Biomarkers of transition from Cyclosporine-induced renal dysfunction to nephrotoxicity

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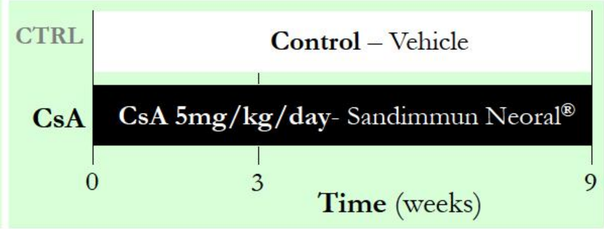
Background and Aims

Calcineurin inhibitors, in particular Cyclosporin A (CsA), remain the cornerstone of immunosuppressive regimens in many transplantation centres worldwide, regardless of drug-induced nephrotoxicity. The pathogenesis of CsA-induced nephropathy remains to be fully elucidated, but it seems to be affected by the duration of drug exposure.

This experimental animal study aimed to clarify the pathways involved in short and long-term CsA-induced nephrotoxicity and the putative biomarkers of transition from cyclosporine-induced renal dysfunction to nephrotoxicity.

Animal Groups and Assays

Groups: The study comprised 24 male Wistar rats, divided in two models: short- and Long-term treatments (3 and 9 weeks, respectively). Each model included two rat groups (n=6 each), receiving orally: Control group – vehicle; CsA group – 5 mg/Kg BW/day. Renal function was assessed on serum, urine and kidney tissue samples, through creatinine, BUN, TBARs and NGAL measures, including clearances. Renal tissue was also used to evaluate the gene expression (qRT-PCR) profile of proliferation/fibrosis markers (TGF-β_1, PCNA Ki67, mTOR, TP53 and NF-κβ) as well as to determine the protein expression by immunohistochemistry of CTGF, KIM-1, mTOR, PCNA, NF-κβ and TGF-β_1. Hematoxilin & eosin, periodic acid of schiff and masson's trichrome staining were used to evaluate glomerular, tubular and vascular kidney lesions. Statistics: ANOVA and Bonferroni Post hoc tests; $p<0.05$ was considered significant.



Results

1 Kidney function

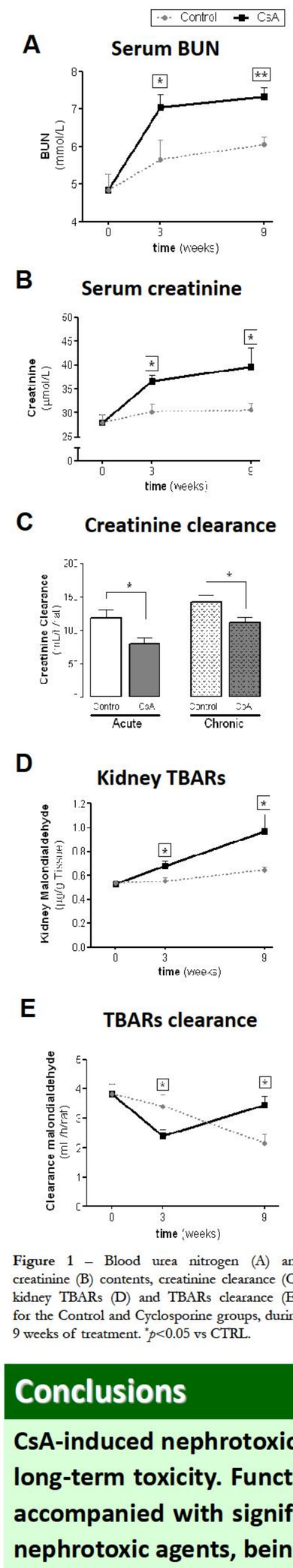


Figure 1 – Blood urea nitrogen (A) and creatinine (B) contents, creatinine clearance (C), kidney TBARs (D) and TBARs clearance (E), for the Control and Cyclosporine groups, during 9 weeks of treatment. $^{*}p<0.05$ vs CTRL.

2 Kidney histology – glomerular (A), tubulointerstitial (B) lesions and collagen stain (C)

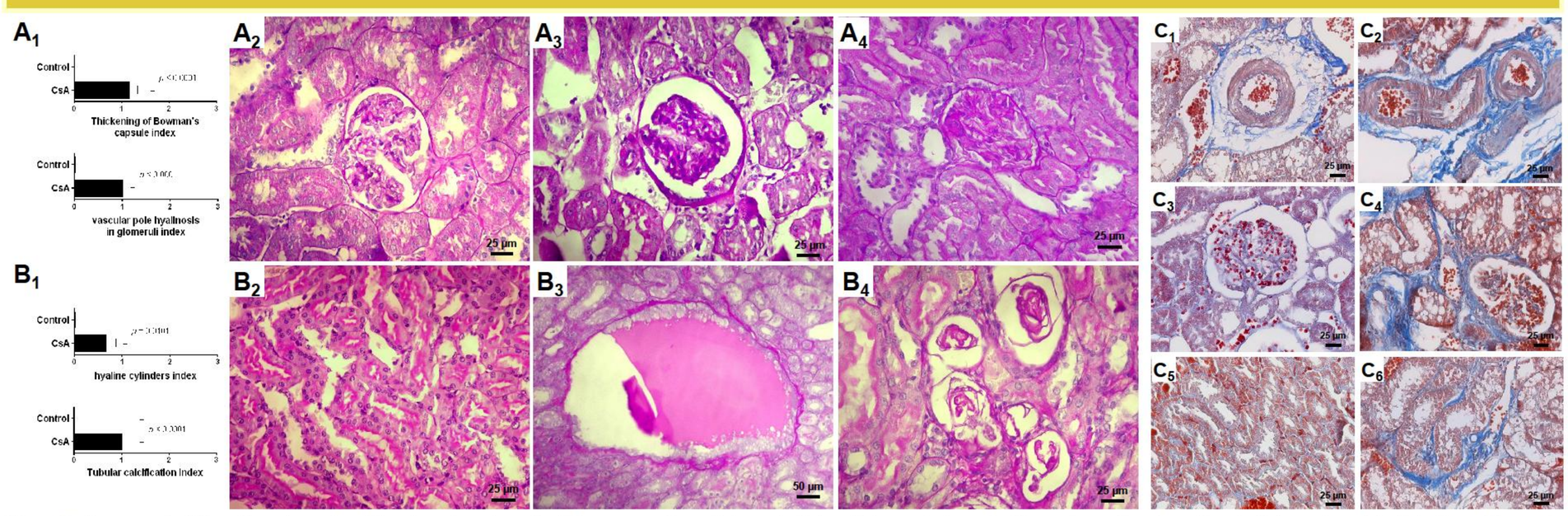


Figure 2 – Representative kidney photomicographs of 9 weeks cyclosporine and vehicle treatment. Score of glomerular lesions (A_1), normal glomerulus Bowman's capsule and vascular pole (A_2), vascular pole hyalinization and bowman's capsule thickening (A_3) and nodular sclerosis (A_4); Score of tubular lesions (B_1), normal distal and proximal tubules (B_2), enormous hyaline cylinder and interstitial fibrosis and tubular atrophy (B_3) and tubular calcification (B_4), with PAS staining. Fibrosis staining with Masson's Trichrome: control (C_1) and CsA (C_2) arteriole, control (C_3) and CsA (C_4) glomeruli, control (C_5) and CsA (C_6) tubules, during 9 weeks of treatment.

3 Kidney gene expression (RT-qPCR) – PCNA, TP53, TGF-β_1, NF-κβ, Mki67 and mTOR

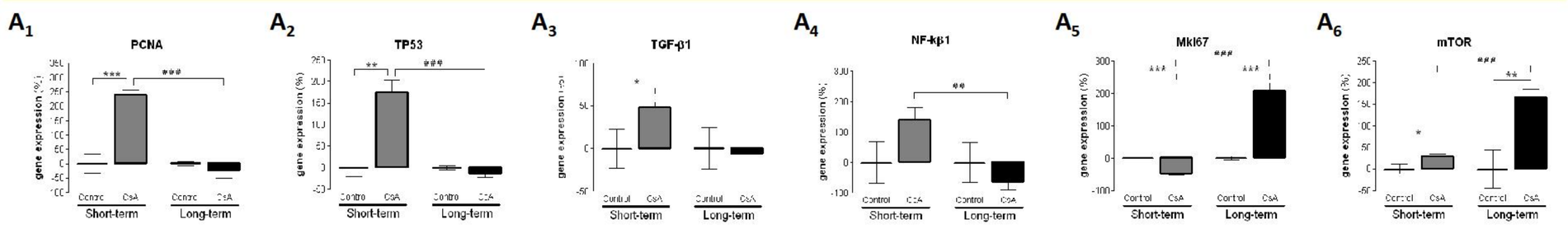


Figure 3 – kidney mRNA levels of proliferating cell nuclear antigen (A_1), tumor protein 53 (A_2), transforming growth factor beta-1 (A_3), nuclear factor kappa beta (A_4), antigen ki-67 (A_5) and mammalian target of rapamycin (A_6). $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs Control; $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ vs CsA 3 weeks.

4 Kidney protein levels – CTGF, Kim-1, NF-κβ and mTOR

Figure 4 – Representative kidney immunostaining at 3 and 9 weeks cyclosporine treatments and 3 weeks vehicle treatment . Connective tissue growth factor (CTGF), kidney injury molecule 1 (Kim-1), nuclear factor kappa beta (NF-Kβ) and mammalian target of rapamycin (mTOR). $^{*}p<0.05$ and $^{***}p<0.001$ vs CTRL; $^{\#\#\#}p<0.001$ vs CsA 3 weeks. Quantitative Immunohistochemical score (QIC) = % of staining area * staining intensity * 0.1.

Conclusions

CsA-induced nephrotoxicity is aggravated over time and the results indicate that distinct mechanisms and biomarkers are involved in regulating short or long-term toxicity. Functional impairment starts earlier but it is aggravated with time, while renal lesions only appeared after the long-term exposure, accompanied with significant mRNA up-regulation of Mki67 and mTOR. These findings reinforce the rationale for the early substitution of CsA by less nephrotoxic agents, being mTOR inhibitors a valid choice, in order to prevent chronic CsA-induced nephrotoxicity.

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