ELSEVIER

Cilostazol and Diltiazem Attenuate Cyclosporine-Induced Nephrotoxicity in Rats

M. Gokce, M.F. Yuzbasioglu, E. Bulbuloglu, H. Oksuz, S. Yormaz, O. Altınoren, M. Kutlucan, I. Coskuner, E. Silay, and I.T. Kale

ABSTRACT

Aim. Cyclosporine (CsA), an important agent used in organ transplantation to prevent rejection, displays nephrotoxicity as the most important side effect limiting usage. In this study, we sought to evaluate the effects of cilostazol and diltiazem to counter the nephrotoxicity induced by the calcineurin inhibitor CsA.

Materials and methods. Animals were randomly divided into seven groups, each consisting of eight animals: sham, controls, cilostazol, diltiazem, CsA, CsA plus diltiazem, and CsA plus cilostazol treatment. At the end of a 60-minute ischemic period, we administered the drugs after reperfusion for 7 days thereafter. CsA (10 mg/kg/d) was intraperitoneally for 7 days; cilostazol (10 mg/kg/d) orally by catheter for 7 days; diltiazem (5 mg/kg/d) intraperitoneally for 7 days. At the end of the 7-day treatment period, blood and tissue samples were harvested for biochemical, and serological evaluation.

Results. Ischemia-reperfusion injury significantly increased malondialdehyde (MDA) levels as well as decreased catalase (CAT) activities and superoxide dysmutase (SOD) content. The lowest MDA mean level was observed in the diltiazem and, the highest in the control group. The lowest CAT mean levels were noted in the CsA and diltiazem groups with highest CAT content was in the CsA and cilostazol groups. The lowest SOD mean level occurred in the sham group; the highest, in the CsA group.

Conclusion. Cilostazol and especially diltiazem were effective to mitigate renal ischemia-reperfusion injury.

CYCLOSPORINE A (CsA) is currently one of the most important immunosuppressive agents for a wide range of organ transplantations, including kidney, liver, heart, lung, pancreas, and intestine.[1] Its most important limiting side effect is nephrotoxicity.[2] Although the underlying mechanism of CsA-induced nephrotoxicity is unclear, reactive oxygen species (ROS) have been implicated extensively in the toxicity.[3] The acute and chronic nephrotoxic effects of CsA are attenuated by administration of various antioxidants.[4]

Cilostazol, a specific type-III phosphodiesterase inhibitor, is widely used to treat ischemic symptoms due to peripheral vascular disease. Recent studies have reported that cilostazol suppresses proinflammatory cytokine production and improves disturbances of local microcirculation.[5] This platelet aggregation inhibitor and vasodilator

From the Department of General Surgery and Renal Transplantation Center (M.G., M.F.Y., E.B., S.Y., I.T.K.), School of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey; Department of Anaesthesia and Reanimaton (H.O., M.K., I.C., E.S.), School of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey; Department of Nephrology and Renal Transplantation Center (O.A.), School of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey.

The study was supported by grants from Kahramanmaras Sutcuimam University Research Department, Kahramanmaras, Turkey.

Address reprint requests to Yüzbaşioğlu MF, MD, Ascociate Professor, Department of General Surgery and Organ Transplantation, School of Medicine, Kahramanmaras Sutcuimam University, Kahramanmaras, Turkey. E-mail: f_yuzbasioglu@hotmail.com

0041-1345/12/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2012.04.025

has been prescribed to treat ischemic symptoms and peripheral vascular disease.[5] Apart from its antiplatelet and vasodilating properties, cilostazol inhibits tumor necrosis factor-α production. In addition, it has been reported to inhibit endothelial neutrophil adhesion by decreasing the expression of adhesion molecules.[6,7] Anti-inflammatory and tissue protective effects of cilostazol have been reported using in vivo and in vitro models.[8,9]

The possible benefits of cilostazol or diltiazem on CsA-induced nephrotoxicity have not yet been sufficiently tested. Therefore, we investigated the protective effects of cilostazol and diltiazem on CsA-induced nephrotoxicity in a rat model.

MATERIALS AND METHODS

Animals

The experimental protocol used in this study approved by our Animal Ethics Review adhered to The National Institutes of Health guidelines for the use of experimental animals; 56 Wistar albino male rats housed in individual cages in a temperature-controlled room with alternating 12-hour light-dark cycles were acclimatized for a week before the study. Food was removed 8 hours prior to the study, but all animals were allowed free access to water and a rat chow diet.

Experimental Design

All assays were performed by an investigator blinded to the study group assignment. Rats were anesthetized with intramuscular injection of 60 mg/kg ketamine hydrochloride (Ketalar, Eczacibaşi, Turkey). The shaved abdominal region was sterilized with povidone iodine solution via midline incision, the abdominal viscera were retracted to the right side. After the left renal hilus was dissected, we occluded the renal vascular pedicle using a microvascular clamp (REDA Instrumente, 13111-06, Tuttlingen, Germany) with the intestine replaced into the abdominal cavity. At the end of a 60-minute ischemic period, reperfusion was established by removing the clamp. After 7 days, a left nephrectomy was performed by relaparotomy. Animals were randomly divided into seven groups, each consisting of six animals: group 1 (sham group, $n = 8$) rats were subjected to the same surgical procedures except for the renal ischemia-reperfusion (I/R). Group 2 (control group, $n = 8$) rats underwent 60 minutes of left renal ischemia. Group 3 animals (I/R + cilostazol group, $n = 8$) were administered cilostazol (Pletal 100 mg; Abdi Ibrahim, Turkey; 10 mg/kg/d) orally by catheter for 7 days. Group 4 animals (I/R + diltiazem group, $n = 8$) were administered diltiazem (Diltizem-L 25 mg; Mustafa Nevzat, Turkey; 5 mg/kg/d) intraperitoneally for 7 days. Group 5 animals (I/R + CsA group, $n = 8$) were administered CsA (Sandimmun 50 gm/mL solution for infusion, Novartis Pharma, Turkey; 10 mg/kg/d) intraperitoneally for 7 days. Group 6 animals (I/R + CsA and diltiazem group, $n = 8$) were administered CsA plus diltiazem for 7 days. Finally, group 7 animals (I/R + CsA and cilostazol group, $n = 8$) were administered CsA plus cilostazol for 7 days.

Antioxidant Study

To determine tissue antioxidant levels, we obtained $1 \times 1\text{-cm}^2$ tissue samples from the left kidney. The samples were preserved in a deep freezer (−80°C) until examination. The tissues were homogenized with three volumes of ice-cold 1.15% KCl. The activities of antioxidant enzymes and the levels of lipid peroxidation were measured in supernates obtained after centrifugation at 14,000 rpm. Superoxide dysmutase (SOD) activity was measured according to the method described by Fridovich,[10] (CAT) by the decrease in hydrogen peroxide concentration at 230 nm using the method of Beutler.[11] Lipid peroxidation expressed as malondialdehyde (MDA) was measured according to procedure of Ohkawa et al.[12] Protein concentrations were determined according to the Lowry method.[13]

Statistical Analysis

All variables were expressed as mean values and standard deviations. Differences between groups were evaluated by Kruskal-Wallis variance analysis followed by a post hoc Mann-Whitney U test. P values $< .05$ were considered statistically significant. All data were entered into and processed by SPSS 11.0 for Windows statistical package (SPSS Inc, Chicago, Ill, USA).

RESULTS

The I/R procedure significantly increased MDA levels and decreased CAT activities as well as SOD levels ($P < 0.05$). MDA levels were lower among diltiazem; CsA plus diltiazem, and cilostazol groups compared with the sham group and in all groups versus the control group ($P < .05$). The lowest MDA mean level was in the diltiazem and the highest value was in the control group (Fig 1).

Our findings showed CAT levels to increase in the CsA plus Cilostazol CsA; and CsA plus diltiazem groups compared with the diltiazem group. The lowest CAT mean level was in the CsA plus diltiazem and the highest value in the CsA plus cilostazol group (Fig 2).

Mean SOD levels were increased in all groups. SOD levels in all groups showed a similar range of values except for the CsA group. The lowest SOD mean level occurred in the sham and the highest in the CsA group (Fig 3).

DISCUSSION

The use of the calcineurin inhibitors has led to major advances in the field of transplantation due to the reduced rates of acute rejection episodes and excellent outcomes. However, the chronic nephrotoxicity of these drugs is the Achilles' heel of current immunosuppressive regimens.[14]

CsA induced nephrotoxicity is associated with nodular hyaline deposits in the afferent arterioles. Eventually, these deposits narrow the vascular lumen.[15] The other facit of the nephrotoxicity is an endothelial injury secondary to vasoconstriction-associated ischemia. In addition, it has been suggested that CsA increases platelet aggregation and activates prothrombotic factors. The mechanisms underlying thrombotic microangiopathy after renal transplantation have been recently reviewed by Ponticelli.[16]

Cilostazol, a platelet aggregation inhibitor and vasodilator, was developed to treat ischemic symptoms and peripheral vascular disease. Its antiplatelet action is mainly due to inhibition of phosphodiesterase III (PDE III), which also results in reduced smooth muscle cell growth and extracellular matrix synthesis. These effects possibly reduces reste-

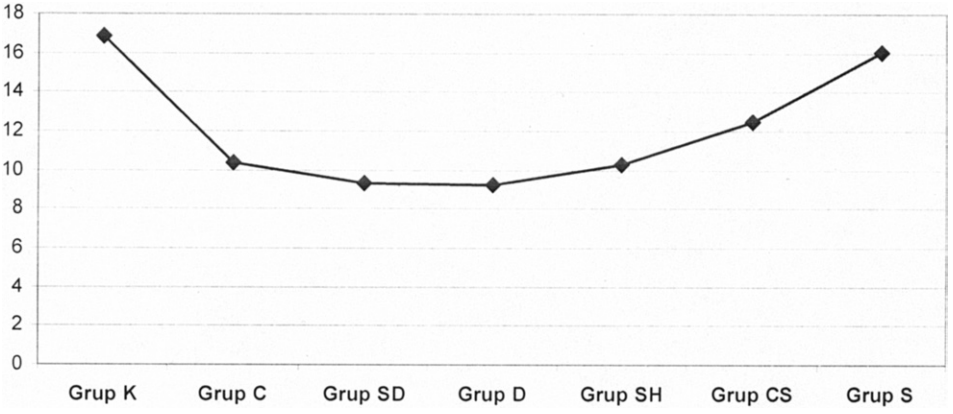

Fig 1. Comparison of malondialdihyde; lowest, diltiazem group; highest, control group.

nosis.[17,18] In a preclinical study, Ishizaka et al evaluated the effects of locally administered cilostazol on neointimal formation in balloon-injured rat carotid arteries. The intimal area in the injured carotid was significantly smaller among the cilostazol versus the control group.[19]

Cilostazol acts as a selective inhibitor of PDE III, an enzyme that breaks down cyclic adenosine monophosphate. The vasodilatory and antiplatelet actions of cilostazol are due mainly to the inhibition of PDE III with subsequent elevation of intracellular cAMP levels. The higher cAMP levels stimulate the production of cAMP-dependent protein kinase, resulting in reduced levels of intracellular Ca within platelets, which, in turn, suppresses platelet activity.[20]

In our experimental study, MDA levels were lower among the cilostazol-A compared with the control group ($P < .05$) and nearly at those of the sham group, indicating a protective, healing effect of cilostazol. In contrast, the lowest MDA level was noted among the diltiazem-treated group, suggesting that cliostazol was effective but not as effective as diltiazem. 5-y However, long-term comparisons of these two agent is needed to derive a conclusion about their individual benefits.

Arteriolar hyalinosis is commonly regarded as irreversible, although it has been reported that complete regression of severe CsA-associated arteriolopathy and remodeling of arterioles can occur after stopping or reducing the CsA dose.[21,22] These manuevers can ameliorate CsA toxicity[23]; however, they can be associated with an increased risk of acute rejection episodes, which are a predictor of long-term graft survival.[24] Based on the intracellular actions of CsA, calcium-dependent mechanisms may be important during the development of CsA-related nephrotoxicity.[25] Previous studies have demonstrated that the calcium-channel inhibitor diltiazem produces not only functional, but also morphological improvement in CsA-induced acute nephrotoxicity.[26] Diltiazem is also effective to prevent ischemic damage.[27] In vitro analysis of isolated perfused kidneys revealed antagonizing effects of diltiazem on the vasoconstriction induced by CsA. A reduction of neurohumoral factors, such as angiotensin, thromboxane, norepinephrine, and endothelin, may contribute to the beneficial effects of diltiazem.[28] Neumayer et al demonstrated that diltiazem treatment reduced posttransplant acute renal failure as well as the incidence of acute rejection episodes.[27]

Lipid abnormalities are common after transplantation. The role of hyperlipoproteinemia in the pathogenesis of atherosclerosis has been clearly established both in healthy individuals and kidney transplant recipients.[29] Transplant recipients are at increased risk for cardiovascular events; more than 55% of deaths with a functioning graft are due to cardiovascular causes.[30,31] Hyperlipidemia has been proposed to be an independent risk factor predisposing to

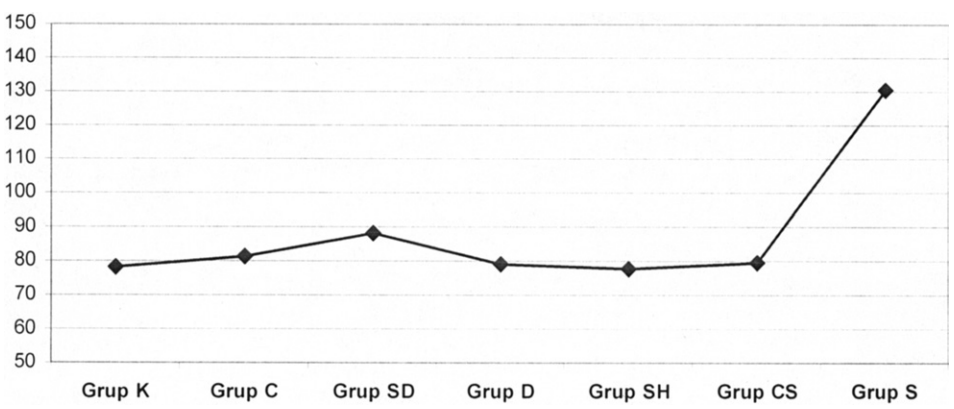

Fig 2. Comparison of superoxide dysmutase; lowest, sham group; highest, cyclosporine group.

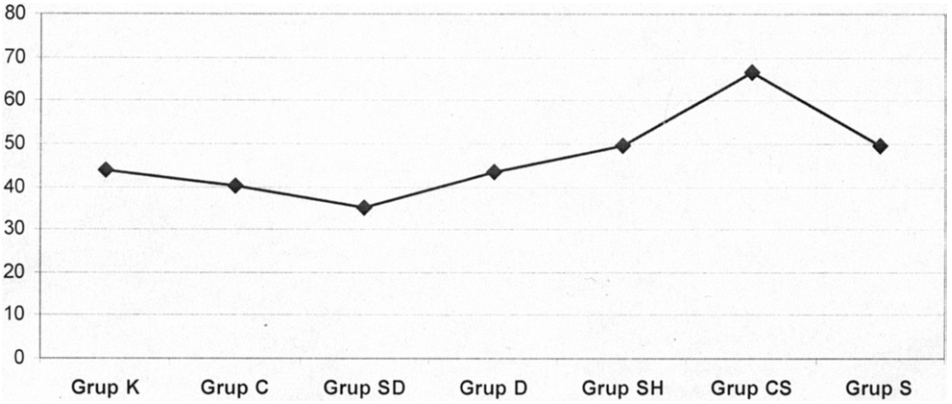

Fig 3. Comparison of catalase; lowest, cyclosporine and diltiazem group; highest, cilostazol and cyclosporine group.

chronic rejection, chronic allograft nephropathy, and coronary arterial occlusive disease. Studies in rats have shown cilostazol to decrease serum triglycerides and to increase high-density lipoprotein cholesterol.[32] In addition, clinical observations have suggested benefical effects of cilostazol on lipid metabolism: namely, reduced plasma triglycerides and increased high-density lipoprotein cholesterol. Preclinical studies have suggested that some of the lipid-lowering effects of cilostazol may be due to increased lipoprotein lipase activity.[33] The other possitive effect of cilostazol is inhibition of platelet aggregation but not accompanied by bleeding. Comparing the effects of aspirm (ASA), ticlopidine, and cilostazol on bleeding time in 10 healthy adults, Tamai et al[34] reported the former two gents to prolong bleeding time with ASA increasing the maximal bleeding rate. In contrast, none of the quantitative bleeding time parameters were altered by cilostazol.

The results of this study clearly showed that 7-day therapy with the selective type-III phosphodiesterase inhibitor cilostazol dramatically reduced I/R. The protection is believed to relate to the pharmacological effects of the PDE III inhibitor including vasodilation, inhibition of platelet activation and aggregation, inhibition of thrombosis, increased blood flow to the limbs, improvement in serum lipids with lowering of triglycerides, and elevation of high-density lipoprotein cholesterol, as well as inhibition of vascular smooth muscle cell growth.

In conclusion, the potent and selective PDE III inhibitor, cilostazol, significantly inhibits the CsA induced renal IR in rats. Our results demonstrate that cilastazol reverses the effects of calcinorin inhibition. Also, the use of cilostazol could be clinically helpful for the therapy of renal ischemia reperfusion damage. Our findings further support the beneficial short-term effects of diltizaem and ciliostazol supplementation in CsA induced renal IR rats. Additional studies are necessary to investigate whether cilostazol is also protective in new immunosuppressive drugs.

REFERENCES

1. Flechner SM, Payne WD, Van-Buren, et al: Transplantation 36:268, 1983)

2. Meyers BD, Sibley R, Newton L, et al: Kidney Int 33590, 1988)

3. Adeagbo AS, Patel D, Iddrissu A, et al: NS-398, a selective cyclooxygenase-2 blocker, acutely inhibits receptor-mediated contractions of rat aorta: role of endothelium. Eur J Pharmacol 458:145, 2003

4. Tariq M, Morais C, Sobki S, et al: N-acetylcysteine attenuates cyclosporin-induced nephrotoxicity in rats. Nephrol Dial Transplant 14:923, 1999

5. Kambayashi J, Liu Y, Sun B, et al: Cilostazol as a unique antithrombotic agent. Curr Pharm Des 9:2289, 2003

6. Shin HK, Kim YK, Kim KY, et al: Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and cytokines via lectin-like oxidized low-density lipoprotein receptor-1 activation prevention by cilostazol. Circulation 109:1022, 2004

7. Omi H, Okayama N, Shimizu M, et al: Cilostazol inhibits high glucose-mediated endothelialneutrophil adhesion by decreasing adhesion molecule expression via NO production. Microvasc Res 68:119, 2004

8. Kin KY, Shin HK, Choi J, et al: Inhibition of lipopolysaccharide-induced apoptosis by cilostazol in humanumbilical vein endothelial cells. J Pharmacol Exp Ther 300:709, 2002

9. Clayton RA, Dick CAJ, Mackenzie A, et al: The effect of selective phosphodiesterase inhibitors, alone and in combination, on a murine model of allergic asthma. Respir Res 5:4, 2004

10. Fridovich I: Superoxide radical: An endogenous toxicant. Ann Rev Pharmacol Toxicol 23:239, 1983

11. Beutler E: Red Cell Metabolism. 2nd ed. New York: Grune & Stratton; 1975

12. Ohkawa H, Ohishi N, Tagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351, 1979

13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 193:265, 1951

14. Naesens M, Kuypers DRJ, Sarwal M: Calcineurin inhibitor nephrotoxicity. Clin J Am Soc Nephrol 4:481, 2009

15. Mihatsch MJ, Kyo M, Morozumi K, et al: The side-effects of ciclosporine-A and tacrolimus. Clin Nephrol 49:356, 1998

16. Ponticelli C: De novo thrombotic microangiopathy. An underrated complication of renal transplantation. Clin Nephrol 67:335, 2007

17. Takahashi S, Oida K, Fujiwara R, et al: Effect of cilostazol, a cyclic AMP phosphodiesterase inhibitor, on the proliferation of rat aortic smooth muscle cells in culture. J Cardiovasc Pharmacol 20:900, 1992

18. Pan, Arauz E, Krzanowski JJ, et al: Synergistic interactions between selective pharmacological inhibitors of phosphodiesterase isozyme families PDE III and PDE IV to attenuate

proliferation of rat vascular smooth muscle cells. Biochem Pharmacol 48:827, 1994

19. Ishizaka N, Taguchi J, Kimura Y, et al: Effects of a single local administration of cilostazol on neointimal formation in balloon-injured rat carotid artery. Atherosclerosis 142:41, 1999

20. Kimura Y, Tani T, Kanbe T, et al: Effect of cilostazol on platelet aggregation and experimental thrombosis. Arzneim-Forsch/Drug Res 35:1144, 1985

21. Collins BS, Davis CL, Marsh CL, et al: Reversible cyclosporine arteriolopathy. Transplantation 54:732, 1992

22. Morozumi K, Thiel G, Albert FW, et al: Studies on morphological outcome of cyclosporine-associated arteriolopathy after discontinuation of cyclosporine in renal allografts. Clin Nephrol 38:1, 1992

23. Burke JF, Pirsch JD, Ramos EL, et al: Long term efficiency and safety of cyclosporine in renal transplant recipients. N Engl J Med 331:358, 1994

24. Kumano K, Endo T, Koshiba K: Functional and morphological changes in rat kidney induced by FK506 and its reversal by various vasodilators. Nippon Hinyokika Gakkai Zasshi 83:650, 1992 Japanese no K. Endo

25. Rodicio JL, Morales JM, Alcázar JM, et al: Calcium antagonists and renal protection. J Hypertens Suppl 11:S49, 1993 (Review)

26. Weir MR, Suthanthiran M: Supplementation of immunosuppressive regimen with a calcium channel blockers. Clin Immunother 2:458, 1994

27. Neumayer HH, Kuzendorf U, Schreiber M: Protective effects of calcium antagonists in human renal transplantation. Kidney Int 41(suppl 36):S87, 1992

28. Guijarro C, Massy ZA, Kasiske BL: Clinical correlation between renal allograft failure and hyperlipidemia, Kidney Int 48:56, 1995

29. Kobashigawa JA, Kasiske BL: Hyperlipidemia in solid organ transplantation, Transplantation 63:331, 1997

30. Lindholm A, Albrecthsen D, Frodin L, et al: Ischemic heart disease: major cause of death and graft loss after renal transplantation in Scandinavia, Transplantation 60:451, 1995

31. Isoniemi H, Nurminen M, Tikkanen MJ, et al: Risk factors predicting chronic rejection of renal allografts. Transplantation 57:68, 1994

32. Stringer MD, Görög PG, Freeman A, et al: Lipid peroxides and atherosclerosis, BMJ 298:281, 1989

33. Tani T, Uehara K, Sudo T, et al: Cilostazol, a selective type III phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats. Atherosclerosis 152:299, 2000

34. Tamai Y, Takami H, Nakahata R, et al: Comparison of the effects of acetylsalicylic acid, ticlopidine and cilostazol on primary hemostasis using a quantitative bleeding time test apparatus. Haemostasis 29:269, 1999