CARDIORENAL SYNDROME TYPE 1: INFLAMMATORY EFFECTS ON RENAL TUBULAR CELLS

Grazia Maria Virzì[1,2], Alessandra Brocca[1,2], Massimo de Cal[1,2], Chiara Bolin[3], Dinna N. Cruz[1,2], Giorgio Vescovo[3] and Claudio Ronco[1,2]

[1]Department of Nephrology, Dialysis and Transplant; [2]IRRIV-International Renal Resarch Institute Vicenza; [3]Internal Medicine
St Bortolo Hospital, Vicenza, Italy

INTRODUCTION AND AIMS

Heart performance and kidney function are closely interconnected and comunication between these organs occurs through a variety of pathways. This special connection is well recognized and was classified as Cardiorenal Syndromes (CRS). In particular, CRS1 is characterized by a rapid worsening of cardiac function leading to AKI. The pathophysiology of CRS1 is very complex and poorly understood. It is postulated that the concurrent dysfunction of heart and kidneys is due to a cellular and molecular crosstalk between these organs with activation of the immune response, inflammation and activation of apoptotic pathways. We conducted a pilot study to examine the possible role of the immune-mediated and inflammatory mechanisms in the pathogenesis of CRS1. The main objective was to analyze in vitro that plasma from patients with CRS1 was able to trigger a response in renal tubular cells (RTCs).

METHODS

We enrolled 29 patients with Heart Failure (HF) (age 73.6±9.5 yrs); these patients had HF of any cause and subsequently not developed AKI (HF group). In addition, we enrolled 11 patients (74.0±13.1 yrs) with CRS Type 1 (CRS Type 1 group). Patients who had AKI prior to the episode of AHF, or had any other potential causes of AKI, were excluded from the study. AKI was defined by AKIN criteria. The cause of AKI was presumed to be related to the AHF after exclusion of other plausible causes based on review of clinical course.
15 healthy volunteers (62±6 yrs) without HF or AKI were recruited (Control group).
Blood samples were collected from recruited patients on admission into Internal Medicine ward. We also collected a blood sample within 24h of AKI for patients who developed CRS Type 1. The blood sample was collected in EDTA tube and subsequently centrifuged. Plasma was immediately separated from the blood cells and stored at -80°C until use.
The protocol and consent forms were approved by our Ethics Committee. Control samples from healthy volunteers were processed in the same manners.
Plasma from different groups were incubated with RTCs for 24h and, subsequently, cell apoptosis was evaluated by different methods. Quantitative levels of TNF-α, IL-6, IL-18, NGAL, soluble I form of cell adhesion molecule (sICAM) and RANTES (Regulated on Activation Normal T cell Expressed and Secreted) in cells supernatant were performed by Human Instant enzyme-linked immuno-sorbent assay (ELISA) kit (eBioscience).
Statistical analysis was performed using the SPSS 15 software.
A p-value of <0.05 was considered statistically significant.

RESULTS

RTCs treated with CRS1 plasma showed higher DNA ladder formations, suggesting presence of apoptotic events (Figure 1).

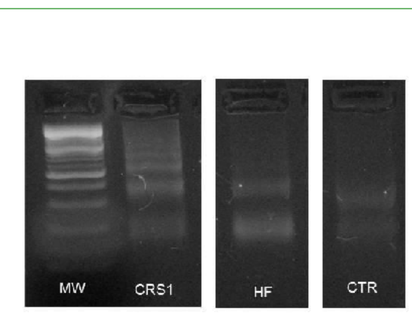

Figure 1: Cell apoptosis by Apoptotic DNA Ladder Kit at 24hours in cultured human Renal Tubular Cells
In RTCs incubated for 24 hours with CRS1 plasma, the results showed DNA ladder formation with different molecular weight fractions, suggesting the presence of apoptotic events.

Indeed, a quantitative analysis of apoptosis using AnnexinV/Propidium Iodide (PI) showed significantly higher apoptosis rates in CRS1 compare to HF and CTR ($p<0.05$). In addition, the proportion of late-stage apoptotic cells (AnnexinV+/PI+) increased significantly from 5.2% in CTR to 8.7% in HF and 30.5% in CRS1 at 24h. The increase of apoptosis indicated by AnnexinV/PI was confirmed by Caspase-3 levels: 2.63±0.24 ng/ml in CRS1 vs 1.75±0.17 in HF and 0.97±0.14 in CTR (Figure 2).

TNF-α and sICAM levels in supernatant were significantly elevated both in HF and in CRS1 compare with CTR (both $p<0.05$). However, no significant difference was found between supernatant TNF-α and sICAM levels among these 2 groups. Furthermore, in CRS1 patients IL-6, IL-18, NGAL, RANTES levels in supernatant were significantly higher compared with HF patients and CTR ($p<0.05$) (Table).

	CTR	HF	CRS Type1	p
IL-18 pg/ml	47.9 (36.9-57.2)	106.8 (94.6-114.2)	379.8 (332.5-481.4)	p<0.001
RANTES pg/ml	82.6 (72.8-104.5)	389.0 (316.4-424.6)	559,3 (473.4-580.6)	p<0.001
sICAM pg/ml	0,7 (0.65-0.76)	1.8 (1.4-2.1)	1.9 (1.6-2.3)	NS
NGAL ng/ml	14.5 (12.1-20.1)	156.4 (137.4-186.8)	255.1 (210.8-318.6)	p<0.001
IL-6 pg/ml	12.6 (10.8-14.5)	197.6 (184.2-279.9)	409.9 (395.9-425.5)	p<0.001
TNF-α pg/ml	8.1 (6.9-13.1)	34.1 (29.6-38.1)	38.1 (31.9-40.9)	NS

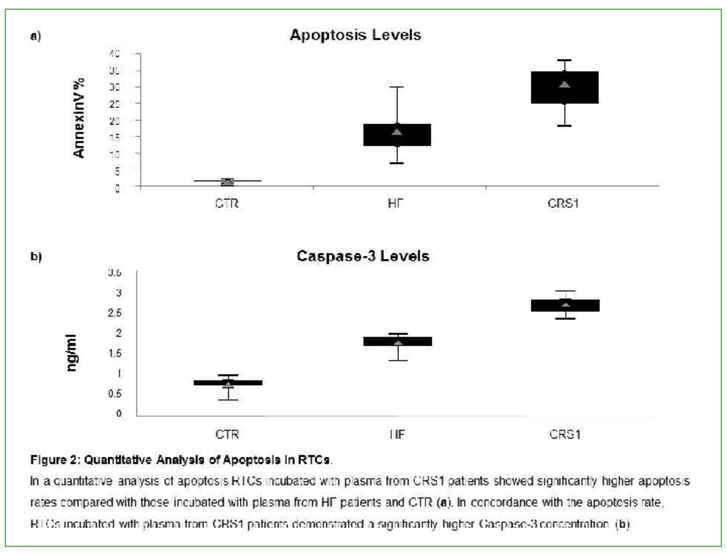

Figure 2: Quantitative Analysis of Apoptosis in RTCs.
In a quantitative analysis of apoptosis RTCs incubated with plasma from CRS1 patients showed significantly higher apoptosis rates compared with those incubated with plasma from HF patients and CTR (**a**). In concordance with the apoptosis rate, RTCs incubated with plasma from CRS1 patients demonstrated a significantly higher Caspase-3 concentration (**b**).

CONCLUSIONS

In conclusion, the inflammatory pathway could be proved an important contribution of the development of CRS1 and we can speculate that cytokines or other mediators may play a role in the mechanism of CRS1 and may be essential for the damage of distant organs.

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