Functional Effects on Glomerular Hemodynamics of Short-Term Chronic Cyclosporine in Male Rats

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Abstract

We evaluated the effects of chronic cyclosporine (CsA) administration on the determinants of nephron filtration rate (SNGFR) using micropuncture techniques (mp) in male Munich-Wistar rats. Animals received CsA (30 mg/kg SQ) in olive oil daily for 8 d before mp. Controls (PFC) were pair fed. SNGFR, glomerular capillary hydrostatic pressure gradient (ΔP), nephron plasma flow (SNPF), plasma protein oncotic pressure (π_A), and glomerular ultrafiltration coefficient (LpA) were quantitated in each experiment. CsA was associated with a lower SNGFR due to decreases in SNPF and a major reduction in ΔP but no decrease in LpA. Plasma volume expansion (PVE) caused SNGFR, ΔP, and SNPF to increase in both CsA and PFC without eliminating the differences between CsA and PFC. CsA/PVE rats responded normally to angiotensin II (AII) infusion indicating that the low ΔP associated with CsA is not due to unresponsiveness to AII. Prior renal denervation caused SNGFR and SNPF to increase in CsA-treated animals but failed to alter the reduction in glomerular capillary pressure after CsA or to eliminate the glomerular hemodynamic differences between treated animals and pair-fed controls. This constellation of glomerular hemodynamic abnormalities suggests that the renal effect of short-term chronic CsA administration is mediated primarily by a reduction in the afferent effective filtration pressure resulting from an imbalance between pre- and postglomerular vascular resistances.

Introduction

Cyclosporine A (CsA) is a potent immunosuppressive agent with undeniable utility in the prevention of organ allograft rejection (1, 2). Its use is encumbered, however, by a host of toxicities, the most problematic of which is an adverse effect on renal function (1–5). CsA nephrotoxicity is characterized by early reversible decreases in glomerular filtration rate (GFR) and renal blood flow (RBF), with later progression to irreversible injury and chronic renal insufficiency (1, 2, 4–6).

The effect of CsA on renal function in animal models varies with respect to species or strain of animal as well as dose, mode of administration, and duration of treatment with CsA (7, 8). Multiple investigators have reported reno-vascular effects of CsA, associating it with elevated reno-vascular resistance (RVR), diminished renal blood flow, and functional renal impairment (1, 4, 5, 9–12). The elevation in RVR associated with CsA has variously been attributed to excess alpha$_1$ adrenergic activity (11, 13, 14), over-stimulation of the renin-angiotensin system (RAS) (15–19), or diminution of vasodilatory prostaglandins (16–22).

Relatively little information is available regarding the effect of CsA on the individual determinants of glomerular filtration at the single nephron level. In one published study, measurements obtained by micropuncture in rats receiving a single acute intravenous infusion of CsA demonstrated a reduction in single nephron glomerular filtration rate (SNGFR) due to elevation of both afferent and efferent arteriolar resistances with an elevated transglomerular hydrostatic pressure gradient, decreased nephron plasma flow, and depressed glomerular ultrafiltration coefficient (15), effects reminiscent of those accompanying acute infusion of angiotensin II (AII) (23).

In contrast to the acute infusion of CsA, we applied micropuncture techniques to evaluate glomerular hemodynamics in a model of short-term chronic CsA nephrotoxicity. Initial studies, performed in euvolemic rats, revealed an effect on SNGFR due primarily to a decrement in effective filtration pressure and, less importantly, nephron plasma flow. These results, being physiologically analogous to the simultaneous effects of excess renal nerve stimulation with RAS blockade, suggested two further sets of studies, one in which we evaluated the response of CsA treated animals to acute AII infusion, and another in which rats were subjected to renal denervation before being treated with CsA.

Glossary

CsA	Cyclosporine A or cyclosporine treated rat.
SNGFR	Single nephron glomerular filtration rate.
MP	Micropuncture.
PFC	Pair-fed control.
EUV	Euvolemia.
PVE	Plasma volume expansion.
AII	Angiotensin II.
DNX	Denervation of left (ipsilateral) kidney.
INX	Denervation of right (contralateral) kidney.
BP	Mean arterial pressure.
GFR	Whole kidney glomerular filtration rate.
P_G	Glomerular capillary hydrostatic pressure.
P_B	Bowman's space hydrostatic pressure.
P_T	Proximal tubular pressure.
HP_E	Efferent arteriolar hydrostatic pressure.
ΔP	$P_G - P_B$.
LpA	Glomerular ultrafiltration coefficient.
C_A	Systemic plasma protein concentration.
C_E	Efferent arteriolar plasma protein concentration.
π	Plasma protein oncotic pressure.

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EFP	Effective filtration pressure, $\Delta P - \pi$.
SNPF	Single nephron afferent plasma flow.
SNBF	Single nephron afferent blood flow.
SNFF	Single nephron filtration fraction.
AR	Preglomerular vascular resistance.
ER	Efferent arteriolar vascular resistance.

Subscripts

A	Afferent.
E	Efferent.

Methods

General procedures

Studies were performed on male Munich-Wistar rats weighing between 250 and 300 g. Each animal was obtained from a continuous colony bred and housed at the San Diego Veterans Administration Medical Center. Animals were allowed free access to tap water. During an 8-d protocol period, those animals treated with CsA and their pair-fed counterparts which were ultimately subjected to micropuncture under standard euvolemic and volume expanded conditions each ate 16 g/d of standard rat chow (Ralston-Purina Co., St. Louis, MO). This dietary protocol was determined after preliminary studies documented a curtailment of food intake from 21±2 to 16±2 g/d in rats begun on CsA, and was intended to eliminate the effect of diet as a confounding influence on renal function when comparing treated (CsA) to control (PFC) animals. Animals subjected to renal denervation prior to beginning CsA were fed ad lib. throughout and their respective controls were matched daily for food intake. CsA-treated animals received cyclosporine A (Sandoz Pharmaceuticals, Morris Plains, NJ) (30 mg/kg body wt) in olive oil administered daily by subcutaneous injection for eight consecutive days before micropuncture. The subcutaneous route was chosen because it has been documented to yield a pharmacokinetic profile similar to oral administration (24), albeit with greater bioavailability, and is technically easier than gavage. PFC animals were injected with oil. 14 animals were subjected to left-sided (DNX) and six to right-sided (INX) renal denervation 5 d before beginning CsA or pair feeding.

On the day of micropuncture, rats were anesthetized with intraperitoneal injection of Inactin (100 mg/kg body wt). Tracheostomy was thereafter performed (PE 240), and catheters (PE 50) placed in right jugular vein, left femoral artery, and urinary bladder. The left kidney was prepared for micropuncture in a manner described previously from this laboratory (25). Mean arterial pressure was monitored throughout the experiment with a P23dB Gould-Statham pressure transducer and recorded on a Statham chart recorder (Statham Instruments, Inc., Oxnard, CA). Body temperature was regulated by use of a servo-controlled heating unit attached to a heating table and rectal temperature probe.

[^{3}H]Inulin, 85 μCi/ml, was dissolved in Ringer's saline and delivered by continuous intravenous infusion at 1.5 cm^3/h beginning 60 min before micropuncture and continuing throughout. Additional saline and/or plasma infusions were given to yield complete euvolemic (EUV) or plasma volume expanded (PVE) protocols as follows: EUV animals received 1.5 cm^3 of Ringer's saline with [^{3}H]inulin and 1 cm^3/100 g body wt of donor rat plasma during a 1-h equilibration period followed by a continuous infusion consisting of 1.5 cm^3 saline with [^{3}H]inulin and 0.15 cm^3/100 g body wt plasma per hour thereafter. This protocol was intended to replace plasma losses associated with surgical preparation and micropuncture. PVE animals received 1.5 cm^3 of saline with [^{3}H]inulin and 2.5 cm^3/100 g body wt of plasma during the 1 h equilibration period and 5.1 cm^3 of saline plus 0.15 cm^3/100 g body wt of plasma per hour thereafter. When infused, these admixtures allowed hematocrits (Hct) to remain relatively constant within an experimental period.

Renal denervation

5 d before beginning CsA or pair-feeding, DNX and INX animals underwent denervation of left and right kidneys, respectively. This was accomplished with sterile surgery under Brevital anesthesia by locating the renal nerves with stereomicroscopic guidance and painting them with a solution of 10% phenol in EtOH. To confirm that denervation had been accomplished, both kidneys from each of these animals were removed after micropuncture, snap frozen in liquid nitrogen, and later assayed for tissue norepinephrine content.

Micropuncture studies of glomerular hemodynamics

Micropuncture experiments were performed according to standard protocols as previously described by this laboratory (25). At the beginning and end of each experimental period, blood was obtained from the femoral artery catheter for the determination of Hct, afferent protein concentration (C_A), and systemic [^{3}H]inulin concentration. Hydrostatic pressures in glomerular capillaries (P_G), Bowman's space (P_{BS}), proximal tubules (P_T), and efferent arterioles (star vessels) (HP_E) were measured directly with a servo-nulling pressure sensor (Instrumentation for Physiology and Medicine, San Diego, CA) connected to a 1–3-μm-tip glass pipette filled with 1.2 M NaCl. Multiple measurements were made in each period and time averaged values were recorded with a P23dB Gould-Statham pressure transducer and chart recorder.

Five timed samples of tubular fluid were collected from late proximal convolutions by use of 9–11-μm-tip sharpened glass pipettes, each filled with lightly Sudan black-stained mineral oil, a small amount of which was injected through the pipette tip into the tubule at the beginning of the collection to block downstream flow of tubular fluid. In some EUV animals, tubular pressure was continuously monitored by an upstream pressure pipette and controlled by means of a syringe attached via flexible tubing to the back of the collection pipette. Care was taken during the collection of tubular fluid so as to prevent the collection process from altering P_T, and hence the hydrostatic force governing filtration at the glomerulus.

Blood was obtained from efferent arterioles (star vessels) by direct puncture (13–16-μm pipettes) for use in determining efferent protein concentration (C_E). Timed urine collections were made from each kidney to assess the rate of whole kidney GFR and urine flow.

Analytical methods

Systemic and efferent arteriolar plasma protein concentrations were measured by a micro-adaptation of the Lowry protein method (26) as described previously in publications from this laboratory (25). Catecholamine content of renal tissue from DNX and INX animals was determined by use of a sensitive radioenzymatic assay (27).

Specific protocols

Glomerular hemodynamics in EUV. Single period micropuncture studies, as described above, were completed under EUV conditions in seven CsA and six PFC animals.

Glomerular hemodynamics in PVE before and during AII. Full sets of measurements were obtained in each of two experimental periods after PVE in six CsA and six PFC animals. A systemic infusion of human angiotensin II (60 ng/min per kg body wt) was begun at the end of the first period in each two period study and continued throughout the remainder of the experiment. 20 min were allowed to elapse for the animals to reequilibrate between the two experimental periods.

The effect of DNX on glomerular hemodynamics after CsA. Single period micropuncture studies were performed under euvolemic conditions in seven DNX-CsA, seven DNX-PFC, and six INX-CsA rats.

Calculations and mathematical models

SNGFR was determined from the clearance of [^{3}H]inulin during timed tubular fluid collections. The transglomerular hydrostatic pressure gradient (ΔP) was calculated as $P_G - P_B$. Plasma oncotic pressure (π) was determined from plasma protein concentration (C), employing the

modified equation of Landis and Pappenheimer (28), $\pi = 1.76C + 0.28C^2$.

Afferent and efferent effective filtration pressures (EFP's) were defined as $\Delta P - \pi_A$ and $\Delta P - \pi_E$, respectively. Nephron filtration fraction (SNFF) was defined as $1 - (C_A/CE)$, nephron plasma flow (SNPF) as SNGFR/SNFF, and nephron blood flow (SNBF) as SNPF/(1 − Hct).

Afferent and efferent arteriolar vascular resistances (AR and ER, respectively) were defined by the equations $AR = (BP - P_G)/SNBF$, $ER = (P_G - HP_E)/(SNBF - SNGFR)$.

For purposes of computing the glomerular ultrafiltration coefficient (LpA), we employed a standard model of the glomerular capillary as a nondimensionalized right circular cylinder with negligible resistance to axial flow such that $SNGFR = LpA * \int_0^1 EFP(x)dx$, where x is the axial position along the non-dimensionalized capillary, $EFP(x) = \Delta P - \pi(x)$, and C(x), from which $\pi(x)$ is derived, satisfies the following differential equation with the boundary conditions $C(0) = C_A$ and $C(1) = C_E$: $(LpA * EFP(x) * C^2(x))/(SNPF * C_A) - d/dx\ C(x) = 0$.

We also used this model to predict unique values for SNGFR, given hypothetical glomeruli for which each of the four determinants of SNGFR (ΔP, SNPF, LpA, and C_A) are given as independent variables. This application of the model was employed to analyze the theoretical impact on SNGFR if individual determinants were altered in isolation from their mean values in one experimental group to their mean values in another experimental group. The relative importance of changes in individual determinants on overall changes in SNGFR was thus quantified.

Statistical analysis

Comparison between groups was by unpaired Student's *t* test with Bonferroni correction for multiple group comparisons (29). Analysis was performed using individual nephron values for SNGFR, SNPF, SNBF, AR, and ER for each nephron from which tubular fluid was collected. The individual values for SNPF, SNBF, AR, and ER were calculated using individual SNGFR measurements and mean values for pressures, Hct, and protein concentrations recorded during the same experimental period. For intergroup comparison, individual measurements of P_G, P_B, P_T, and HPE were entered separately into the analysis.

Results

CsA and PFC animals fared equally with regards to overall growth during the 8 d before micropuncture, with an average weight change of −2±2 g per animal for each group. At the time of micropuncture, CsA rats weighed, on average, slightly less than PFC rats but the difference was not statistically significant (258±6 vs. 269±9 g, $0.5 > P > 0.2$). Likewise, there was no significant difference in left kidney weight between CsA and PFC animals (0.947±0.026 vs. 1.004±0.026 g, $0.2 > P > 0.1$). As would be expected, EUV was associated with a higher Hct than PVE for both CsA and PFC animals (0.49±0.01 vs. 0.44±0.01, and 0.50±0.01 vs. 0.43±0.01, respectively). Pretreatment with CsA, however, did not affect the Hct when measured in EUV or PVE. Excepting the DNX and INX groups, there were no statistically significant differences within any group between left and right kidneys with regard to urine flow or GFR.

Tissue norepinephrine content was diminished by > 90% in the left kidney of each DNX and > 70% in the right kidney of each INX animal included in the analysis when the respective contralateral innervated kidneys were used as controls. Right kidneys were technically more difficult to denervate for anatomic reasons.

The results of micropuncture studies are summarized in Table(s) I and II.

Table I. Micropuncture Results in Euvolemia and Volume Expansion

Group	Hct	BP	P_G	P_B	ΔP	π_A	EFP_A	SNGFR	SNPF	SNBF	SNFF	LpA	AR	ER	GFR_L
	%	*mmHg*							*nl/min*			*nl/s/mmHg*	*10^9·dyn·cm/s^5*		*ml/min*
1 CsA/EUV	49±1	108.5±4.5	40.3±1.5	16.5±1.5	23.6±0.9	16.7±0.5	6.9±1.0	25.2±1.2	104±7	206±13	0.26±0.02	0.136±0.017	31.1±3.5	10.4±0.7	0.53±0.09
2 PFC/EUV	50±1	118.3±2.5	49.0±1.9	11.8±0.9	37.2±1.5	19.7±1.0	17.5±1.8	31.8±1.1	121±5	244±11	0.29±0.02	0.055±0.006	24.1±1.5	13.8±0.7	0.90±0.07
3 CsA/PVE	44±1	96.3±1.4	53.5±1.1	19.1±0.7	34.4±1.1	18.9±0.7	15.4±1.5	38.9±1.5	134±7	237±12	0.32±0.02	0.085±0.014	15.6±1.1	15.1±1.0	1.21±0.14
4 PFC/PVE	43±1	117.0±3.1	56.4±1.5	14.2±0.9	42.2±1.2	18.2±0.7	24.0±1.2	45.3±1.6	177±11	312±20	0.28±0.02	0.051±0.011	16.8±1.1	12.9±1.1	1.45±0.14
5 CsA/PVE/AII	47±1	122.5±5.6	61.0±1.7	19.6±1.5	41.4±1.7	19.6±0.4	21.1±1.7	33.2±1.7	97±7	180±12	0.36±0.02	0.050±0.011	29.2±2.1	25.0±2.0	1.00±0.13
6 PFC/PVE/AII	45±1	137.0±2.1	59.9±2.4	13.7±1.2	46.3±1.3	15.8±0.5	30.3±1.7	40.3±2.3	96±5	174±10	0.44±0.02	0.040±0.010	39.0±2.8	27.9±1.7	1.47±0.06
1 vs. 2			*	‡	*	*	*	*	‡	‡		*		*	*
1 vs. 3	*	‡	*		*	‡	*	*	*	*		‡	*	*	*
2 vs. 4	*		*		‡		‡	*	*	*			*		*
3 vs. 4		*		*	*		*	*	*	*					
3 vs. 5		*	*		*		*	‡	*	*			*	*	
4 vs. 6		*			‡	*	‡		*	*	*		*	*	
5 vs. 6		‡	*	‡	‡	*	‡	‡					*		*

Refer to Glossary for definition of terms. All values expressed as mean±SEM. * $P < 0.05$ by unpaired Student's *t* test after Bonferroni correction. ‡ $P < 0.05$ by unpaired Student's *t* test.

Table II. Micropuncture Results After Subacute Renal Denervation

Group	Hct	BP	P_G	P_B	ΔP	π_A	EFP_A	SNGFR	SNPF	SNBF	SNFF	LpA	AR	ER	GFR_L
	%			mmHg					nl/min			nl/s/mmHg	$10^9 \cdot dyn \cdot cm/s^5$		ml/min
CsA/DNX	51±1	94.3±2.4	45.7±1.3	14.7±1.0	30.9±0.7	15.8±0.4	14.8±1.4	31.4±1.3	106±7	215±15	0.30±0.02	0.066±0.005	20.5±1.5	13.6±0.8	0.73±0.11
CsA/INX	49±1	86.6±3.2	45.2±1.1	18.7±0.7*	25.5±1.0*	14.7±0.6	11.6±1.2	23.6±1.3*	88±4*	171±9*	0.29±0.02	0.061±0.010	21.1±1.9	14.9±1.1	0.43±0.11
PFC/DNX	51±1	122.0±6.1*	49.1±1.6	12.7±0.9	36.4±1.1*	16.1±1.0	20.3±1.9*	49.7±1.3*	146±8*	289±14*	0.36±0.03	0.084±0.017	20.9±1.3	10.9±0.7*	1.15±0.08*

Refer to Glossary for definition of terms. All values expressed as mean±SEM. * $P < 0.05$ vs. CsA/DNX by Student's t test with Bonferroni correction for multiple group comparisons.

Glomerular hemodynamics in EUV. Under EUV conditions, CsA-treated rats manifested a lower SNGFR than did PFC animals (Fig. 1). In all CsA treated rats and in one PFC rat, $\Delta P - \pi_E$ was not significantly greater than zero, so that, within the limitations of measurement, filtration pressure equilibrium was considered to have occurred. In these animals further isolated increases in LpA would not have caused any increase in SNGFR, and only minimum estimates for LpA could be calculated. Favoring the net effect of CsA in lowering SNGFR, the treated group manifested a markedly lower ΔP and slightly lower SNPF than did the PFC group. A lower C_A and higher minimum estimate of LpA in the CsA group served as mitigating influences on the propensity of CsA to lower SNGFR (Fig. 1). The impressive numerical difference in the minimum estimate of LpA between CsA and PFC is somewhat deceptive inasmuch as SNGFR is relatively insensitive to perturbations in LpA for glomeruli which achieve filtration pressure equilibrium, especially when EFP_A is low. In the EUV-CsA group, for instance, a 2-mmHg further reduction in ΔP from 23.6 to 21.6 mmHg would result in a 25% further reduction in SNGFR whereas LpA would have to decline from 0.136 to 0.059 nl/s per mmHg to reduce SNGFR by the same amount. Additionally, some of the difference in LpA between the CsA and PFC groups may be the result of a computational artifact as will be discussed later. CsA influenced ΔP both by lowering P_G and elevating P_B, although the effect on P_B failed to remain statistically significant after application of the stringent Bonferroni correction to the multiple-group data. This reduction in ΔP associated with CsA caused an almost 60% reduction in EFP_A and was the single determinant most responsible for the decrement in SNGFR associated with CsA in EUV animals. The relative contributions of changes in the individual determinants of SNGFR to the net observed effect of CsA on SNGFR are illustrated in Fig. 2.

Glomerular hemodynamics in PVE. When compared to the results obtained in EUV, 2.5% plasma volume expansion was associated with higher values for SNGFR, P_G, and SNPF, independent of prior treatment with CsA (Fig. 1). Blood pressure was not affected by PVE in the PFC group, but appeared to be lowered in CsA animals subjected to PVE.

Plasma volume expansion in CsA treated animals allowed SNGFR to attain values greater than those observed in the PFC/EUV group, indicating a functional component to the nephrotoxic effect of CsA observed in EUV. A parallel increase in SNGFR was observed in the PFC group, however, so that PVE did not eliminate the difference in SNGFR between CsA and PFC. Comparing the individual determinants of SNGFR between CsA/PVE and PFC/PVE, CsA was again associated with a lower ΔP and SNPF while no significant differences in LpA or C_A were detected. In contrast to EUV, however, where differences in ΔP were primarily due to differences in P_G, in PVE, treatment with CsA was associated with a lower ΔP due mostly to higher values for P_B. Although the effect of CsA on SNPF was more pronounced in PVE than EUV, the CsA associated decrement in EFP_A remained of greater importance to the overall influence of CsA on SNGFR (Fig. 2).

Response to AII infusion in CsA rats. CsA/PVE rats responded to the infusion of a modest pressor dose of AII with significant increases in BP, P_G, and ΔP, while SNPF, LpA, and SNGFR tended to decline. PFC/PVE animals responded similarly, but in comparison to the CsA group manifested lesser

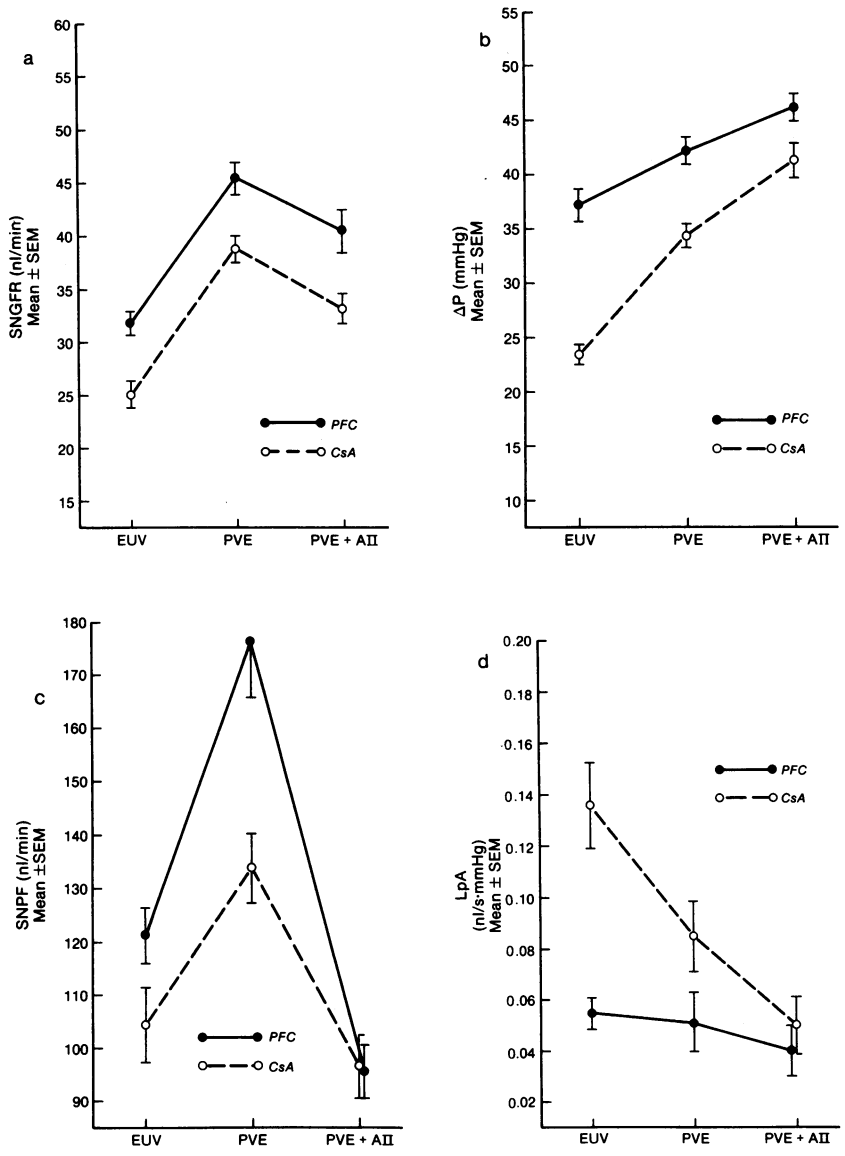

Figure 1. The effects of CsA in EUV, PVE, and PVE with AII infusion on (*a*) SNGFR, (*b*) ΔP, (*c*) SNPF, and (*d*) LpA are shown. Data are expressed as mean±SEM. Refer to Glossary for definitions and to Table I for statistical significance.

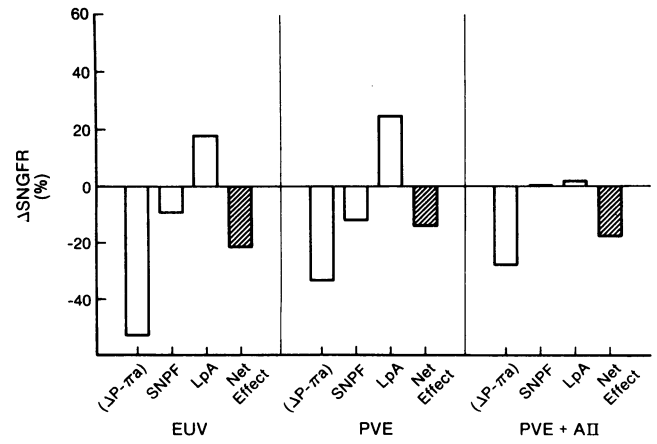

Figure 2. Predicted effects on SNGFR when the experimentally derived mean values for individual determinants of SNGFR for CsA treated animals are imposed, one at a time, on a hypothetical nephron with determinants derived from the respective PFC group means. Refer to Glossary for definitions.

changes in ΔP and LpA and a greater decrement in SNPF so that the differences between CsA and PFC with respect to each determinant of SNGFR were less distinguishable after AII infusion (Fig. 1).

Response to DNX. Among animals receiving CsA, DNX improved SNGFR via increases in ΔP and SNPF while not affecting LpA (Fig. 3, Table III). The increase in ΔP with DNX was entirely the result of a lower P_B (in contrast to the comparison between EUV/CsA and EUV/PFC where ΔP varied largely due to changes in P_G.) The relative importance of differences in the various determinants of SNGFR to the net effect of DNX on nephron filtration rate are illustrated in Fig. 4.

When comparing CsA/DNX with pair-fed DNX rats, CsA treatment was associated with a significantly lower BP and ΔP, while differences in P_G and P_B failed to achieve statistical significance. Among DNX rats, CsA was also associated with lower SNGFR and SNPF but no difference in LpA. The relative importance of these differences is illustrated in Fig. 5.

The persistent effects of CsA on glomerular hemodynamics

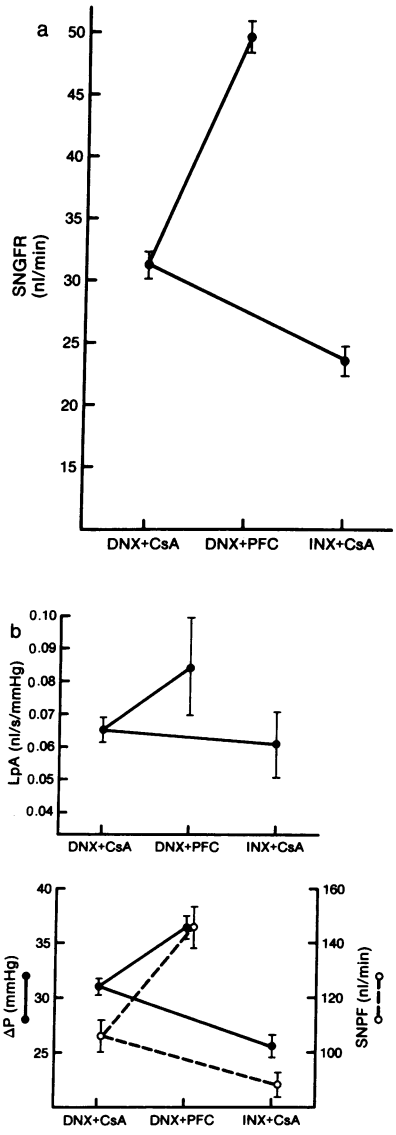

Figure 3. The DNX/CsA group is compared with DNX/PFC and INX/CsA with regards to (*a*) SNGFR and (*b*) the individual determinants of SNGFR. Data are expressed as mean±SEM. Refer to Glossary for definitions and Table II for statistical significance.

after DNX as well as the different means by which CsA caused ΔP to decline in innervated vs. denervated kidneys suggest that the mechanism by which DNX allowed SNGFR to increase

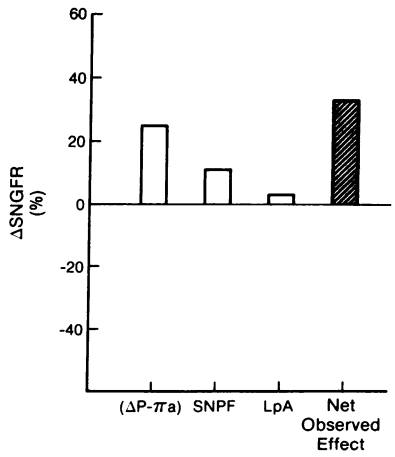

Figure 4. Theoretical effect of DNX on SNGFR in CsA-treated animals when the various determinants of SNGFR are changed, one at a time, from their mean experimentally derived values in the INX group to the respective DNX group mean. Refer to Glossary for definitions.

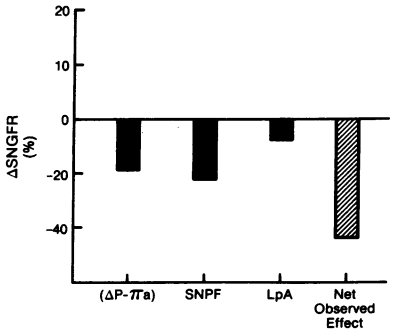

Figure 5. Predicted effects on SNGFR when the experimentally derived mean values for individual determinants of SNGFR for CsA/DNX animals are imposed, one at a time, on a hypothetical nephron with determinants derived from PFC/DNX group means. Refer to Glossary for definitions.

was not merely the converse of that by which CsA caused it to decrease. While these studies do imply a functional elevation in tubular pressure associated with CsA that may be ameliorated by DNX as well as a lessened vasoconstrictor response to chronic CsA after DNX, the significant remaining differences between the CsA/DNX and PFC/DNX groups suggest that the glomerular hemodynamic effects of CsA are not solely attributable to adrenergic overdrive.

Discussion

When administered to humans, cyclosporine may impair renal function by mechanisms which are either functional and reversible, or structural and relatively permanent (3, 4, 6, 30). CsA-induced nephrotoxicity tends to be reversible in inverse proportion to cumulative administered dose or duration of treatment with CsA. Furthermore, the functional effects of a single intravenous bolus of CsA differ from the effects associated with short-term chronic administration. We employed a short-term chronic model in these animal studies because such a model may be the best analogue to the clinically encountered reversible form of CsA nephrotoxicity.

In both EUV and PVE, CsA treatment was associated with clear-cut and multifactorial glomerular hemodynamic abnormalities. Various mechanisms contributed to the net diminution in SNGFR by altering both afferent effective filtration pressure and nephron plasma flow. Under euvolemic conditions, for instance, CsA was associated with a modest decline in mean arterial pressure which was accompanied by a decrement in efferent arteriolar, but not preglomerular, resistance so that glomerular capillary hydrostatic pressure was reduced. Additionally, elevated Bowman's space pressures in animals subjected to CsA therapy contributed to the lower glomerular hydrostatic pressure gradient in this group. Finally, the net renal vascular response to CsA resulted in a decrease in nephron plasma flow. Hence, several simultaneous abnormalities combined to yield the overall glomerular hemodynamic circumstance associated with CsA in euvolemia.

The demonstrated improvement in SNGFR among CsA-treated animals after PVE suggests a functional component to this model of CsA nephrotoxicity. Our protocol for PVE should have eliminated any discrepancies in plasma volume between CsA and PFC animals which may have persisted despite pair-feeding. The fact that PVE failed to eliminate the glomerular hemodynamic differences between CsA and PFC reinforces the role of CsA as a primary influence on renal function in this model.

Despite the histological localization in rat models of CsA induced renal damage predominantly to the epithelium of the

proximal tubule (31), the weight of evidence now available from physiological studies suggests that CsA impairs glomerular filtration via effects on the renal vasculature. A number of animal models have shown that CsA, whether administered by acute infusion (8, 11, 14, 15) or given daily for several days (8–11, 14), leads to a decline in renal blood flow and an increase in renal vascular resistance. However, of the authors reporting marked decreases in renal blood flow with chronic administration of CsA, only Murray and Paller (11, 14) report having pair-fed their animals. Furthermore, Perico et al. (18), who noted no difference in food intake between CsA-treated rats and controls, also noted no difference between the two groups with respect to renal blood flow after 45 d of treatment with a moderate dose of CsA (25 mg/kg per d). Additionally, Winston (32) has reported a single-kidney rat model in which any difference in renal blood flow between CsA treated and control animals was eliminated when the control animals were pair fed. Our findings with regards to the effect of CsA on renal blood flow concur in general with the prevailing opinion in the published literature, namely that CsA causes renal blood flow to decrease. Our results, however, are less striking than those reported elsewhere inasmuch as the significance of the difference in SNPF between CsA and PFC groups only survived the Bonferroni correction to Student's *t* test after PVE. Moreover, CsA was associated with lower blood pressures than PFC such that in no circumstance were we able to demonstrate an association, at the single nephron level, between CsA and elevated overall vascular resistances (AR + ER). The propensity towards lower BP and SNPF unaccompanied by any decrease in AR + ER may follow from an upregulating of renal vascular tone and/or an inability of the CsA treated kidney to undergo autoregulatory vasodilation as suggested by Kaskel (10).

Despite the absence of any significant difference in overall nephron vascular resistance between CsA and PFC animals (except after AII infusion) the two EUV groups did differ with regards to the partitioning of resistance between afferent and efferent arterioles such that treatment with CsA led to a significant increase in the ratio, AR/ER. This altered balance between AR and ER in CsA-treated EUV animals resulted in a lower P_G which, when combined with a higher Bowman's space pressure and comparable systemic plasma oncotic pressure led to a greater than 50% decrement in EFP_A for CsA treated vs. PFC/EUV animals. A significant difference in EFP_A between CsA and PFC groups persisted despite PVE or DNX such that, regardless of volume status and independent of renal nerves, CsA-associated differences in glomerular filtration rate were more dependent on changes in afferent effective filtration pressure than on changes in nephron plasma flow.

The notion that functional CsA nephrotoxicity may center around afferent arteriolar vasoconstriction has been propounded by others (33, 34). In support of this notion, English et al. (33), using morphometric analysis of vascular perfusion casts, have demonstrated a progressive reduction in the caliber of afferent glomerular arterioles in rats treated chronically with CsA. Our glomerular hemodynamic measurements in EUV rats may be interpreted as a physiologic correlate to those observations despite a nonstatistically significant numerical difference in preglomerular resistance between CsA and PFC given the failure of CsA treated kidneys to maintain a normal P_G in the face of a decrement in BP falling well within the range of normal autoregulation.

Previous reports regarding the effect of CsA on transglomerular ultrafiltration pressure are not plentiful. On the basis of elevated blood pressures and similar plasma oncotic pressures in heart transplant patients treated with CsA vs. azathioprine, Tomlanovich et al. (35) speculated that the former might exhibit a higher renal EFP. Schurek et al. (36) reported measuring glomerular ultrafiltration pressures by stop-flow micropuncture techniques in the isolated perfused rat kidney after treatment in vivo with CsA, but did not report values for pressures or vascular resistances. Barros and colleagues (15) reported a substantial rise in P_G with no change in P_T as ascertained by direct micropuncture in rats before and after the acute parenteral infusion of CsA (50 mg/kg body wt i.v.). In their experiments, SNPF declined by almost 60% while ΔP rose due to increases in both AR and ER with the latter predominating. The discrepancies between these data and our own may provide insight into the disparate means by which CsA tends to impair renal function depending upon the mode of administration and duration of treatment. This propensity of chronic CsA to lower the transglomerular capillary hydraulic pressure gradient is consistent with recently reported preliminary data from Barros and colleagues (37).

Renal vasoconstriction has been linked to impaired renal excretory function in a variety of animal models of acute renal failure (38). In many of these models, the glomerular ultrafiltration coefficient declines along with plasma flow and glomerular filtration rate (38, 39). Thus, it is surprising that treatment with CsA caused no apparent decrease in LpA. In fact, under EUV conditions LpA appears to be higher in CsA-treated animals when compared with pair-fed controls. There are several possible explanations for this finding. An increase in the minimum estimate of LpA associated with CsA may be more apparent than real due to problems inherent in attempting to calculate LpA for glomeruli with extremely low afferent effective filtration pressures. LpA is computed from a complex mathematical function of ΔP, π_A, SNGFR, and SNPF. Although not amenable to verification because of inherent restrictions on sample size, it is reasonable to assume that, within any experimental period, a sample set of ΔP measurements tends to be distributed symmetrically about the true mean ΔP for that period. When a sample set of values for LpA is calculated, *ceteris paribus,* from a sample set of ΔPs, random variation among the ΔPs will lead to a systematic upward bias in the mean calculated LpA. This bias is negligible when ΔP is high, but as ΔP approaches π_A, it could lead to a significant overestimation of LpA. Additionally, as ΔP declines towards π_A, randomly distributed perturbations in tubular pressure caused by the technical process of collecting tubular fluid will lead to a systematic overestimation of SNGFR and hence to an overestimation of LpA. Thus, we may have overestimated the difference in LpA between the two EUV groups. These explanations notwithstanding, it still appears that administration of CsA did not cause LpA to decline. In fact, CsA may have protected the kidney against a reduction in LpA induced by pair feeding, since the PFC/EUV group exhibited a lower mean LpA than prior euvolemic controls reported from this laboratory (40).

Multiple humoral and autonomic effector mechanisms have been implicated as potential mediators of functional CsA nephrotoxicity. Our studies are phenomenologic in the sense that micropuncture provides information regarding the end-organ response to a particular stimulus without directly implicating an intermediate pathway via which the stimulus exerts its effect. Certain inferences may be drawn, however, when the

glomerular hemodynamic response to CsA is compared to that known to occur when potential intermediary systems (i.e., renal nerves, renin-angiotensin system, intrarenal prostaglandins, etc.) are independently manipulated.

CsA and renal nerves. There exists a large body of evidence that links the renal adrenergic nervous system to the control of renal hemodynamics, and evidence has accrued to implicate the renal nerves as mediators of functional CsA nephrotoxicity. The role of renal adrenergic activity in modulating the determinants of SNGFR has been investigated by micropuncture performed in euvolemic rats during electrical renal nerve stimulation (RNS) (41–43). These studies have demonstrated that RNS causes a form of renal vasoconstriction which is distributed between afferent and efferent vessels such that SNPF declines substantially while P_G remains unperturbed or decreases slightly. Additionally, Kon and Ichikawa (42) reported a reduction in LpA (K_f) and P_T during RNS while Pelayo and Blantz (43) reported no change in either of these parameters in euvolemic rats. The glomerular hemodynamic effects of chronic CsA mimic those of acute RNS in some, but not all aspects. Both CsA and RNS produce renal vasoconstriction. The preservation of LpA in the face of renal vasoconstriction, as observed with CsA, has also been reported with RNS (43). When CsA causes vasoconstriction, this effect appears to be confined to the afferent arteriole. Furthermore, CsA, but not RNS, is associated with elevated P_T.

Moss et al. recorded an increase in renal and genitofemoral nerve traffic in rats during an acute infusion of CsA, raising the possibility that CsA nephrotoxicity might be mediated by heightened sympathetic nervous system activity (13). Murray and Paller (11, 14) reported that the impact of acute CsA administration on renal hemodynamics was ameliorated by prior renal denervation and, similarly, that the effects of chronic CsA could be abolished by the concurrent administration of prazosin, an alpha-1 antagonist, again suggesting an important role for renal nerves in the genesis of CsA-induced renal vasoconstriction.

We also found improved SNGFR due to favorable differences in ΔP and SNPF when kidneys were subjected to denervation before CsA therapy. However, while the most important effect of CsA on EUV animals was to lower EFP_A, an outcome brought about in large part through a decline in P_G, DNX did not affect P_G in CsA-treated animals. After comparing the differences between DNX and INX animals to those between CsA/EUV and PFC/EUV animals, we suspect that the path by which renal denervation leads to improved renal function is not the mere reversal of that leading to impaired function as observed in EUV animals.

Renin-angiotensin system. The evidence suggesting that CsA administration leads to excess renal sympathetic nerve activity could be reconciled with the discrepant effects of CsA and RNS if, in addition to causing increases in renal nerve activity, CsA were to modulate the effects of that activity on certain intrarenal hormones. Under normal circumstances, the glomerular hemodynamic effects initiated by RNS occur via multiple pathways. Afferent arteriolar vasoconstriction, for instance, may be mediated directly by either alpha-1 adrenergic stimuli or AII, whereas increases in efferent arteriolar resistance and decreases in LpA are primarily dependent upon AII (23, 44). Furthermore, RNS may lead to the generation and release of AII by pathways which are either dependent on or independent of intrarenal prostaglandins (45). If CsA were to increase sympathetic renal nerve activity while selectively inhibiting the AII limb of the sympathetic response, unopposed alpha-1 effects would remain, resulting primarily in preglomerular vasoconstriction.

Several approaches have been taken to study the interaction of CsA with the renin-angiotensin system. Plasma renin activity (PRA) has been measured in humans and animals receiving CsA. Chronic treatment with CsA in humans has consistently been associated with diminished PRA (5, 35, 46). The acute infusion of CsA into rats, on the other hand, causes PRA to rise (11, 47), whereas the PRA response to chronic CsA administration in animals is less clear cut. The majority of animal studies devoted to this matter have reported PRA to be elevated by the chronic administration of CsA (11, 17–19, 48). In only one of these studies (11), however, was dietary intake matched between treated and untreated animals, and in none of them is it certain that the stimulus for renin secretion was other than volume depletion. In another of these studies (19) in which CsA was administered to spontaneously hypertensive rats with stable hypertension, initial elevations in PRA diminished progressively with time and increased dose of CsA. Furthermore, when the capacity for renin synthesis and secretion was assessed in a two-kidney one-clip model of hypertension, clipped rats treated chronically with CsA manifested significantly greater renal renin content but much lower PRA than did untreated clipped rats, suggesting that CsA may impair renin release (49). In vitro studies using renal cortical slices (16) and juxtaglomerular cells in primary culture (50) have demonstrated increases in the rate of renin synthesis concommitant with the addition of CsA to the medium.

Other investigators have explored the physiologic role of the RAS in CsA nephrotoxicity (10, 11, 15). Armed with the prior knowledge that CsA elevates renal vascular resistance, it would not be unreasonable to presuppose that any relevant difference between CsA treated and untreated animals with regards to activity of the RAS should attribute an increase in the level of activity to CsA. Hence, in each of these studies, attempts were made to modify the renal hemodynamic effects of CsA by co-administering an angiotensin converting enzyme inhibitor (CEI). As a result of such experiments it appears that AII mediates the hemodynamic consequences of acute (15), but not chronic (10, 11) CsA administration. Such a conclusion is compatible with the micropuncture data of Barros (15) who reported changes in the determinants of SNGFR after acute CsA infusion that are almost identical to those known to result from a parenteral infusion of AII (39).

Consistent with the observation that converting enzyme inhibition will not abrogate the renal hemodynamic effects of short-term chronic CsA administration, the results of our initial micropuncture experiments performed in EUV animals are compatible with the notion that CsA might induce a state of relative AII insufficiency. Since we were able to demonstrate a normal glomerular hemodynamic response to AII infusion in CsA-treated animals, it is apparent that this postulated "insufficiency state" does not occur as a result of insensitivity to the hormone. The fact that the differences between CsA and PFC animals with respect to each individual determinant of SNGFR were less distinguishable after AII infusion provides further indirect evidence in support of the hypothesis that CsA-treated animals lack an effective means for maintaining the proper balance between pre- and postglomerular vascular resistances, a role generally subserved by intrarenal AII.

In addition to altering the relationship between the renal nerves and RAS, CsA has been demonstrated to interfere with glomerular prostaglandin synthesis (17, 18–20, 22). Such interference may contribute to the glomerular hemodynamic effects of CsA since an impediment to the normal synthesis of prostacycline will interrupt one limb of the connection between renal nerve stimulation and AII production (45) thereby reducing the influence of the renal nerves over ER and LpA, while an inability to normally increase the synthesis of PGE_2 may inhibit the capacity of the kidney to moderate the vasoconstrictive effect of excess sympathetic nerve activity on the pre-glomerular blood vessels leading to an alteration of the balance between AR and ER.

Summary. In this short-term chronic model, treatment with CsA produced a functional decrease in nephron filtration rate mediated by decreases in hydrostatic filtering force and nephron plasma flow, but no reduction in the glomerular ultrafiltration coefficient. These findings differ qualitatively from those reported to occur after the acute infusion of CsA. Chronic CsA caused the glomerular transcapillary hydrostatic pressure gradient to decrease by lowering intracapillary pressure and raising the pressure in Bowman's space. Prior renal denervation ameliorated the effect of CsA on SNGFR without reversing all of the primary hemodynamic abnormalities caused by CsA, implying that the functional glomerular hemodynamic effects of chronic CsA are not coterminous with those of excess renal nerve activity. Instead, the constellation of glomerular hemodynamic abnormalities associated with CsA in this animal model bears the phenomenologic footprint of excess renal nerve stimulation or impaired autoregulatory capacity modified by some mechanism as to prevent a normal concomitant efferent arteriolar response (often associated with AII).

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