NGF ENHANCES THE NEPHROTOXIC EFFECTS EXERTED BY CYCLOSPORINE A IN TUBULAR CELLS

Donatella Vizza, Anna Perri, Danilo Lofaro, Pina Toteda, Simona Lupinacci, Francesca Leone, Paolo Gigliotti, Teresa Papalia and Renzo Bonofiglio

Research Center "Kidney and Transplantation" - Department of Nephrology Dialysis and Transplantation, Annunziata Hospital Cosenza – ITALY-

CENTRO DI RICERCA RENE E TRAPIANTO

BACKGROUND AND AIM

Chronic Allograft Dysfunction (CAD) affects almost all kidney transplantation patients in the long time and causes about the 50% of graft loss. Despite the crucial role played by Calcineurin Inhibitors (CNIs) in renal acute rejection prevention, the chronic drug nephrotoxicity induced by CNIs therapy contributes to CAD pathogenesis through molecular mechanisms not yet completely understood. Calcineurin inhibition results in the inactivation of Nuclear Factor of Activated T-cells (NFAT), a transcriptional factor involved also in Nerve Growth Factor (NGF) synthesis and expression, suggesting a potential link between CNIs and NGF (1). NGF promotes cell growth, differentiation, survival and death by two different receptors: TrkA and p75. A specific cell-surface TrkA-p75 ratio seems to be directly responsible for either proliferative/survival effects (TrkA) or apoptotic response (p75) (2). Our recent studies demonstrate that NGF and its receptors exert a critical role in progressive renal disease (3) and in an experimental model of diabetic rodent. Moreover, in our cohort of 79 renal transplant patients, we found significantly higher NGF serum levels compared to patients with CKD, independently of renal function. However nothing is known about NGF expression in kidney transplantation. The aim of our study is to investigate the role of NGF and its receptors in the nephrotoxicity induced by CNIs in kidney trasplant.

MATERIALS AND METHODS

We evaluated in human proximal tubular epithelial cells, HK-2, treated with Cyclospirin A 10 nM (CsA) or NGF 100ng/ml, alone or in combination, vitality cell, gene and protein expression of NGF receptors, apoptosis and cell cycle regulators by MTT assay, real time RT- PCR-assay and Western blot analysis, respectively

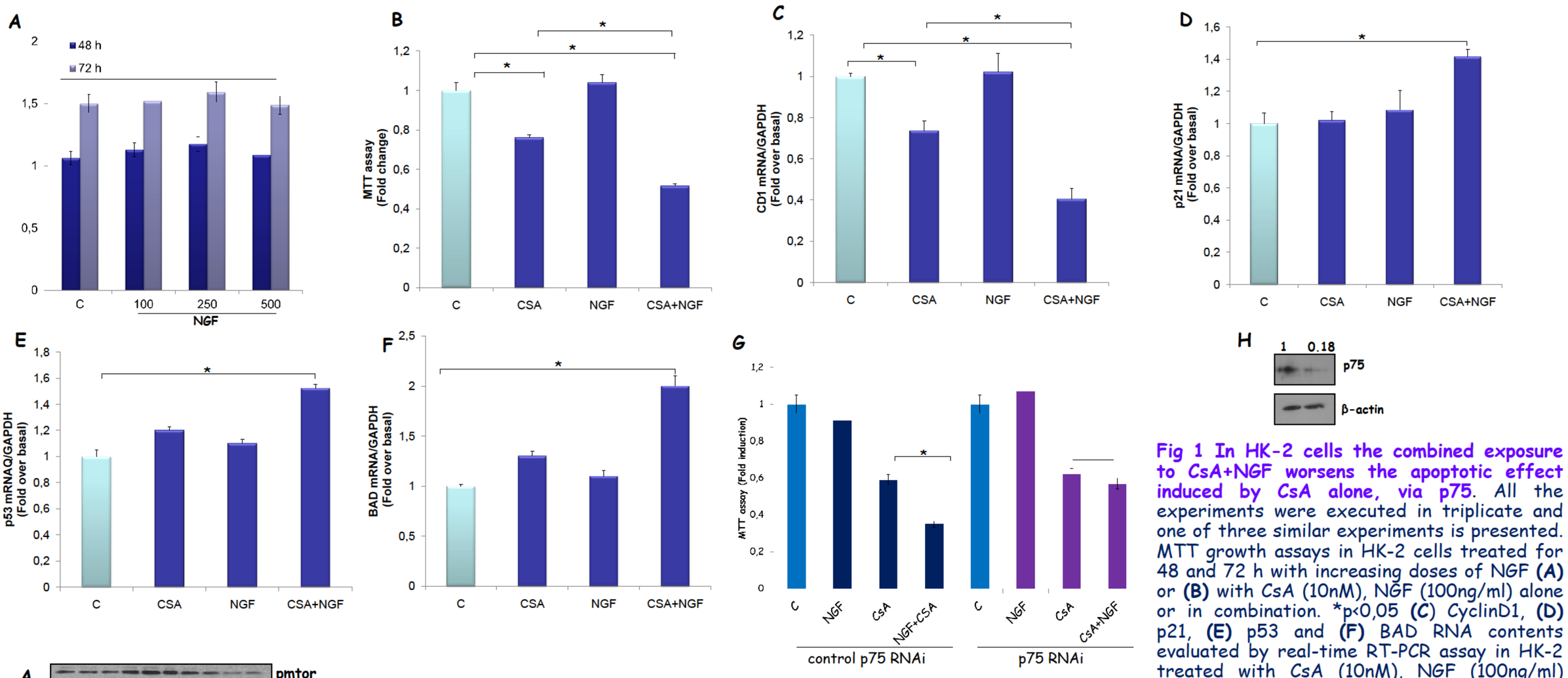

Fig 1 In HK-2 cells the combined exposure to CsA+NGF worsens the apoptotic effect induced by CsA alone, via p75. All the experiments were executed in triplicate and one of three similar experiments is presented. MTT growth assays in HK-2 cells treated for 48 and 72 h with increasing doses of NGF **(A)** or **(B)** with CsA (10nM), NGF (100ng/ml) alone or in combination. *p<0,05 **(C)** CyclinD1, **(D)** p21, **(E)** p53 and **(F)** BAD RNA contents evaluated by real-time RT-PCR assay in HK-2 treated with CsA (10nM), NGF (100ng/ml) alone or in combination. **(G)** MTT growth assay in HK-2 cells transfected with siRNA targed human p75 mRna sequence or with a control siRNA **(H)**, and then untreated (C) or treated for 48 h with CsA (10nM), NGF (100ng/ml) alone or in combination.

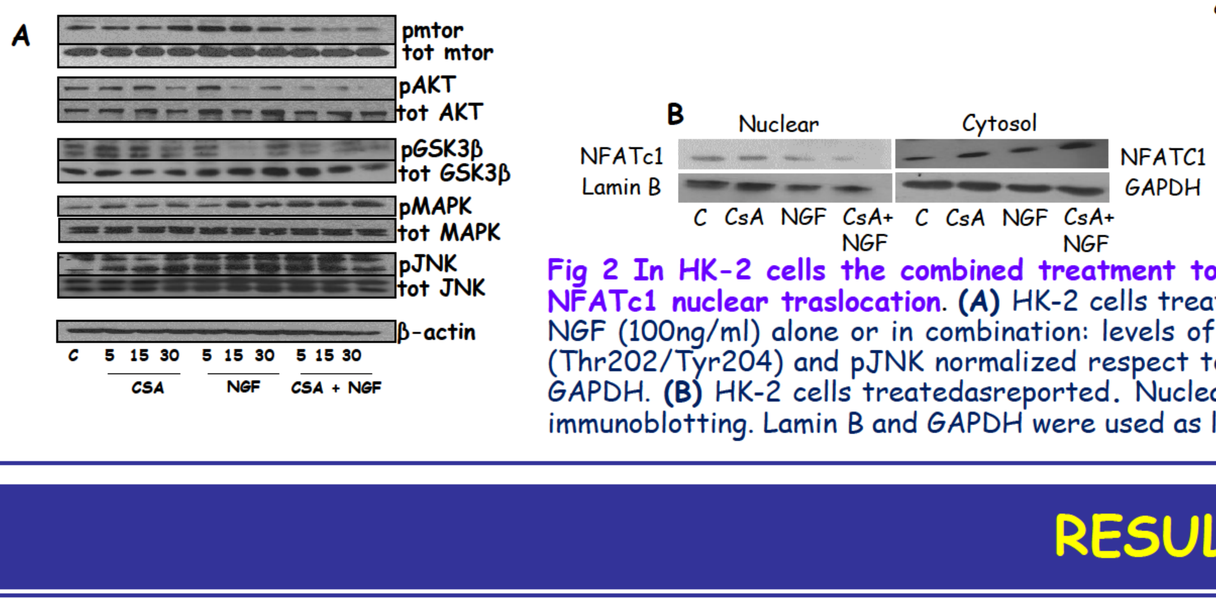

Fig 2 In HK-2 cells the combined treatment to CsA+NGF induce a double inhibition on NFATc1 nuclear traslocation. **(A)** HK-2 cells treated for 5,15,30 min with with CsA (10nM), NGF (100ng/ml) alone or in combination: levels of pmTor, pAkT (Ser473), pGSK3B, pMAPK (Thr202/Tyr204) and pJNK normalized respect to total non-phosphorylated protein and to GAPDH. **(B)** HK-2 cells treatedasreported. Nuclear and cytosolic fraction were analyzed by immunoblotting. Lamin B and GAPDH were used as loading.

RESULTS

Our in vitro studies reveals that increasing doses of NGF did not influence HK2 cells vitality as well as p75 and TrKA expression levels. Instead, the combined chronic exposure to NGF and CsA, induced a more pronounced apoptotic process respect to CsA treatment alone. In the same experimental condition, we observed that HK2 cells significantly increase p75 expression together with a down-regulation of TrkA respect to CsA alone. In HK2 cells treated with NGF+CsA, chemical inhibition of p75-silencing approaches (siRNA) down-regulated the apoptotic signal, highlighting the contribution of NGF-p75 in mediating HK2 apoptosis. Finally, we found that upon co-treatment, NFATc1, one of the two major isoforms of NFAT mainly expressed in HK2 cells, underwent to a double nuclear translocation inhibition by: i) calcineurin inhibition; ii) activation of kinases JNK and GSK3β through p75 up-regulation and TrkA down-regulation transduction pathways respectively.

CONCLUSIONS

Our study demonstrate that the combined treatment CsA+NGF enhance the nephrotoxic effects exerted by CsA, through a molecular mechanism inducing a significant cell-surface p75 receptor up-regulation leading to a massive apoptotic process.

REFERENCES

1.Rana OR, Saygili E, Meyer C, Gemein C, et al (2009) Regulation of nerve growth factor in the heart: the role of the calcineurin-NFAT pathway J Mol Cell Cardiol.
2.Bonofiglio R, Antonucci MT, Papalia T, Romeo F et al (2007) Nerve growth factor (NGF) and NGF-receptor expression in diseased human kidneys. J Nephrol.
3.Antonucci MT, Bonofiglio R, Papalia T, Caruso F et al. (2009) Nerve growth factor and its monocyte receptors are affected in kidney disease. Nephron Clin Pract.
4.Micera A, Puxeddu I, Aloe L, Levi-Schaffer F (2003) New insights on the involvement of Nerve Growth Factor in allergic inflammation and fibrosis. Cytokine & Growth Factor Reviews

50ERA-EDTA
7--MP
Cell signalling
Donatella Vizza
DOI: 10.3252/pso.eu.50era.2013
Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 15-21, 2013
PosterSessionOnline.com