O108

2-hour Oral Session

Immunosuppressive effects of chloroquine and hydroxyl-chloroquine on purified human CD4+ T-cells

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Objectives

The anti-malarial drugs chloroquine and hydroxy-chloroquine have immunomodulatory potency which is therapeutically harnessed in rheumatic diseases. However, the exact mechanisms of action on immune cells have not been fully described. Consequently, we assessed the influence of chloroquine on human $CD4^+$ T-cells.

Methods

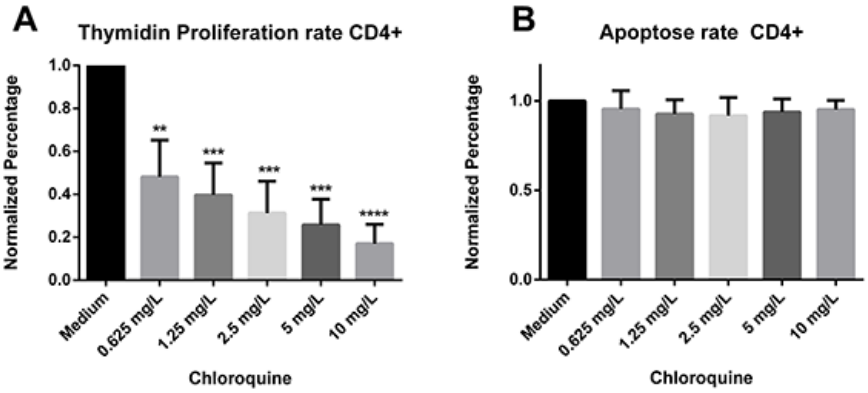
Highly purified human $CD4^+$T-cells of eight apparently healthy donors were pre-incubated with chloroquine or hydroxy-chloroquine concentrations of up to 10 µM, which leads to intracellular accumulation of drug levels comparable to oral therapy in rheumatic diseases. Using microbeads, coated with agonistic anti-CD3/anti-CD28 antibodies, $CD4^+$ T-cells were activated in in the presence or absence (as controls) of the indicated concentrations of chloroquine and hydroxychloroquine. Proliferation was measured by incorporation of [3H]-thymidine and by dilution of the cell proliferation dye eFluor670 CPD using flow cytometry. Effector cytokine production in the cell`s supernatant was measured after 24 hours (IL-2) and 72 hours (IL-4, IL-10, IL-13) using luminex multiplex technology. After 72 hours, cell viability was assessed by Annexin V / Propidium Iodide staining. For analysis of intracellular signalling pathways, Jurkat reporter cell lines were used expressing GFP under control of either the NFAT, NF-kB or AP-1 promoter. These cells were pre-incubated as described above and stimulated using anti-CD3/CD80 expressing T-cell stimulator cells. After 24 hours, GFP fluorescence was measure as readout for promoter activity. Rapamycin (100 nM), Cyclosporin (10 µM) and the PKC inhibitor GÖ6983 (1 µM) were included as control for the specificity of the reporter constructs.

Results

Following activation, proliferation of exposed T-cells was already suppressed at a concentration of 0.6 mg/L chloroquine (p=0.002) or hydroxy-chloroquine (p=0.005). This effect, which was more pronounced at higher concentrations, was due to specific inhibition of activation, since no decrease in cellular viability could be detected. Similarly, effector cytokines were significantly decreased at 5mg (IL-2, p=0.003) or 10 mg/L chloroquine (IL-4 p=0.007; IL-10, p=0.038, IL-13, p=0.049). In re-stimulation experiments, we determined that the effects of chloroquine were reversible after removal of the substance. Using Jurkat reporter cell lines expressing GFP under control of either the NFAT, NF-kB or AP-1 promoter, we observed that chloroquine specifically inhibited AP-1 promoter activity. No influence on the NFAT and NF-kB signalling was observed. First experiments suggest that this effect was mediated by the inhibition of phosphorylation of c-Jun.

Conclusion

In conclusion, we describe immunosuppressive functions of chloroquine on purified $CD4^+$ T-cells and propose that inhibition of AP-1 activity is the main molecular mechanism of this effect.



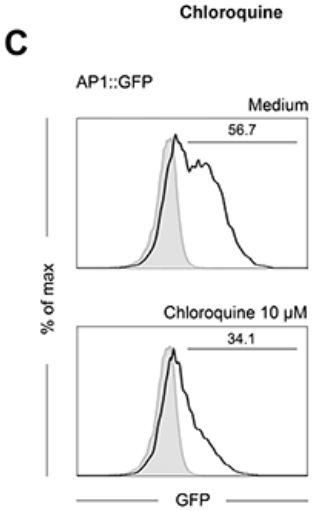


Figure I: Chloroquine effects on T-cell function

A.) Cell proliferation, measured by incorporation of [3H]-thymidine

B.) Cell viability, assessed by Annexin V / Propidium Iodide staining

C.) Jurkat reporter cell line expressing GFP under control of AP-1 promoter. GFP fluorescence was measure as readout for promoter activity.