Other *in vitro* effects of nettle root

Immunomodulatory effects

Increased lymphocyte proliferation was demonstrated for isolated nettle root polysaccharides as well as for aqueous lyophilized extracts, and a methanolic and an ethanolic nettle root extract (Wagner et al., 1989, Koch, 1995). Likewise, pure *Urtica dioica* agglutinin (UDA) affected lymphocyte proliferation in a dose-dependent manner (Wagner et al., 1994). UDA is a complex mixture of at least 11 isolectins that differ with respect to their amino acid composition (Van Damme et al., 1988). The gene for nettle lectin encodes both a lectin (chitin-binding domain) and a chitinase domain (Lerner and Raikhel, 1992). Sixteen different genes were identified which encode seven putative UDA-isolectins. In extracts of nettle root six isolectins were detected by mass spectrometry (Does et al., 1999a). Capillary electrophoresis has recently been described as a new method to quantify the individual isolectins (Ganzera et al., 2005). The impact of extracted UDA as mitogen for murine thymocytes and spleen T lymphocytes was first demonstrated by LeMoal and Truffa-Bachi (1988). The late T lymphocyte proliferation induced by UDA correlated well with (i) the observed late interleukin-2 production and interleukin-2 receptor expression, and (ii) the long-lasting cyclosporin A-sensitive early activation period. A reduction in prostaglandin synthesis is probably involved in T cell activation by nettle lectins (Aussel et al., 1987). UDA and its subfractions increased mitosis dose-dependently in the lymphocyte transformation test (Wagner et al., 1989). In contrast UDA isolated from freshly harvested roots showed initially a strong suppression of T lymphocyte proliferation. Using a cytofluorimetric analysis Le Moal and others (1992a) showed that UDA bound uniformly to all T lymphocytes, but might activate only a subpopulation of T cells. An UDA-sensitive and an UDA-refractory subset among splenic mouse T lymphocytes was identified. Compared to Con A, the kinetics of proliferation was delayed for UDA. Likewise, its transcription of the genes encoding different cytokines was delayed, in particular that of IL-2, IL-3 and IFNγ gene expressions. These

particular kinetics corresponded to an unusually high level of IL-3 and IFNγ and a low level of IL-4 and IL-5 gene transcripts (LeMoal et al., 1992b). Likewise, interferon was dose-dependently released by UDA from human lymphocytes (Willer, 1992). Willer (1992) also found a significant induction of TNFα release by a particular polysaccharide. It has been suggested that UDA fulfills the criteria for a superantigen (Galelli and Truffa-Bachi, 1993). Detailed kinetic analysis of the functional status of Vß8 T cells in UDA-treated mice indicated that a lectin that activates T cells in a Bß-specific manner can induce *in vivo* tolerance in peripheral T cells by the induction of specific clonal deletion and anergy of responding cells (Galelli et al., 1995). However, the mechanisms responsible for these processes are not yet clear. The demonstration that a single injection of UDA is efficient at causing a deletion of UDA-reactive cells may indicate that such treatment represents a useful model for studying the mechanisms in superantigen-induced deletion of peripheral T cells in diseases caused by T cells of known Vß type. UDA injection resulted in the deletion of a large fraction of Vß8.3-bearing mature T-cells without affecting the Vß8.3-bearing immature thymocytes. In contrast to classical superantigens, a thymic atrophy was not observed with UDA (Delcourt et al., 1996). Musette and others (1996a) used the Vß8.3-specific superantigenic lectin UDA to delete the Vß8.3+T cells in MRL 1pr/1pr mice. UDA-treated animals did not develop overt clinical signs of lupus or nephritis. There is evidence that UDA may alter the production of autoantibodies in a sex-dependent manner. In another study Musette and others (1996b) showed that the Jß segment of the T cell receptor-ß chain, but not the CDR3 region, participated in superantigen binding, presumably by influencing the quaternary structure of the T cell receptor-ß chain. The interaction between UDA and MCH-I molecules may demonstrate their implication in lectin-binding (Rovira et al., 1999). It seems likely, that the UDA superantigenic properties may arise from the simultaneous fixation of glycans on TCR and MHC molecules of T cells and antigen-presenting cells, respectively (Saul et al., 2000). The well defined spacing between the two binding sites of UDA may be a

key factor in determining the specificity for Vß8.3+T lymphocytes. UDA was shown to be a potent inducer of gelatinase B. Since high circulating gelatinase B levels are associated with specific pathologies including shock syndromes, UDA toxicity may be partially mediated or influenced by gelatinase induction (Dubois et al., 1998). UDA and interleukin-2, the *in vitro* production of which is enhanced by this lectin, exhibited obvious preference for hyperplastic prostate cells of surgical specimens. Future studies need to prove the clinical relevance of this observation (Kayser et al., 1995).

Uptake studies with oral radioactively labelled UDA were performed in mice. Radioactivity was detected in the blood (9%), stomach (22%), gut (18%), liver (15%) and kidney (24%) (Geiger et al., 1996). Unmetabolized UDA was found in urine and faeces. Another study evaluated the oral uptake and excretion of 20 mg purified UDA in volunteers and patients by using a sensitive and reliable ELISA test. 30 to 50% of the lectin was excreted in the faeces, whereas less than 1% of the total amount of UDA was found in urine. The data confirm the extreme stability of UDA in the digestive tract and its partial uptake and renal clearance (Samtleben et al., 1996).

Antiviral effect

UDA inhibited cytopathicity induced by human immunodeficiency viruses types 1 and 2, cytomegaly virus, respiratory synthytial virus and influenza A virus. N-acetylglucosamine-specific UDA was a potent inhibitor of syncytium formation between persistently HIV-1 and 2-infected HUT-78 cells and CD4+ Molt/clone 8) cells. The plant lectins did not interfere with HIV-1 adsorption to MT-4 cells and RSV- and influenza virus adsorption to HeLa and MDCK cells, respectively (Balzarini et al., 1992). UDA may represent the prototype of a new conceptual class of carbohydrate-binding agents with unusually specific and targeted drug resistance profile. It forces HIV to escape drug pressure by deleting the indispensable glycans on its GP120, thereby obligatorily exposing previously hidden immunogenic epitopes on its

envelope (Balzarini et al., 2005). Acute infection and cell-to-cell transmission of feline immunodeficiency virus were assayed by the syncytia formation assay and inhibited by aqueous nettle root extract. High extract concentrations were associated with cytotoxic effects (Uncini Manganelli et al., 2005). Although a 50% ethanolic nettle root extract showed no inhibitory effect against HIV-1-induced infections in MT-2 cells (Bedoya et al., 2002), *Urtica dioica* agglutinin potently inhibited dendritic cell human immunodeficiency virus type 1 infection and dendritic-cell-directed HIV-1 transfer (Turville et al 2005).

Fungistatic effect

The growth of several phytopathogenic and saprophytic chitin-containing fungi (*Botrytis cinerea*, *Collectotrichum lindemuthanium*, *Phoma betae*, *Phycomyces blakesleeanus*, *Septoria nodorum*, *Trichoderma hamatum* and *viride*) was inhibited by UDA in vitro. Chitin-negative *Phytophthora erythroseptica* was insensitive to UDA. UDA potentiated the antifungal activity of chitinases, which are an ubiquitous class of antifungal plant proteins. The nettle lectin may, thus be a promising candidate for application in the genetic engeneering of disease-resistant crops (Broekaert et al., 1989). In-vitro antifungal assays on germinated spores of various fungi revealed that growth inhibition occurred at a specific phase of fungal growth and was temporal, suggesting that the fungi had an adaptation mechanism (Does et al., 1999b).

Chemopreventive effect

The HL-60 cell line was established from a patient with acute myeloid leukemia. In culture, it can be induced to differentiate terminally into granulocyte- or monocyte/macrophage-like cells by various substances. An ethyl acetate extract[1] of nettle root had an ED_{50} value of 4 µg/ml and belonged to 17 of 398 tested crude extracts with chemopreventive potential (Suh et al., 1995). Willer (1992) suggested that a particular polysaccharide may contribute to the chemopreventive effect. Aqueous nettle extract resulted in significant inhibition on adenosine

deaminase activity in prostate cancer cells which may explain the beneficial effect of nettle root in prostate cancer (Durak et al., 2004).

[1]100 g was soaked with 400 ml (2 x 200 ml) of methanol overnight. Each methanol-soluble fraction was filtered and concentrated to about 100 ml. Then 5-10 ml water and 200 ml hexane s were added and shaken. When the mixture separated into two layers, the lower methanolic layer was removed and 30 ml water added. Ethyl acetate was then added (1:1) and shaken. The ethyl acetate fraction (lower layer) was separated from the mixture, dried and used for biological tests.

Cardiovascular activity

Aqueous and methanolic nettle root extracts[2] and purified fractions produced hypotensive responses probably through a vasorelaxing effect mediated by the release of endothelial nitric oxide, the opening of potassium channels and, through a negative inotropic action (Testai et al., 2002).

[2]800 g fresh root were extracted by 6 l of water and the whole crude extract after lyophilization was suspended in methanol 3 l at room temperature. The insoluble fraction was filtered off and the fraction soluble in methanol, gave after evaporation of the solvent in vacuum, 15 g dried crude extract which was further fractionated by chromatography

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