SL19 *Agrostemma githago* L. Isolation of the toxic compounds and new approaches to their mode of action

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In the course of our investigation of *Agrostemma githago* L. var. *githago*, a well known toxic member of the Caryophyllaceae family, to date three triterpenoid saponins have been isolated with gypsogenin (3β-hydroxy-olean-12-en-23-al-28-oic acid) as aglycone (1). A combination of these particular saponin derivatives with a formyl function in triterpene position 4 (3 µg/ml) together with agrostin, a glycoprotein (M_r: 27 kDa), showed comparable toxicity against an endothelial ECV-304 cell line. In order to reproduce our results, we isolated Agrostin (3), a ribosome-inactivating protein (RIP type 1) from the seed of *A. githago*. After extraction, filtration, centrifugation and $(NH_4)_2SO_4$-precipitation, crude extracts were dialysed against 5 mM sodium-phosphate buffer (pH 6,5) and applied to a Sephadex column (Sephadex G 50/75) and to a CM-cellulose column. M_r values were determined by polyacrylamide-gel-electrophoresis and quantification of the isolated protein was determined by the Bradford- and the BCA-protein assay. Subsequent antigen-antibody-testing was undertaken for identification and quantification of the protein. Fluorescent microscopy imaging is used for intracellular detection of stained Agrostin. In order to obtain both active compounds from the seed material, we isolated an active Agrostemma-saponin from the seeds of *A. githago* (2). Repetition of our in vitro experiments with both isolated substances revealed the expected toxicity. No analogy could be drawn between the observed induction of RIP-toxicity of Agrostin and the induction of apoptosis by FAS-C-terminal tripeptide through Agrostemma-saponin, suggesting that these peptides use a different mechanism to penetrate through the cell membrane.

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SL20 Cyclotides - plant defense peptides with anticancer lead potential

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Several members of the Violaceae and the Rubiaceae plant families produce peptides of about 30 amino acids with a remarkable 3-dimensional structure, including a head-to-tail cyclised backbone and three disulfide bonds arranged as a cysteine knot. These peptides, referred to as cyclotides (1), have a potential role in the plant host defense system (2).

We have developed specific methods for isolation and structure elucidation of cyclotides. A fractionation protocol is used for the isolation of highly purified cyclotide fractions and for the removal of substance classes know to interfere with bioassays e.g., tannins (3-4). Examples of the methods used for structure elucidation, i.e. mass spectrometry sequencing and homology modelling, are presented in this poster.

In addition, we show that cyclotides from *Viola* sp. have cytotoxic activity in human cell lines using a fluorometric microculture cytotoxic assay (FMCA) (5). Activity profile of cyclotides differs significantly from those of anticancer drugs in clinical use today, indicating a new mode of action (6). The dose response curves show a very sharp profile, a phenomenon also described for a similar host defense peptide family, called defensins (7). A likely mode of action, formation of pores in the cell membranes, is discussed.

The spectacular biological and chemical stable structure of the cyclotides and a possible new mode of cytotoxic action, represent an interesting starting point in the design of new anticancer leads.

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