves post-transplant renal function in patients receiving cadaveric kidney transplantation (27), and shows a renal protective effect against warm renal ischemia (28).

As a result, high plasma ET-1 and low plasma NO associated with abnormal renal histology were found in the ACN, and the PAF antagonist Ginkgo glycosid EGb 761 improved the renal histology of the ACN, decreased ET-1 levels, and increased NO levels. Therefore, it may be suggested that a PAF antagonist may be suitable for clinical use in renal transplantation because of its beneficial effects.

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