Immunosuppression by hydroxychloroquine: mechanistic proof in *in vitro* experiments but limited systemic activity in a human clinical pharmacology study

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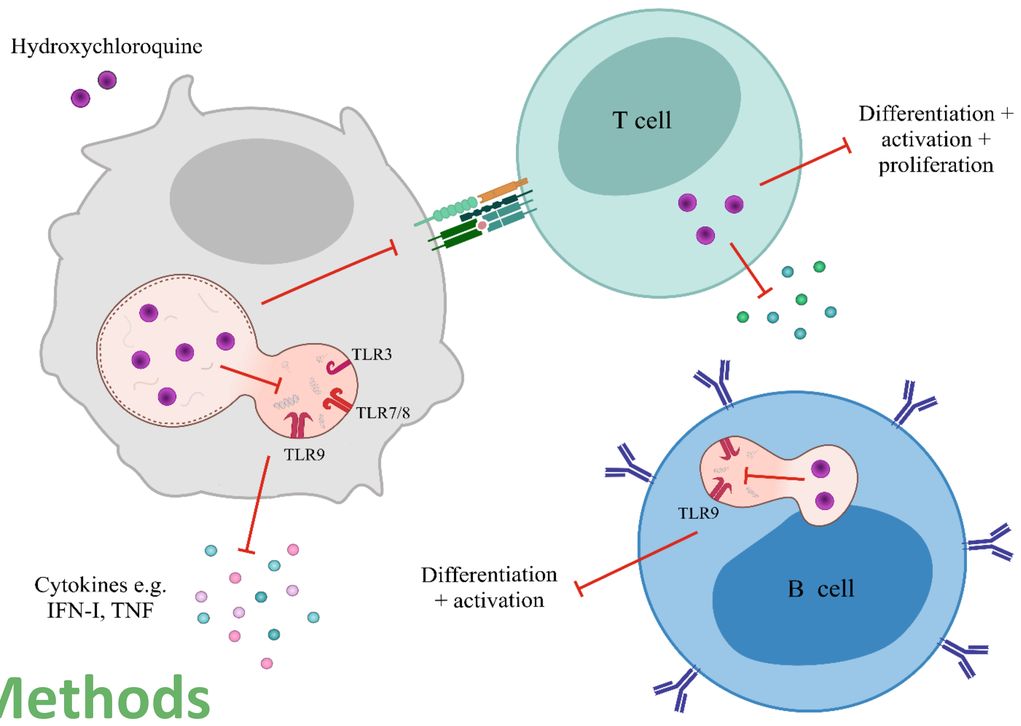
Introduction

Because of its wide range of immunosuppressive properties, the antimalarial drug hydroxychloroquine (HCQ) is also used for the treatment of autoimmune diseases such as systemic systemic lupus erythematosus and rheumatoid arthritis. Even though HCQ is used as treatment for these autoimmune diseases since 1955, limited literature is available on the relationship between HCQ concentration and its immunosuppressive effect.

Aim

Driven by the recent interest in HCQ as potential treatment for COVID-19, we aimed to evaluate the immunosuppressive effects of HCQ on human immune cells.



Methods

A single-blind, randomized, placebo-controlled multiple dose study in 40 healthy male volunteers was conducted. Subjects were randomized to receive either HCQ (plaquenil) or placebo tablets, in a 1:1 ratio. A cumulative dose of 2400 mg HCQ over 5 days, the then-standard dosing regimen for moderate to severe SARS-CoV-2 patients, was used to study the following endpoints:

- T cell activation and cytokine production
- TLR3/TLR7/TLR9/RIG-I-induced cytokine production
- T and B cell proliferation

The *in vitro* effect of HCQ (10-10,000 ng/mL) was evaluated in human PBMCs, using the same endpoints.

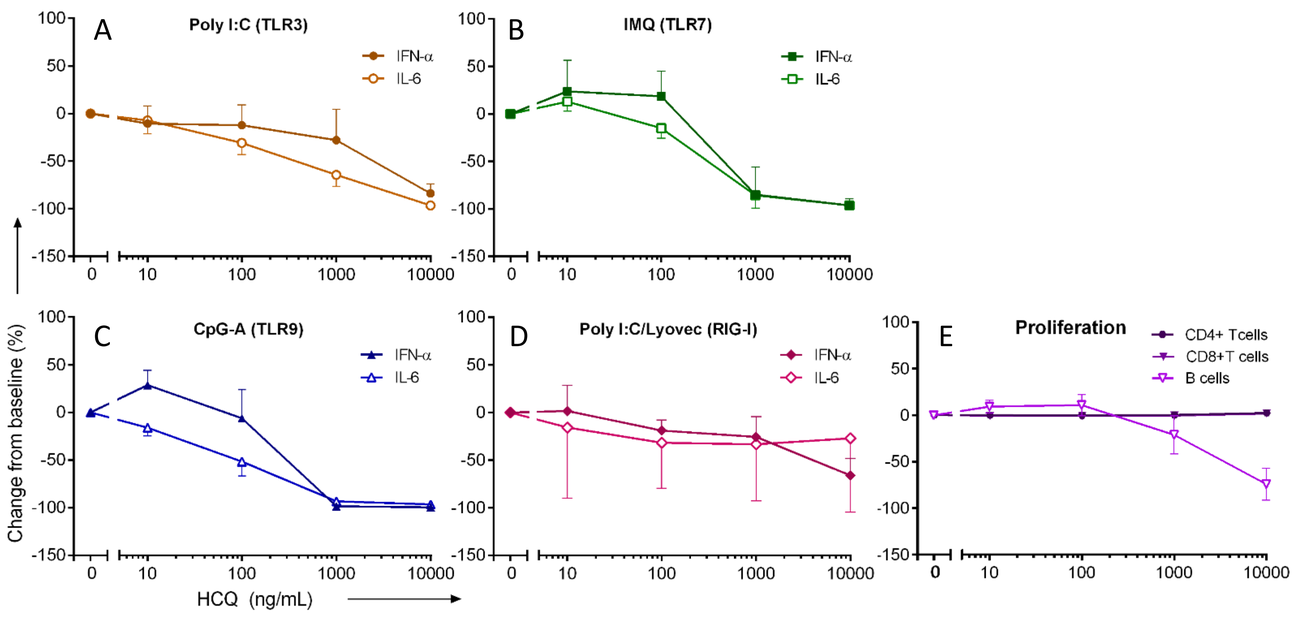


Figure 2A,B,C,D: In vitro TLR/RIG-I mediated IRF activation (IFNa production) and NFkB activation (IL-6 production) 2E: In vitro CD4+ and CD8+ T cell and CD19+ B cell proliferation

Results

In vitro

- TLR/RIG-I mediated cytokine production was inhibited by HCQ with IC50s >100 ng/mL, and Emax reaching 100% inhibition
- HCQ did not affect T cell activation or proliferation, but did inhibit B cell proliferation at concentrations >100 ng/mL.

Clinical study

- Peak HCQ plasma concentration ranged from 75-200 ng/mL
- HCQ treatment did not affect T cell activation or B cell and T cell proliferation
- No strong *ex vivo* effect of HCQ treatment were found on TLR/RIG-I mediated IRF/NFκB activation, except for a significant suppression of the imiquimod response (TLR7) and mild suppression of CpG and polyI:C responses (TLR9 and TLR3)

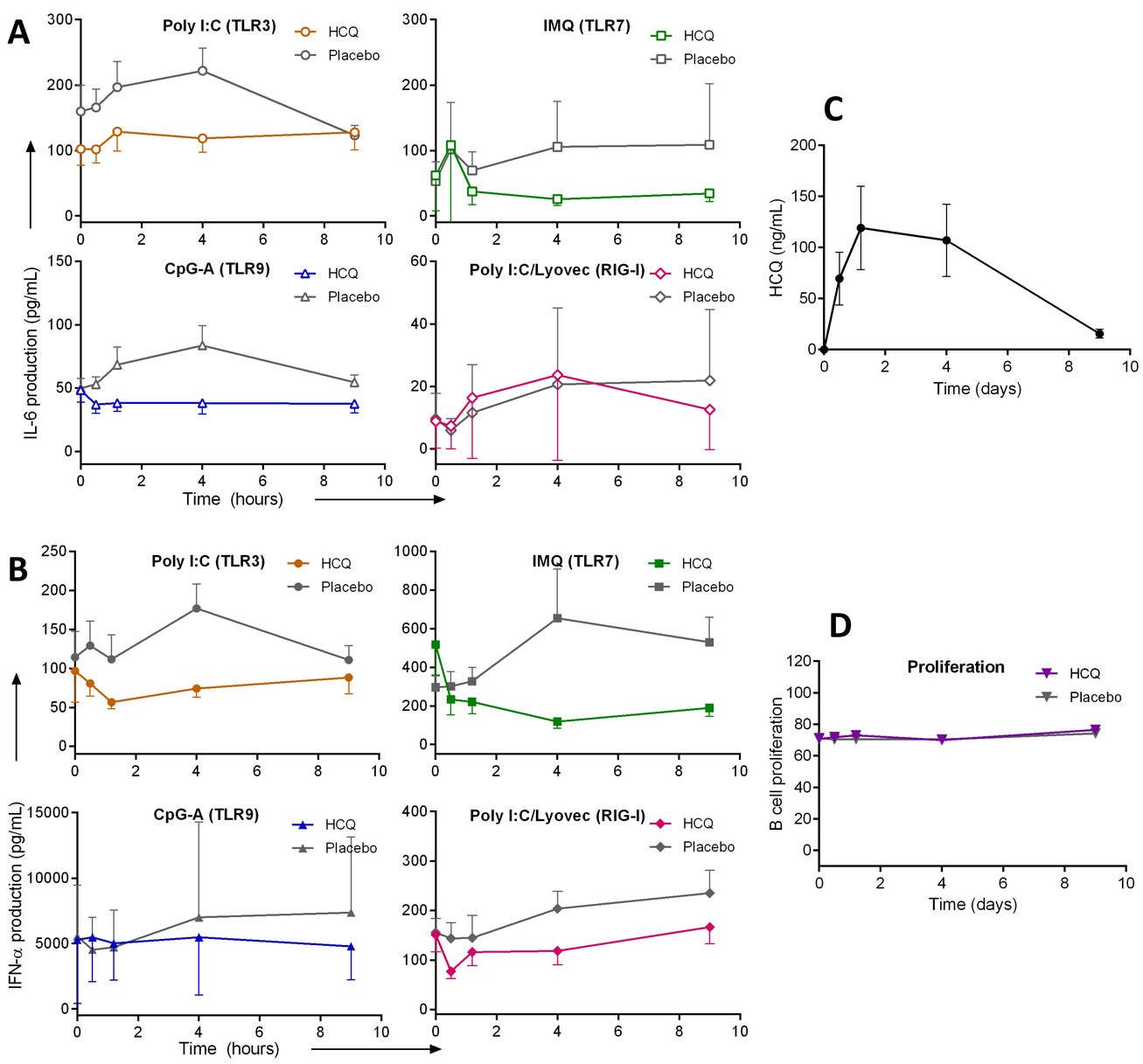


Figure 1A,B: TLR/RIG-I mediated IL-6 production (A) and IFN-α production (B). 1C: HCQ plasma concentration at 3, 27, 99 and 171 hours post-dose. Subjects were dosed with 400 mg HCQ at time points 0, 12, 24, 48, 72 and 96 hours (cumulative dose of 2400 mg) 1D: CD40/CpG-induced B cell proliferation

Conclusions

- HCQ has clear immunosuppressive effects on human immune cells at concentrations exceeding the circulating HCQ concentrations under conventional use in COVID-19
- Based on HCQ's physico-chemical properties, local drug concentrations may be higher than circulating concentrations, potentially resulting in significant local immunosuppression.

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