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Cyclosporine reduces basolateral, but not apical, nitric oxide secretion in medullary thick ascending limb cells

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Abstract Cyclosporine (CsA) reduces nitric oxide (NO) production in medullary thick ascending limb (mTAL) cells. We postulated that CsA affected NO secretion in a vectorial manner in polarized renal epithelial cells. The experiments were performed in a model of mTAL subcultured cells. The expression of iNOS in mTAL cells was confirmed by RT-PCR. The cells were grown on a non-permeable filter. Nitrite was measured by the modified Griess method. Transepithelial resistance was measured to ensure the integrity of the tight junction. CsA (100 ng/ml) reduced NO production by 22% in mTAL cells. The inhibitory effect was limited to the basolateral side (control: 165 ± 11; plus CsA: 93 ± 17 nM/10^6 cells, $P < 0.001$) without affecting apical NO secretion. The transepithelial resistance through the epithelial monolayer remained unchanged in CsA-treated cells. CsA reduced basolateral NO secretion without affecting apical secretion. The results suggest that CsA might affect intrarenal hemodynamics at the peritubular level.

Key words Cyclosporine · Nitric oxide · Thick ascending limb cells

Introduction

Nitric oxide (NO) plays a major role in intrarenal hemodynamics [3, 6]. Recent studies indicate that tubular epithelial cells may constitutively generate NO. *iNOS* mRNA has been shown to be mainly expressed in rat kidney medullary thick ascending limb (mTAL) cells and medullary collecting ducts without any NO inducer [8, 11, 14]. These results demonstrated constitutive expression of *iNOS* mRNA in rat medulla and suggested that NO may play a role in hemodynamic regulation in this part of the kidney [3]. An *iNOS* mRNA has been identified in a model of polarized medullary thick ascending limb (mTAL) cultured cells from mouse kidney [16].

Cyclosporine (CsA) is a potent immunosuppressive agent widely used in the prevention of transplant organ rejection. The major drawback of this immunosuppressive agent is its nephrotoxicity [12]. Although the exact mechanism of CsA nephrotoxicity is still unknown, CsA has been shown to enhance renal arterial vasoconstriction and decrease renal blood flow [4], in part by altering the balance between vasodilating and vasoconstricting mediators, such as endothelin and NO/EDRF [7]. Our previous study suggested that CsA inhibited NO production by mTAL cell [16]. As the mTAL cell is a polarized epithelial cell [9, 11], the question arises whether CsA modulates the production of NO in a polarized manner. To address this issue, we investigated the effects of CsA on the production of NO in a model of cultured mouse mTAL cells that retain the specific functions of the parent cells from which they were derived [15].

Materials and methods

The experiments were carried out on cultured cells derived from isolated mTAL microdissected from the kidney of 1-month-old normal mice [15]. Cultured mTAL cells were routinely grown in a

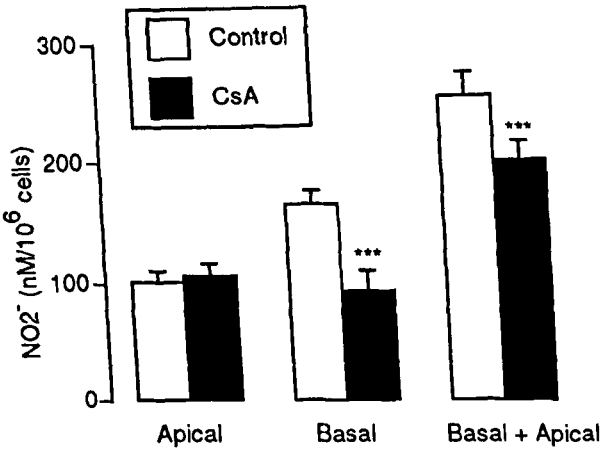

Fig. 1 Effects of CsA on the production of NO by cultured mTAL cultured cells grown on non-permeable filters. The release of NO_2^- was measured from apical and basal medium on sets of confluent mTAL cells incubated without or with CsA (100 ng/ml) for 6 h at 37 °C. Values represent the mean ± SEM from six separate experiments. ***$P < 0.001$ vs control (*C*) values. *Black bars*, CsA, *white bars*, control

modified culture medium (DMEM : HAM's F12, 1:1 vol/vol; 60 nM sodium selenate; 5 μg/ml transferin; 2 mM glutamine; 5 μg/ml insulin; 50 nM dexamethasone; 1 nM triiodothyronine; 10 ng/ml epidermal growth factor; 2 % fetal calf serum; 20 mM HEPES, pH 7.4) at 37 °C in a 5 % $C0_2$-95 % air atmosphere. The transepithelial electrical resistance (R_T) was measured on confluent cell layers grown on collagen-coated permeable filters using the Millicell Electrical Resistance System (ERS, Millipore Corporation, Bedford, Mass., USA) [13].

The stable end product of NO oxidation, nitrite (NO_2^-), was measured in the medium by the Griess method to determine the amount of NO produced by cultured mTAL cells [16]. Briefly, 1 ml culture medium was mixed with 1 ml Griess reagent (0.1 % naphthylethylenediamine dihydrochloride, 1 % sulfanilamide in 5 % concentrated H_3PO_4, vol:vol). After 15 min of incubation at room temperature, the colorimetric reaction was read by spectrophotometry at 546 nm (Beckman DU-600). A standard curve was generated by addition of various concentrations of sodium nitrite (0.01–0.30 μg/ml) to the culture medium.

The effects of various agents on the production of NO by cultured mTAL cells were tested. Confluent cells were incubated with 10–300 ng/ml CsA (Sandoz, Basel, Switzerland), 10^{-7} M FK506 (Fujisawa, Osaka, Japan) or 10^{-7} M rapamycin (Wyeth-Ayerst Research, Princeton, N.J., USA) for 6 h at 37 °C. All measurements were performed in duplicate. Results are expressed as nM NO_2^- per 10^6 cell as means ± SEM from (*n*) experiments performed in duplicate or triplicate. Significant differences from paired and unpaired experiments were analyzed by Student's *t*-test.

Results

iNOS isoform in microdissected mouse mTAL segments and cultured mTAL cells was detected by RT-PCR using specific primers as described in a previous study [16]. The release of NO_2^- was measured on confluent mTAL cells incubated with various concentrations of CsA for 6 h at 37 °C. CsA decreased production of NO in a dose-dependent manner and reached its maximal inhibitory effect with 100 ng/ml CsA, as previously described [16]. We tested the effects of FK506 and rapamycin, two other immunosuppressive agents similar to CsA [2], on the production of NO. Compared with untreated cells, 100 ng/ml (corresponding to 0.8×10^{-7} M) CsA significantly reduced NO_2^- released by mTAL cells by 43 % (control: 334 ± 14; plus CsA: 192 ± 15 nM/10^6 cells, $P < 0.001$ vs control). FK506 (10^{-7} M) also reduced, but to a lesser extent (292 ± 8 nM/10^6 cells, $P < 0.01$ vs control), the production of NO, whereas rapamycin (10^{-7} M) had no effect (354 ± 11 nM/10^6 cells). These results indicated that CsA had a more potent inhibitory effect than FK506 and rapamycin on the production of NO by cultured mTAL cells. To test the polarized effect of CsA in mTAL cells, the cells were grown on non-permeable filters. CsA (100 ng/ml) reduced NO production by mTAL cells grown on the filters (control: 254 ± 10; CsA: 200 ± 18 nM/10^6 cells, $P < 0.001$ vs control). The effect was restricted to the basolateral side (control: 165 ± 11; CsA: 93 ± 17 nM/10^6 cells, $P < 0.001$ vs control), but not apical NO secretion (control: 101 ± 11; CsA: 106 ± 9 nM/10^6 cells, NS vs control) (Fig. 1). The transepithelial electrical resistance (R_T) from cells grown on filters was higher in CsA-treated cells (213 ± 5 $\Omega.cm^2$, $n = 6$) as compared to untreated cells (153 ± 9 $\Omega.cm^2$, $n = 6$). These results indicated that 100 ng/ml CsA did not alter the integrity of the tight junctions.

Discussion

The intrarenal production of NO was thought to be an important factor in regulation of the renal hemodynamics and transport physiology [5]. Mohaupt and Morrisey [8, 11] had demonstrated the steady production of NO in the mTAL segment. The exact mechanism of the NO action on intrarenal hemodynamics is still unknown. It is postulated that the locally produced NO acts at the peritubular level and affects the adjacent vascular tone [16]. The peritubular vessel, adjacent to the mTAL segment, is the key smooth muscle containing blood vessel in regulating renal medullary perfusion [3]. NO is a major mediator in regulating the renal medullary circulation [6]. We suggest that NO, produced by the mTAL cells, is released mostly into the basolateral side of the polarized renal epithelial cells. The basolateral releasing NO subsequently affects the peritubualar vessel and plays an important role in maintaining medullary perfusion.

The major drawback of CsA is its nephrotoxicty [12]. Our previous report indicated that CsA can reduced NO production in renal mTAL epithelial cells. The

question arises if the CsA affects NO production in a polarized manner. Our experiment suggested that CsA affects NO production in a vectorial manner. Basolateral NO end-product was reduced without affecting apical NO production. The results suggest that CsA affects only basolateral NO release in the absence of apical effect.

As a gas, NO can diffuse freely through the cell membrane. However, the different environmental factors between apical and basolateral sides of epithelial cells could affect the diffusion of NO. We had shown that basolateral NO_2^- production is much higher than the apical production. It is also likely that NO_2^- goes through the cell membrane via an anion channel. A recent study has indicated that the chloride channel could transport the nitric oxide derivative [10]. NO_2^- could pass through the chloride channel, which is specifically localized in the basolateral membrane of mTAL cells. Furthermore, nitrite was also shown to have an effect on the regulation of ion transport in renal epithelial cells and regulation of renal function [1]. Further investigation is indicated to confirm the accessibility of the NO and its derivatives in polarized renal epithelial cells.

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References

1. Adragna NC, Lauf PK (1998) Role of nitrite, a nitric oxide derivative, in K-Cl cotransport activation of low-potassium sheep red blood cells. J Membr Biol 166: 157–167
2. Andoh TF, Burdmann EA, Fransechini N, Houghton DC, Bennett WM (1996) Comparison of acute rapamycin nephrotoxicity with cyclosporine and FK506. Kidney Int 50: 1110–1117
3. Brezis M, Heymen SN, Dinosur D (1991) Role of nitric oxide in renal medullary oxygenation. J Clin Invest 88: 390–395
4. Devarajan P, Kaskel FJ, Albeit LA, Moore LC (1989) Cyclosporine nephrotoxicity: blood volume, sodium conservation, and renal hemodynamics. Am J Phyiol 256:F71–F78
5. Haynes WG, Hand MF, Dockrell ME, Eadington DW, Lee MR, Hussein Z, Benjamin N, Webb DJ (1997) Physiological role of nitric oxide in regulation of renal function in humans. Am J Physiol 272:F364–371
6. Lahera V, Navarro-Cid J, Cachofeiro V, Garcia-Estan J, Ruilope LM (1997) Nitric oxide, the kidney, and hypertension. Am J Hypertens 10: 129–140
7. McNally PG, Feehally J (1992) Pathophysiology of cyclosporin A nephrotoxicity: experimental and clinical observations. Nephrol Dial Transplant 7: 791–804
8. Mohaupt MG, Elzie JL, Ahn KY, Clapp WL, Wilcox CS, Kone BC (1994) Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. Kidney Int 46: 653–665
9. Mohaupt M, Schoecklmann HO, Schulze-Lohoff E, Sterzel RB (1994) Altered nitric oxide production and exogenous nitric oxide do not affect the proliferation of rat mesangial cells. J Hypertens 12: 401–408
10. Mo L, Hellmich HL, Fong P, Wood T, Embesi J, Wills NK (1999) Comparison of amphibian and human ClC-5: similarity of functional properties and inhibition by external pH. J Membr Biol 168: 253–264
11. Morrissey JJ, McCracken R, Kaneto H, Vehaskari M, Montani D, S K (1994) Localization of an inducible nitric oxide synthase mRNA in the normal kidney. Kidney Int 45: 998–1005
12. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M (1984) Cyclosporine-associated chronic nephropathy. N Engl J Med 311: 699–705
13. Riccaldi D, Robic D, Bens M, Cluzeaud F, Wu MS, Bourbouze R, Vandewalle A (1995) Cultured proximal cells derived from transgenic mouse provide a model to study drug toxicity. Kidney Int 48: 722–730
14. Tojo A, Gross SS, Zhang L, Tisher CC, Schmidt HH, Wilcox CS, Madsen KM (1994) Immunocytochemical localization of distinct isoforms of nitric oxide synthase in the juxtaglomerular apparatus of normal rat kidney. J Am Soc Nephrol 4: 1438–1447
15. Wu MS, Bens M, Cluzeaud F, Vandewalle A (1994) Role of F-actin in the activation of Na(+)-K(+)-Cl- cotransport by forskolin and vasopressin in mouse kidney cultured thick ascending limb cells. J Membr Biol 142: 323–336
16. Wu MS, Yang CW, Bens M, Yu HM, Huang JY, Wu CH, Huang CC, Vandewalle A (1998) Cyclosporin inhibits nitric oxide production in medullary ascending limb cultured cells. Nephrol Dial Transplant 13: 2814–2820