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Alleviation of experimental cyclosporin A nephrotoxicity by low dose aspirin in the rat

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Abstract—Groups of male Sprague–Dawley rats received either cyclosporin A (CsA; 25 mg/kg by gavage), low dose aspirin (ASP; 20 mg/kg by gavage), a combination of both, or the appropriate drug vehicles daily for 14 days. Renal structure and function were assessed on day 0 (pretreatment) and on days 7 and 14. Compared to pretreatment results, CsA nephrotoxicity was characterized by increased plasma urea and creatinine concentrations and by moderate to severe microcalcification (MC) at the corticomedullary junction by day 14. The development of nephrotoxicity was also associated with a 5-fold increase in urine thromboxane B_2 (TxB_2) excretion by day 10, while that of 6-ketoprostaglandin $F_{1\alpha}$ remained relatively constant. Although both ASP and saline (ASP vehicle) -cotreated animals demonstrated significantly lower plasma urea and creatinine concentrations compared to treatment with CsA alone, the severity of MC observed on day 14, was reduced only in the ASP cotreatment group. Trough whole blood CsA concentrations were similar at around 2400 ng/mL in all experimental groups. In addition, although a 2-fold increase in urine TxB_2 excretion was observed on days 7 and 10 following treatment with CsA/ASP, levels were significantly reduced compared to treatment with either CsA alone or CsA/saline (both $P < 0.05$).

The functional consequences of acute cyclosporin A (CsA*) nephrotoxicity in the rat include reduced renal blood flow and glomerular filtration rate (GFR) which are thought to result, at least in part, from altered renal haemodynamics [1–4]. Previous studies from this and other laboratories have demonstrated that CsA-induced renal vasoconstriction is associated with increased excretion of the vasoconstrictor eicosanoid thromboxane A_2 (TxA_2) although effects on vasodilatory eicosanoids, including prostaglandin E_2 and 6-ketoprostaglandin $F_{1\alpha}$ ($6KPGF_{1\alpha}$), are less clear [5–12].

Aspirin (ASP) is a non-steroidal anti-inflammatory drug with both anti-pyretic and analgesic properties which has recently been used successfully as an antithrombotic agent in the treatment of native coronary disease and extracranial cerebrovascular disease [13, 14]. Its mode of action is through the irreversible inactivation of arachidonic acid cyclooxygenase and the consequent reduction in prostaglandin synthesis. Consequently, ASP prevents the formation of the vasoconstrictor TxA_2 and the vasodilator prostacyclin (PGI_2) in platelets and vascular endothelial cells (VEC), respectively [15, 16]. However, VEC can synthesize new cyclooxygenase enzyme whilst platelets cannot and the fall in PGI_2 synthesis is relatively transient compared with that of TxA_2 synthesis in platelets. Furthermore, the inhibition of platelet cyclooxygenase activity is observed at lower ASP doses than is the case with VEC [16, 17].

Considering the proposed role of TxA_2 in the pathogenesis of experimental CsA-nephrotoxicity the aims of this study were to (1) identify an appropriate ASP dose, without overt toxicity of its own, which decreased the excretion of TxA_2 but maintained that of $6KPGF_{1\alpha}$ and (2) investigate the potential of low dose ASP to ameliorate experimental CsA nephrotoxicity in the rat.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (initial body weight 200–280 g) were obtained from Charles River Ltd (Hythe, U.K.). They were maintained in the Foresterhill Animal Department, Aberdeen University and allowed food and water *ad libitum* throughout the duration of the study.

Drugs. CsA: Sandimmun (100 mg/mL, Sandoz Pharmaceuticals, Basle, Switzerland) was diluted in olive oil (OO) (The Boots Company plc, Nottingham, U.K.) to give a final concentration of 25 mg/mL. ASP: Dispersable ASP tablets (BP 75 mg; The Boots Company plc) were dissolved in 0.9% (w/v) saline (SAL). CsA, ASP or their vehicles were administered to the conscious rat by gavage using a 4FG cannula (Portex Ltd, Hythe, U.K.) at a dose of 0.1 mL/100 g body weight. When animals received both drugs and/or their vehicles, ASP or SAL were administered approximately 30 min after CsA or OO (VEH).

Protocol. Animals were randomised into experimental groups ($N = 6$) and received either CsA alone (25 mg/kg body weight), ASP alone (20 mg/kg body weight) or a combination of both daily for up to 14 days. Untreated animals and those receiving drug vehicles, either alone or in combination, were also studied. Indices of renal function, namely plasma urea and creatinine concentrations, were measured pretreatment and on days 7 and 14 while prostaglandin excretion was estimated pretreatment and following 4, 7, 10 and 14 days of treatment. Renal structure was studied at the end of day 14 of the experimental period.

Measurements. Trough whole blood CsA measurements, measured by radioimmunoassay (CYCLO-trac, Incstar Corp., MN, U.S.A.) using a monoclonal antibody specific for the parent molecule and estimations of plasma urea and creatinine concentrations and renal structure were performed as described previously [9]. Trough plasma salicylate concentrations were measured using a commercial kit based on NADH-dependent-salicylate hydroxylase activity (Beckman Instruments Inc., CA, U.S.A.). Urine thromboxane B_2 (TxB_2), a stable metabolite of TxA_2, and $6KPGF_{1\alpha}$, a stable metabolite of PGI_2, were measured using Biotrak eicosanoid enzyme immunoassay kits following purification using Amprep C2 columns (Amersham International plc, Amersham, U.K.). The cross reactivity of the TxB_2 antibodies with $6KPGF_{1\alpha}$ was 0.15%.

After the rats were killed, renal tissue was fixed in 10% neutral buffered formalin then wax embedded; 5 μm sections were cut and stained with haematoxylin and eosin. Renal histology was scored 'blind' by an experienced

* Abbreviations: CsA, Cyclosporin A; ASP, aspirin; SAL, saline; OO, olive oil; GFR, glomerular filtration rate; VEC, vascular endothelial cells; PTV, proximal tubular vacuolation; MC, microcalcification; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2; $6KPGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PGI_2, prostacyclin; VEH, OO and ethanol (CsA vehicle).

Table 1. The effect of ASP on TxB_2 and $6KPGF_{1\alpha}$ excretion

Group	Days 0	4	7	10	14
Urine TxB_2 excretion (ng/24 hr)					
SAL	23.4 ±6.5	25.6 ±8.3	22.6 ±4.6	35.4* ±6.1	25.9 ±6.2
ASP (20 mg/kg)	20.9 ±4.2	24.9 ±9.3	16.3 ±4.9	14.8† ±3.8	13.9‡ ±6.1
Urine $6KPGF_{1\alpha}$ excretion (ng/24 hr)					
SAL	27.9 ±8.9	32.6 ±10.6	18.9 ±11.6	22.5 ±9.8	23.8 ±7.9
ASP (20 mg/kg)	23.6 ±7.7	25.9 ±5.1	22.6 ±12.2	24.9 ±11.8	28.9 ±4.9

Results are expressed as the mean ± SD from groups of four animals. SAL and ASP treated animals were compared at each time point (*$P < 0.001$). In addition, results were compared to pretreatment values (†$P < 0.0001$; ‡$P < 0.01$) using the Student's t-test for dependent samples. Prostaglandin excretion was measured over a 16–18 hr period.

histopathologist using the following scheme. Proximal tubular vacuolation (PTV) and basophilia were scored on a scale of 0–3 indicating either no changes present or 10, 10–25 or 25–50% tubules effected, respectively. Microcalcification (MC) was scored on a scale of 0–3 indicating either no calcification present, 1 or 2 small areas, a few scattered areas of MC or regular large areas of calcification, respectively.

Statistics. Results were analysed by ANOVA followed by Fisher's test or by Student's t-test. P values of <0.05 were considered significant.

Results

The effect of ASP alone on prostaglandin excretion. Preliminary experiments demonstrated that of the four regimens tested, namely SAL alone or ASP administered at either 6 mg/kg/24 hr, 17.5 mg/kg/48 hr or 20 mg/kg/24 hr, significant effects on prostaglandin excretion rates were only noted over the experimental period at the highest dose tested (Table 1); while excretion rates of $6KPGF_{1\alpha}$ remained relatively constant those of TxB_2 fell by about 25% from day 7 onwards. In addition, all ASP doses tested were without significant effect on renal structure and function (results not shown).

The effect of ASP on CsA nephrotoxicity. As treatment with drug vehicles, either alone or in combination were without significant effect on renal structure and function or prostaglandin excretion these results have been excluded for clarity. In addition, body weight was similar in all experimental groups over the experimental period (results not shown).

CsA nephrotoxicity was characterized functionally by around a 20 and 50% increase in plasma creatinine concentrations noted on days 7 and 14, respectively, ($P < 0.05$ and $P < 0.01$, respectively; Fig. 1). In addition, a significant 200% increase in plasma urea concentrations was also observed on day 14 ($P < 0.01$). The co-administration of ASP, partially but significantly, alleviated the increased urea and creatinine concentrations observed on day 14 following treatment with CsA alone (both $P < 0.01$). Interestingly, rats treated with the CsA/SAL treatment combination also demonstrated plasma urea and creatinine concentrations which were significantly lower than those observed following CsA alone (both $P < 0.01$) and similar to those observed in the CsA/ASP treatment group.

Structurally, CsA nephrotoxicity was characterized by moderate to severe MC at the corticomedullary function by day 14 (Table 2). PTV and significant basophilia were not observed in any experimental animal due to the CsA dose administered. Co-treatment with ASP but not SAL, significantly reduced the severity of MC present at the corticomedullary junction observed following treatment with CsA alone.

Prostaglandin excretion. The development of CsA nephrotoxicity was associated with a significant 3-, 5- and 5-fold increase in urine TxB_2 excretion observed on days 4, 7 and 10 ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively) followed by a slight, approximately 25% fall in mean excretion on day 14. A similar pattern was also observed in rats receiving CsA/SAL (results not shown). In the CsA/ASP treatment group, although a 2-fold increase in urine TxB_2 excretion was observed on days 4, 7 and 10 (all $P < 0.05$), values were significantly reduced compared to treatment with CsA alone at these time points (all $P < 0.05$). Treatment with ASP/VEH resulted in a 50% reduction in TxB_2 excretion noted on day 10 ($P < 0.05$). Treatment with CsA alone, ASP alone or a combination of both was without significant effect on the excretion of $6KPGF_{1\alpha}$ which remained relatively constant between 15 and 33 ng/24 hr over the experimental course (results not shown).

Circulating trough whole blood CsA concentrations were unaffected by the administration of ASP throughout the experimental period and remained at around 2400 ng/mL. Trough circulating plasma ASP concentrations were below the sensitivity limits of the assay (<1 mg/L) used (results not shown).

Discussion

The CsA dose administered in the present study produced a mild nephrotoxicity characterized functionally by increased plasma and urea concentrations and structurally by MC at the corticomedullary junction, the latter an early feature of chronic toxicity. PTV was not observed due to relatively low circulating CsA concentrations. The results of this study clearly demonstrate that the co-administration of ASP at 20 mg/day for 14 days effectively reduced the excretion of TxB_2 from day 4 onwards, without significantly effecting that of $6KPGF_{1\alpha}$, and produced a marked decrease in the MC at the corticomedullary junction noted following treatment with CsA alone. Interestingly, and unexpectedly however, both the CsA/ASP and CsA/SAL treatment

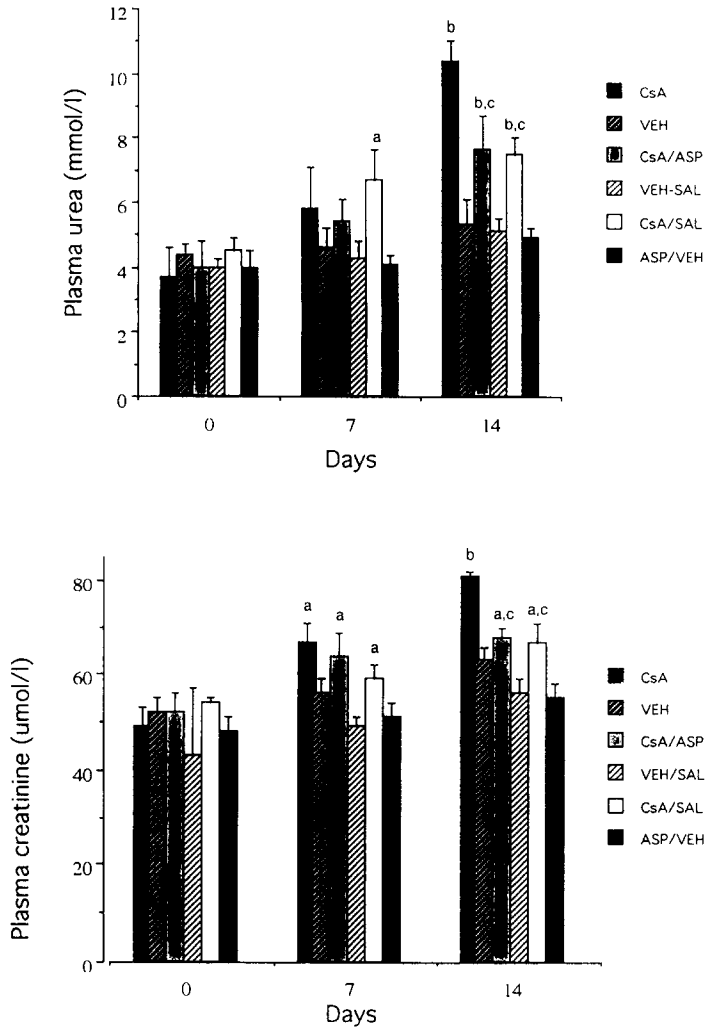

Fig. 1. The effect of CsA or ASP either alone or in combination on plasma urea and creatinine concentrations. Results are expressed as the means ± SD of six determinations,. Treatment groups compared to appropriate vehicle results at each time point. a, $P < 0.05$; b, $P < 0.01$. In addition, CsA only group compared to CsA/SAL and CsA/ASP combination groups on day 14. c, $P < 0.01$. For further details see text.

combinations resulted in the production of a similar and less severe functional nephrotoxicity compared to that produced following treatment with CsA alone. This observation, whilst unexpected, is perhaps consistent with those of Gerkens *et al.* [18] who demonstrated that while low salt intake exacerbated experimental CsA nephrotoxicity in the rat, high salt intake reduced the degree of renal dysfunction by altering the chloride load within the nephron and the tubuloglomerular feedback mechanisms. However, the severity of structural damage and the magnitude of TxB_2 excretion in the CsA/SAL group was similar to that seen with CsA alone suggesting that co-treatment with ASP both reduced TxA_2 production and reduced the severity of MC.

While ASP co-treatment decreased TxB_2 excretion by only 50% from day 4 onwards; reduced renal function was observed only on day 14. Such observations are consistent with previous results [9] which demonstrated increased TxA_2 excretion prior to a reduction in GFR and also that different and/or additional factors are involved in the development of CsA nephrotoxicity. In addition, plasma creatinine concentrations are not as sensitive an indicator of renal insult as either its clearance measurement or estimation of GFR.

The use of low dose ASP in the treatment of cerebral vascular accidents, coronary heart disease and migraine [13, 14, 16, 17] is based on the observation that while VEC can synthesize new cyclooxygenase enzyme the platelet population has to be replaced over a period of 7–10 days in humans before new enzyme can be resynthesized. Theoretically this regime will potentially minimise the effects on the anti-aggregatory and vasodilatory prostaglandin species (e.g. PGI_2) produced by vascular epithelium while reducing synthesis of the proaggregatory and vasoconstrictive eicosanoid TxA_2 by the platelet population. Indeed, in clinical studies the anti-thrombotic, and not the thrombotic, effects of low dose ASP have been shown to predominate [17]. The results of the present study suggest that these properties of ASP can be utilized to reduce the CsA-stimulated increase in TxA_2 production, a

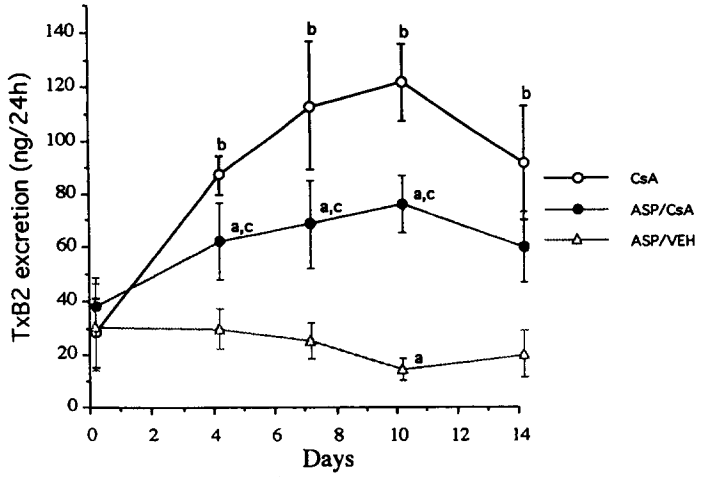

Fig. 2. The effect of CsA or ASP either alone or in combination on urine TxB_2 excretion. Results are expressed as the mean ± SD of six animals. TxB_2 excretion at each time point compared to pretreatment values. a, $P < 0.05$; b, $P < 0.01$. In addition, CsA only group compared to CsA/ASP combination treatment group at each time point. c, $P < 0.05$. For further details see text.

Table 2. The effect of ASP on CsA-induced renal MC

CsA alone	CsA/SAL	CsA/ASP
2,2,3,2,2,3 (2.3)	2,2,1,1,2,3 (1.5)	1,0,0,1,0,1* (0.5)

Individual results from each individual animal are given with the mean score in brackets. MC was scored on a scale of 0–3 indicating either no calcification present, 1 or 2 small areas, a few scattered areas of MC or regular large areas of calcification, respectively. CsA/ASP scores compared to those from both CsA alone and CsA/SAL groups; *$P < 0.05$. For further details see text.

major contributing factor in the pathogenesis of renal dysfunction.

The therapeutic manipulation of the nephrotoxicity observed following CsA administration has important clinical consequences in that this important side effect may yet limit the efficacy of the drug. Clinically, calcium channel antagonists have been used, with some success, to limit the degree of both functional and structural damage caused following CsA-induced vasoconstriction [19]. The results of this study suggests that ASP can potentially act at an earlier stage in the cascade producing toxicity by partially preventing the increased TxB_2 excretion caused by CsA and consequently reducing the degree of vasoconstriction and subsequent ischaemic damage caused to the kidney.

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