A125 Superinduction of gene transcription in human lymphocytes by the isoflavonoid rotenone

J. Gertsch, J. Heilmann and O. Sticher
Institute of Pharmaceutical Sciences, Department of Applied BioSciences, Swiss Federal Institute of Technology (ETH) Zurich Winterthurerstr. 190, CH-8057 Zürich, Switzerland.

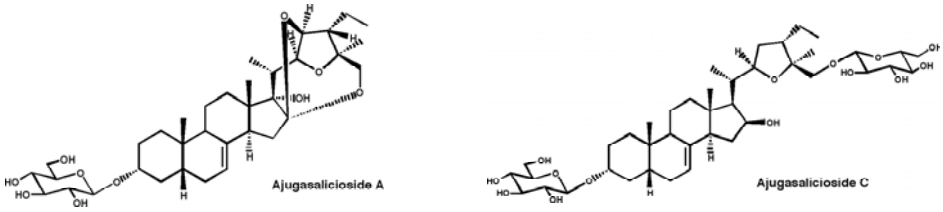
For the first time it is shown that the piscicidal and insecticidal isoflavonoid rotenone, which is a wellknown mitochondrial complex I inhibitor, also is a potent immunomodulator and biologic response modifier. At low concentrations (5 µM), rotenone potently up-regulates phorbol 12-myristate 13-acetate induced mRNA levels of several cytokines (> 15-fold), as well as beta-actin in human lymphocytes and T helper cells in a dose- and time-dependent manner. The up-regulation of beta-actin might be correlated to an increase in variability of actin organization after inhibition of mitochondrial respiration by rotenone, or to its inhibition of spindle microtubule assembly (1, 2). Furthermore, the mRNA expression of NF-ATc, a crucial transcription factor involved in the control of IL-2 gene expression and ubiquitous regulator of cell differentiation was upregulated significantly in PBMCs. These results suggest that derangement of mitochondrial function in T cells itself could lead to the differential modulation of these mRNAs, and that this mechanism may be related to, or even be a cause of the immunomodulation seen in rotenone induced Parkinson's disease (3, 4).
At higher concentrations, rotenone induces apoptosis and specifically inhibits cyclin D1 expression in human leukemia cells or in PMA stimulated primary lymphocyte cultures. The mRNA expression profiles modulated by rotenone have been measured with Reverse Transcription real time PCR (RT-rt-PCR) as described previously (4).

References: 1. Barham, S.S., and Brinkley, B.R. (1976) Cytobios 15: 97-102. **2.** Breitener-Hahn, J. et al. (1984) Cell Tissue Res. 238: 129-134. **3.** Fiszer U. (2001) BioDrugs 15: 351-5. **4.** Gao, H.M. et al. 2002 J. Neurosci. 22: 782-90. **5.** Gertsch, J. et al. (2002) Pharm. Research (in press).

A126 Antileukemic activity of novel sterol glycosides from *Ajuga salicifolia*

P. Akbay [a], J. Gertsch [a], I. Çalış [b], J. Heilmann [a], O. Zerbe [a] and O. Sticher [a]
[a] Department of Applied BioSciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich. Winterthurerstr. 190, CH-8057 Zürich, Switzerland. [b] Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey.

In the flora of Turkey the genus *Ajuga* L. is represented by 11 species (1) some of which are traditionally used. We recently reported novel sterol glycosides (ajugasalicioside A-E) from the aerial parts of *Ajuga salicifolia*, collected from Ankara (2). We tested these compounds for cytotoxicity against KB (HeLa), Jurkat (human T-cell leukemia) and human peripheral mononuclear blood cells (PMBCs). In Jurkat cells, ajugasalicioside A-D showed significant to moderate activity (IC_{50} values 10 µM). Ajugasalicioside C was the most active against Jurkat T cells (IC_{50} = 3 µM), followed by ajugasalicioside A (IC_{50} = 6 µM). Interestingly, ajugasalicioside A induced cell-cell contacts in Jurkat T cell populations similar to phorbol 12-myristate 13-acetate (PMA). To follow up this effect, we measured the possible modulation of ajugasalicioside A on PMA-induced mRNA profiles in Jurkat T cells with RT-rt-PCR (3). We discovered a significant up-regulation of cyclin D1 mRNA expression. Ajugasalicioside A weakly inhibited PMA-induced p65 mRNA levels in a concentration-dependent manner but did not influence I-κBα. Our results suggest a NF-κB independent induction of cyclin D1 by ajugasalicioside.



References: 1. Davis P.H. (1982) Flora of Turkey and the East Aegean Islands, Edinburg. 7. **2.** Akbay P. et al. (2002) Helv. Chim. Acta (in press) **3.** Gertsch J. et al. (2002) Pharm. Res. (in press).