OMEGA-3 FATTY ACIDS FOR THE PREVENTION OF CYCLOSPORIN A-INDUCED PROXIMAL TUBULAR TOXICITY IN VITRO

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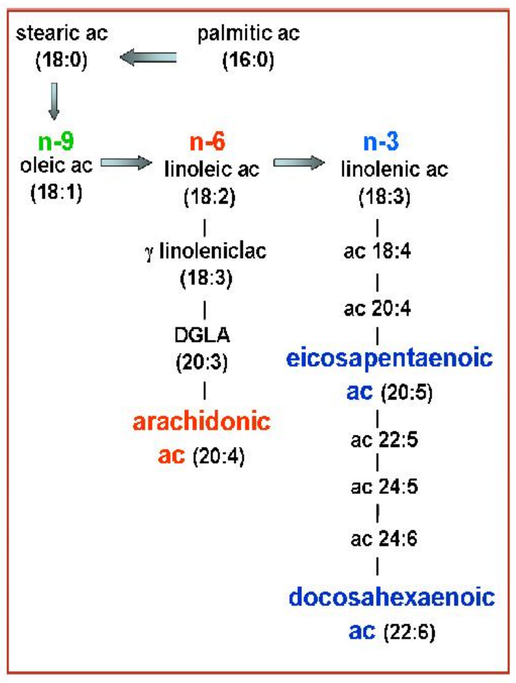
INTRODUCTION

Cyclosporin A (CyA) is a potent immunosuppressive agent widely employed in the prevention of graft rejection and in the treatment of autoimmune diseases. Unfortunately, its use is associated with several side effects among which nephrotoxicity may be of particular concern in kidney transplanted patients.

Polyunsaturated fatty acids (PUFAs) arachidonic (AA, 20:4, n-6) and eicosapentaenoic (EPA, 20:4, ω-3) acids as well as the derivative docosahexaenoic acid (DHA, 22:6, ω-3), have been shown to interfere with the synthesis of a variety of inflammatory factors and events.

Epidemiological and clinical studies clearly demonstrate that PUFAs and their metabolites play an important role as autocrine and paracrine mediators in various patho-physiological conditions at different tissue and organ levels including kidney disease. In this context, we recently demonstrated that in CyA-treated human mesangial cells, EPA and DHA, ω-3 PUFAs, significantly counteracted the up-regulation of pro-fibrotic genes induced by the immunosuppressant.



ω-3 & ω-6 FATTY ACID CASCADE

AIM OF THE RESEARCH

The onset of renal fibrosis is characterized by mesangial cells activation with increased synthesis of pro-inflammatory cytokines, adhesion molecules, and extracellular matrix protein production.

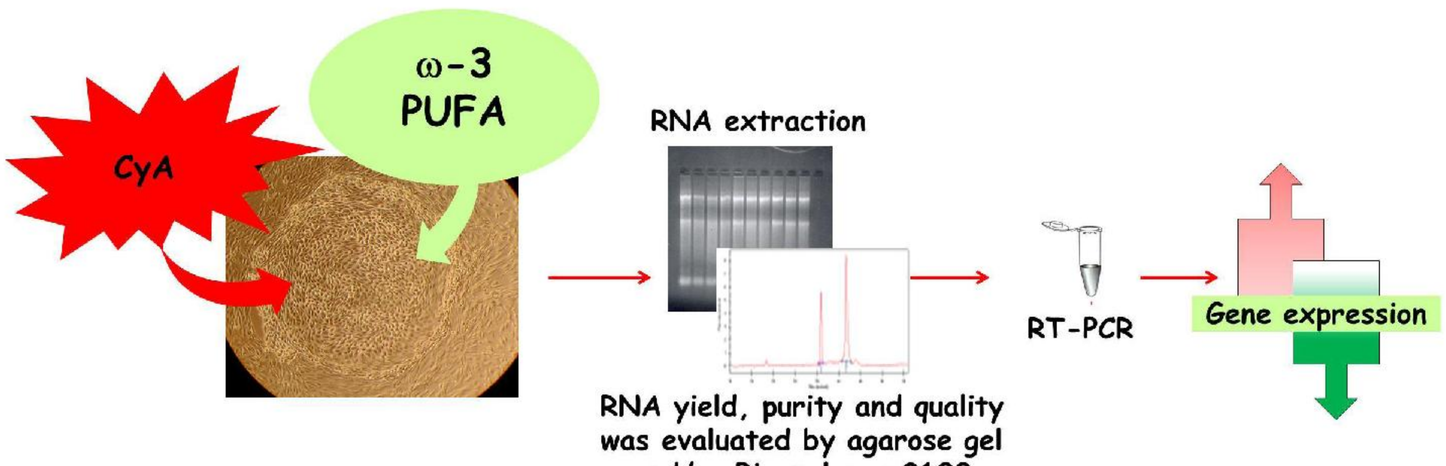
Chronic CyA nephrotoxicity is also characterized by early disturbances of proximal tubule structure and function progressing to tubular atrophy and interstitial fibrosis, universally recognized side effect of CyA therapy.

The aim of the present study was to investigate, employing an in vitro model of human proximal tubular cells, a possible protective action of ω-3 PUFAs in counteracting the adverse effects of CyA.

METHODS

HK-2 cells of human proximal tubular origin were grown in DMEM supplemented with 10% FBS, penicillin 100 IU/ml, and streptomycin 100 μg/ml at 37°C, 95% air and 5% CO_2. Cells were subcultured in 12-well plates, grown to 70–80% confluence and subsequently exposed to CyA at 1 or 5 μg/ml either alone or simultaneously with DHA 50 μM for 24 hrs.

In order to test the possible toxic effect of such treatment, morphology and viability were assessed at light microscopy by Trypan blue exclusion. Gene expression analysis was performed by RT-PCR to evaluate the markers of renal fibrosis FN and COLIV, and of inflammation MCP-1, TGFβ, and CTGF.

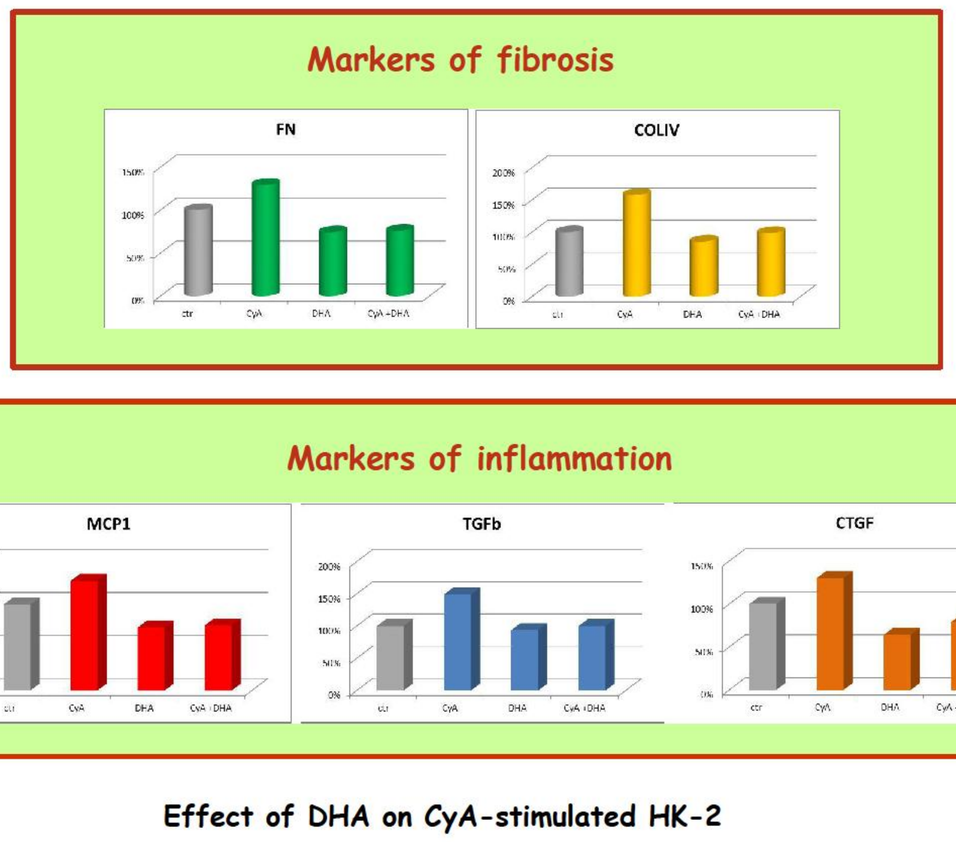


RESULTS

CyA induced a significant up-regulation of all genes considered: FN +129%, COLIV +158, MCP-1 +127%, TGFβ +149%, CTGF +130% ($p<0.05$).

On the contrary, the effect of the ω-3 PUFA DHA treatment resulted in a significant down-regulation: FN –26%, COLIV –15%, MCP-1 –27%, TGFβ –10%, CTGF –36%, with respect to controls (untreated cells).

When DHA was used in simultaneous treatment with CyA, it exerted a significant inhibitory effect on CyA-induced upregulation of all genes: FN –55%, COLIV –60%, MCP-1 –52%, TGFβ –50%, CTGF –50% ($p<0.01$ vs CyA-treated cells).



Effect of DHA on CyA-stimulated HK-2

CONCLUSIONS

The fibrogenic process results from the activation of several systems including both the inflammatory and ECM proliferation pathways. Our in vitro results of cytokine upregulation substantiate clinical reports of CyA-induced kidney toxicity. Moreover, we demonstrated a beneficial effect of the fish oil active component DHA on the pro-inflammatory and pro-fibrotic cytokine imbalance induced by CyA at renal tubular level.

The favourable action of omega-3 PUFA on CyA-altered cytokines profile may suggest their use in kidney transplanted patients for the non-pharmacological management of adverse outcomes such as tubular atrophy, tubular dysfunction, and interstitial fibrosis characteristic of chronic CyA nephropathy.

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