

Antiproteinuric Effects of Green Tea Extract on Tacrolimus-Induced Nephrotoxicity in Mice

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ABSTRACT

Introduction. It has been reported that proteinuria is an early predictive marker in detection of tacrolimus (TAC) nephrotoxicity. The aim of this study was to investigate the anti-proteinuric effects of green tea extract (GTE) on TAC-induced acute nephrotoxicity in mice.

Methods. The mice ($n = 20$) were divided into 4 groups ($n = 5$ per group); control group mice were intraperitoneally (IP) injected with 0.9% saline, TAC group mice were IP injected with TAC 1 mg/kg, and inducible nitric oxide synthase (iNOS) inhibitor group mice were given in addition NG-nitro-L-arginine-methyl ester 12 mmol/L by subcutaneous injection. TAC-GTE group mice were given TAC by IP injection and GTE 100 mg/kg by subcutaneous injection.

Results. The 24-hour urine protein amounts were significantly increased in TAC group mice (36.1 ± 9.9 mg/d) compared with control group mice (13.3 ± 5.4 mg/d) and significantly decreased in TAC-GTE group mice (19.1 ± 6.9 mg/d, $P < .01$) compared with TAC group mice. The nitric oxide (NO) production by TAC was significantly suppressed by GTE and iNOS inhibitor injection. Renal tissue malondialdehyde (MDA) level was significantly increased in the TAC group compared with the control group and was significantly decreased in the TAC-GTE group compared with that of the TAC group. The antioxidant enzyme activities of superoxide dismutase and catalase were significantly suppressed in the TAC group compared with the control group and were restored in the GTE injection group.

Conclusions. GTE treatment has beneficial antiproteinuric effects on TAC-induced acute renal injury in mice.

PROTEINURIA is an early predictive factor in detection of tacrolimus (TAC) nephrotoxicity [1]. The nitric oxide (NO) produced by TAC plays a role of pathogenesis in TAC-induced nephrotoxicity [2]. Green tea extract (GTE), as an antioxidant and free radical scavenger, had a protective effect in cyclosporine-induced nephrotoxicity in our previous studies [3,4]. We investigated the antiproteinuric effects of GTE on TAC-induced acute renal injury in mice.

METHODS

Tacrolimus was purchased from Chong Kun Dang (Tacrobell, Seoul, Korea). GTE was prepared from a hot-water extract of green tea (Boseong, Chonnam, Korea) as reported by Maity et al. [5]. Normal male Institute for Cancer Research (ICR) mice,

weighing between 20 and 25 g, were used in this study. The mice ($n = 20$) were divided into 4 groups ($n = 5$ per group). Control group mice were intraperitoneally (IP) injected with 0.9% saline, TAC group mice were IP injected with TAC 1 mg/kg, and inducible NO synthase (iNOS) inhibitor group mice were given in addition NG-nitro-L-arginine-methyl ester (L-NAME) 12 mmol/

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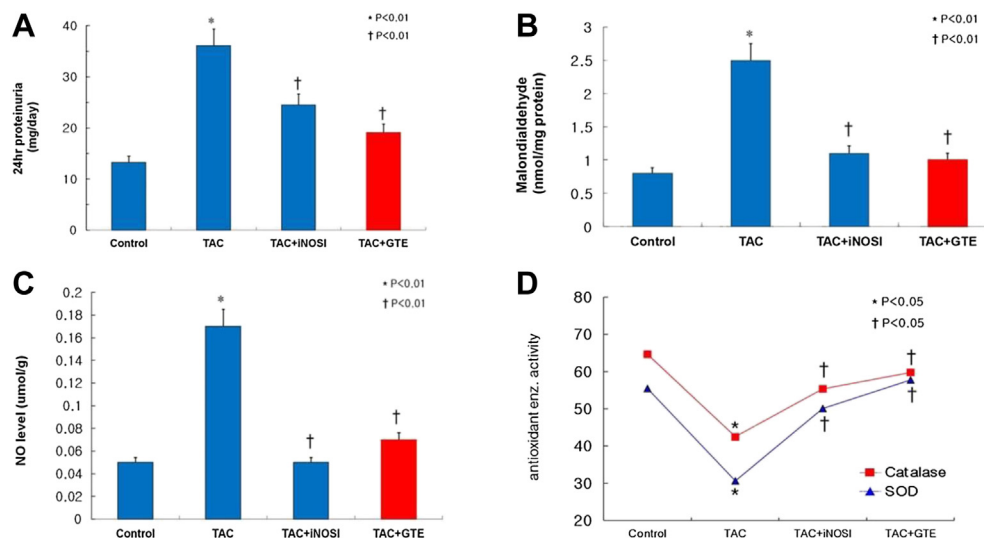


Fig 1. The effects of green tea extracts on 24 hours proteinuria amount (A), malondialdehyde level of kidney tissue (B), nitric oxide level (C) and antioxidant enzyme of catalase and superoxide dismutase (D) on tacrolimus-induced nephrotoxicity in mice. The data were presented during the last CAST meeting.

L by subcutaneous injection. TAC-GTE group mice were given TAC by IP injection and GTE 100 mg/kg by subcutaneous injection. Blood samples were obtained, and the 2 kidneys from each mouse were immediately removed. The concentration of NO was

measured by NO ELISA (enzyme-linked immunosorbent assay) kit. Renal tissue malondialdehyde (MDA) level and antioxidant enzyme activities also were measured. Tissue samples of the mouse kidneys were fixed in 10% buffered formalin embedded in

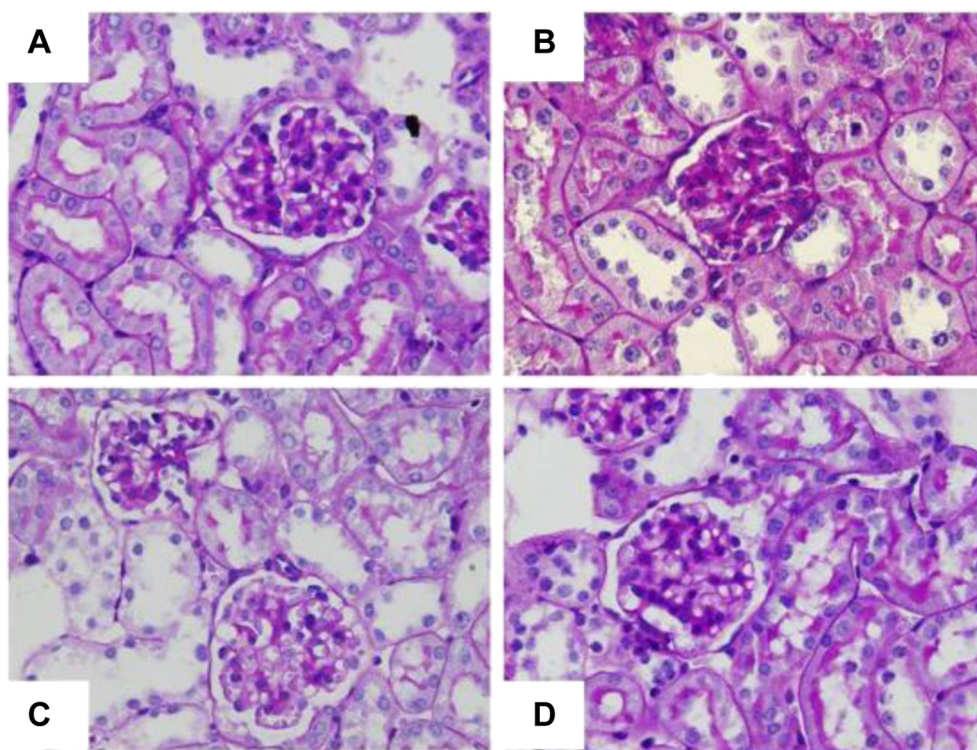


Fig 2. Light microscopic findings of mice kidney (PAS staining, X100). Mice were subjected to 0.9% saline injected group (Control, A), tacrolimus injected group (TAC, B), tacrolimus plus iNOS inhibitor group (TAC+iNOSi, C) and tacrolimus plus green tea extract group (TAC+GTE, D). There are proximal tubular necrosis and mild interstitial inflammation in the kidneys of mice after TAC injection but no significant pathologic changes in TAC plus L-NAME injection and GTE injected group.

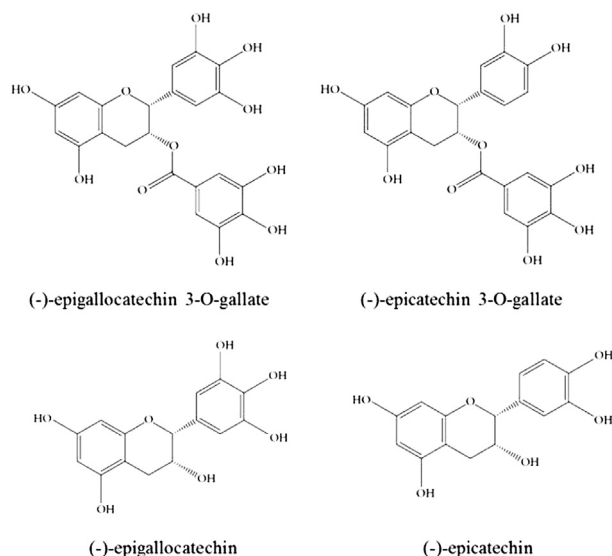


Fig 3. Chemical structure of primary components of green tea.

paraffin and cut into 4- μ m sections. For histopathology examination, the sections were stained with periodic acid-Schiff.

RESULTS

There were no significant differences in serum urea nitrogen and creatinine and serum TAC levels ($P > .05$). The 24-hour proteinuria levels were significantly increased in the TAC group (36.1 ± 9.9 mg/d) compared with the control group (13.3 ± 5.4 mg/d, $P < .01$) and significantly decreased in the TAC-GTE group (19.1 ± 6.9 mg/d, $P < .01$) compared with the TAC group. The NO production by TAC was significantly suppressed by GTE and iNOS inhibitor compared with the TAC group ($P < .01$). The renal tissue MDA level was significantly increased in the TAC group compared with the control group and significantly decreased in the TAC-GTE group compared with that of the TAC group ($P < .01$). The antioxidant enzyme activities of superoxide dismutase and catalase were significantly ($P < .05$) suppressed in the TAC group compared with the control group and restored in the GTE injection group (Fig 1).

There was proximal tubular necrosis and mild interstitial inflammation in the kidneys of mice after TAC injection, but no significant pathologic changes in GTE-injected and L-NAME-injected groups (Fig 2).

DISCUSSION

In this experiment, we showed that the proteinuria of the TAC nephrotoxicity is associated with lipid peroxidation

and NO production. The present study also showed that treatment with GTE has beneficial antiproteinuric effects through antioxidative effects in the kidney from TAC-induced acute nephrotoxicity in mice.

The primary components of green tea are 57% polyphenol, 11% amino acid, 9% free sugar (maltose, fructose, glucose), 16% inorganic substance (potassium, calcium, magnesium), and 1% caffeine. The relative compositions of polyphenol are 44% (-)-epigallocatechin 3-O-gallate, 15% (-)-epigallocatechin, 15% (+)-gallocatechin, 10% (-)-gallocatechin 3-O-gallate, 7% (-)-epicatechin, 5% epicatechin 3-O-gallate, and 4% (+)-catechin. The chemical structure of primary components of green tea is presented in Fig 3. Green tea extract, including (-)-epigallocatechin 3-O-gallate, has antioxidants and antidiabetes activities [6–9]. In this study, GTE also had antiproteinuric activity in TAC-induced acute renal injury in mice. Further studies of GTE should be conducted to investigate the effects of TAC-induced nephrotoxicity in humans.

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