A123 Tumor cell adhesion to laminin is inhibited by antimetastatic sulfated polysaccharides

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Tumor cell metastasis requires the interaction of tumor cells with components of the basement membrane. One of these is the glycoprotein laminin, which is involved in a number of steps of tumor cell invasion and metastasis. Cancer cell lines, which are highly metastatic in mice, were shown to exhibit an enhanced attachment to laminin, spreading on it and migration toward it. Antibodies to laminin inhibited tumor cell metastasis.

Heparin was shown to prolong the survival time of tumor patients. According to manifold *in vitro* and animal experiments, it influences manifold processes involved in tumor metastasis. The aim of the presented study was to examine whether heparin also affects the laminin-dependent steps of metastasis. Further, the activity of partial synthetically sulfated polysaccharides (SP) from non-animal resources should be investigated as a potential alternative to the animal-derived heparin.

To investigate the effects of the test compounds on the tumor cell adhesion, a microplate coated with laminin was incu-bated with the metastatic breast cancer cell line MDA-MB231 in presence or absence of increasing concentrations of the test compounds. After washing, the adhering cells were quantified by means of their lactate dehydrogenase activity.

In contrast to heparin (IC_{50} > 100 µg/ml) and non-sulfated polysaccharides, SP dose-dependently inhibit the adhesion of MDA-MB231 cancer cells to laminin. An essential prerequisite for any effect are sulfate groups covalently bound to the polysaccharide. The inhibitory activity of the SP increases with increasing molecular weight (MW) and degree of sulfa-tion (DS). In addition β-1,3-glucan sulfates are significantly more active than α-1,4/1,6-glucan sulfates, if their MW is lower than 60 kD and their DS lower than 1.0. At high DS (e.g. 1.26) and MW (e.g. 240 kD), however, the α–1,4/1,6- are as active as the β-1,3- glucan sulfates (IC_{50} 0.5 µg/ml). The inhibitory activity of the SP can be neutralized by protamin sulfate. Mechanistic studies suggest that the SP interact with laminin and not with the laminin receptor structures.

Since these algae- and fungi-derived SP are also superior to heparin in other test systems recording interference with tumor metastasis, they represent interesting candidates for the development of new antimetastatic drugs for an adjuvant tumor therapy.

A124 Differentiation between the complement modulating effects of an arabinogalactan-protein from *Echinacea purpurea* and heparin

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Due to the important physiological role of the complement system, complement modulation, either inhibition or stimulation, is an interesting target for drug development (1, 2). Several plant polysaccharides are known to exhibit complement modulating activities. Sometimes these effects are described as complement inhibition, although the basic mechanism is a stimulation of the complement activation. This misinterpretation is due to the observed reduced haemolysis in the widely used haemolytic complement assay, which does not allow to differentiate between complement activators and inhibitors, when it is performed in the usual manner.

The aim of the presented study was to distinguish between real complement inhibitors and complement activating com-pounds by simple modifications of the usual procedure this assay without performing expensive, molecular mechanistic investigations. As practical examples heparin with proven complement inhibiting activity (3) and AGP, a new arabino-galactan-protein type II isolated from pressed juice of the aerial parts of *Echinacea purpure*a (4), as a potential comple-ment activating compound were included in the study.

By means of varying the preincubation time of the test compound with complement, AGP was clearly identified as a stimulator on both the classical and alternative pathway of complement activation. These findings correspond to the results of molecular mechanistic investigations. Selective removal of the arabinose side chains of AGP resulted in considerably reduced activity. Therefore, the three-dimensional structure of the polysaccharide, i.e. a backbone branched by side chains, is supposed to be important for the interactions with the complement system. The complement activating effects of AGP may contribute to the well-established immunostimulating effects of the pressed juice from *Echinacea purpurea*.

References: 1. Marsh, J.E. et al. (1999) Curr. Opin. Nephrol. Hypertens. 8: 557-562. **2.** Figueroa, J.E. et al. (1991) Clin. Microbio. Rev. 4: 359-395. **3.** Edens, R.E. et al. (1993) Complement Profiles. Karger. Basel. **4.** Classen, B. et al. (2000) Carbohyd. Res. 327: 497-504.