Effects of Resveratrol and a novel Resveratrol-salicylate hybrid molecule on activation of human CD4+ T-cells

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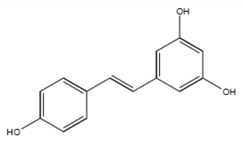
Introduction

T-cells are assumed to play a crucial role in the pathogenesis of systemic autoimmune diseases, e.g. rheumatoid arthritis (RA). Consequently, substances modulating T-cell activity may have therapeutic benefit in rheumatic diseases. Resveratrol (Res) is a natural plant polyphenol (present e.g. in red grapes, red wine) with beneficial effects in *in vitro*, animal and preclinical studies of cancer, metabolic and rheumatic diseases [1]. These effects are due to its anti-inflammatory, anti-carcinogenic and anti-oxidant activities. However, its bioavailability is low, and little is known on its effects on T-cells. Therefore, our aims were to synthesize a novel Res derivative with improved pharmacological properties, the Res-salicylate hybrid molecule C-10 [2], and compare the effects of Res and C-10 on human CD4 + T-cells .

A



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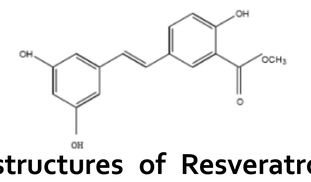

Fig. 1. Chemical structures of Resveratrol (A) and the Res-salicylate hybrid molecule C-10 (B).

Materials and Methods

CD4+ T-cells from healthy donors were pre-incubated with Res (Sigma) or C-10 and stimulated with anti-CD3/ anti-CD28 antibody-coated beads (Gibco). Cytokines in cell supernatants were quantified by ELISA after 24h (IL-2) or 72h (IFN-γ, TNF-α). Cell proliferation was measured by ^{3}H-thymidine incorporation. Phosphorylation of signal transduction molecules (Erk1,2, Akt, STAT-5) was analyzed by western blot or flow cytometry. For analysis of effects of Res and C-10 on different T-cell subsets, CD4+ T-cells were sorted into naive cells, T_H1 and T_H2 cells by flow cytometry. Data are presented as means ± SD. * p<0,05; ** p<0,001; *** p<0,0001.

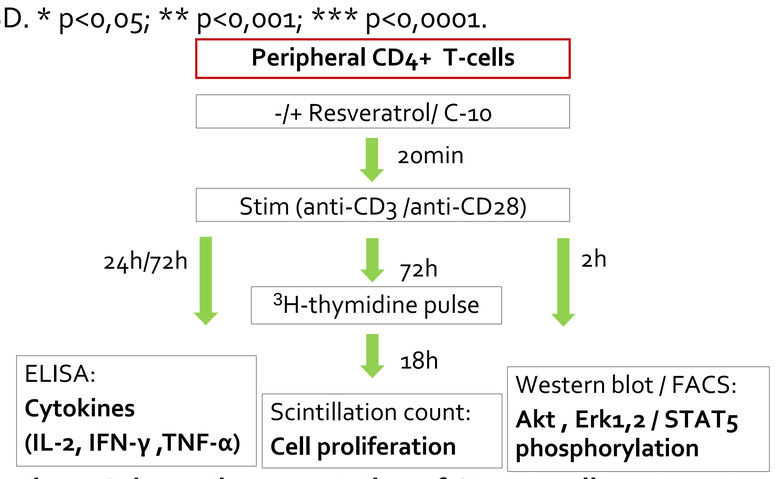

Fig. 2. Schematic presentation of CD4+ T-cell treatment and analysis.

Results

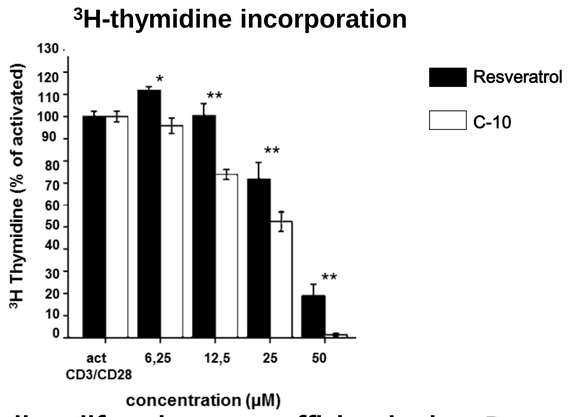

Fig. 3. C10 reduces CD4+ T-cell proliferation more efficiently than Resveratrol.
Both compounds reduced proliferation at 25µM and 50µM. In contrast to Res, C-10 did not increase proliferation at low concentrations.

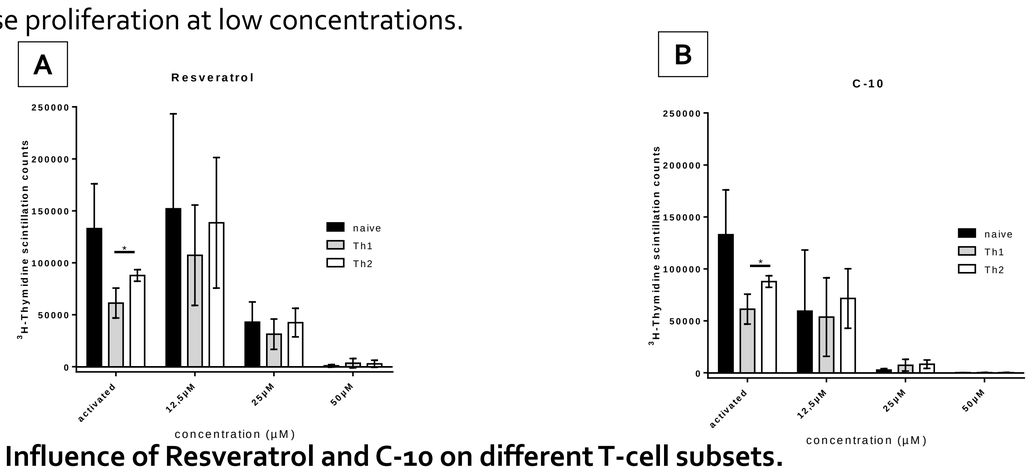

Fig. 4. Influence of Resveratrol and C-10 on different T-cell subsets.
Res (A) + C-10 (B) inhibited proliferation most strongly in naive T-cells. Both compounds reduced proliferation of all subsets at 25µM and 50µM, C-10 being more effective.

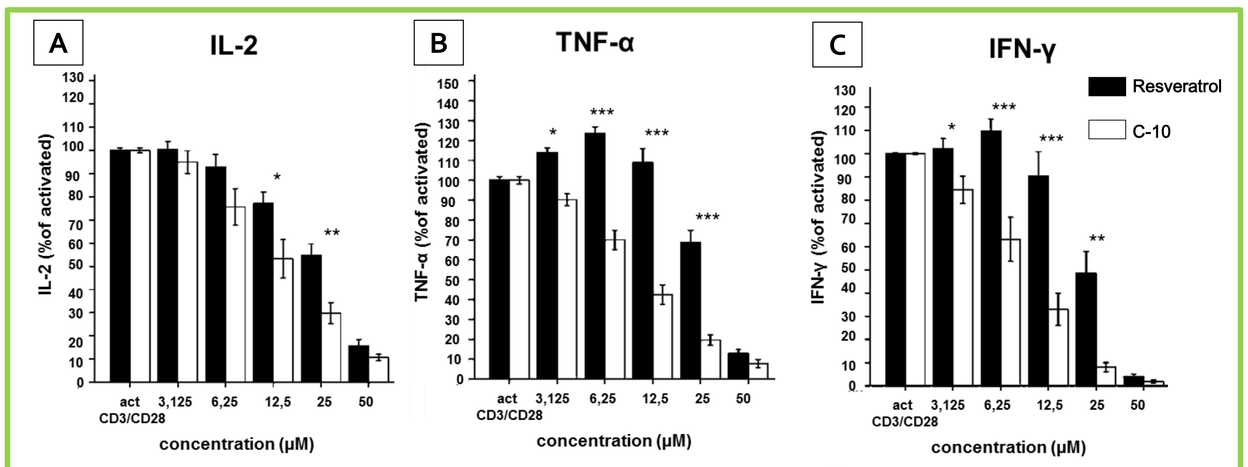

Fig. 5. C-10 reduces cytokine secretion by CD4+ T-cells more efficiently than Resveratrol
Inflammatory cytokine secretion in Res- or C-10-treated CD4+ T-cells was analyzed by ELISA. IL-2 (A), TNF-α (B) and IFN-γ (C) were reduced concentration-dependently.

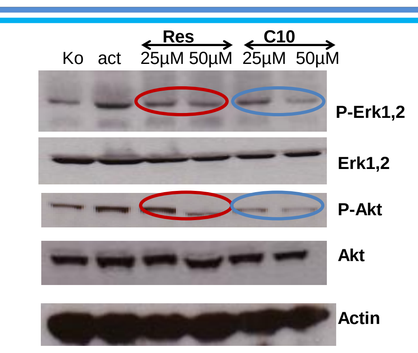

Fig. 6. C-10 reduces phosphorylation of Erk1,2 and Akt more efficiently than Resveratrol.
The kinases P-Erk1,2 (p44/42) and P-Akt were reduced by Res (red ellipses) and C-10 (blue ellipses), C-10 being more effective . Loading control: actin.

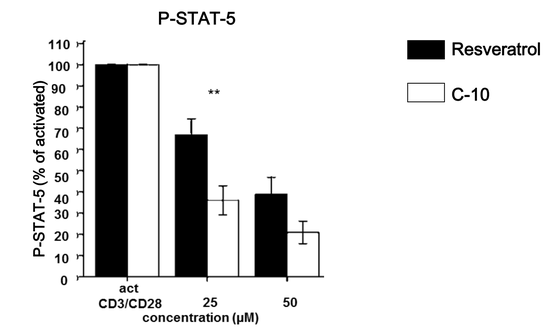

Fig. 7. C-10 reduces STAT-5 phosphorylation more efficiently than Resvertrol.
Flow cytometry showed reduced STAT-5 phosphorylation in Res- and C-10-treated CD4+ T-cells, C-10 being more effective.

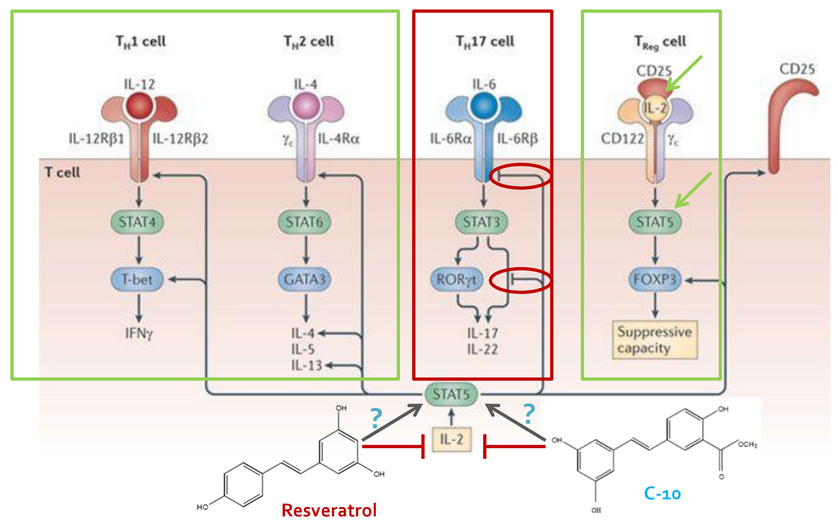

Fig. 8. The role of IL-2 and STAT-5 in T_H differentiation and T_{Reg} homeostasis.
IL-2, via STAT-5, is essential for differentiation and activation of regulatory T-cells (T_{Reg}) and enhances activity of T helper 1 (T_H1) and T_H2 cells. IL-2 and STAT-5 inhibit activity of T_H17 cells. Res and C-10 reduce IL-2 secretion in CD4+ T-cells and may thus influence activity and differentiation of different T-cell subsets (modified after [3]).

Conclusion

- C-10 suppressed inflammatory cytokine secretion and proliferation more effectively than Res.
- Res and C-10 reduced phosphorylation of the signaling molecules Erk1,2, Akt and STAT-5.
- Reduced STAT-5 phosphorylation may be the key mechanism for inhibition of T-cell activation by Res and C-10.

→ **The Res derivative C-10 might be a candidate drug for treatment of RA and other T-cell driven autoimmune diseases.**

References

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