T 01

Novel inhibitors of tumor growth and angiogenesis from human urine

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Angiogenesis, the formation of new capillaries, is critical for the growth of solid, malignant tumors and other diseases associated with pathological angiogenesis including hemangiomas and retinopathy of prematurity. Recognition of the potential therapeutic benefits of controlling pathological angiogenesis has lead to the search for angiogenesis inhibitors.

We have screened human urine for the presence of small-molecular weight substances with antiangiogenic activities. We found several substances with such properties, two of which were identified as genistein and 2-methoxyestradiol. Genistein is present in high quantities in certain diets, 2-methoxyestradiol is an endogenous estrogen metabolite with negligible estrogen activities and previously unknown function.

Genistein (1) and 2-methoxyestradiol (2) inhibit in a potent manner the proliferation and migration of capillary endothelial cells and they inhibit angiogenesis and the growth of malignant tumors *in vivo*. Though both substances also inhibit the proliferation of tumor cells (2,3), they exert major inhibitory activities towards growth of tumor capillaries (2,3). Both substances have negligible side effects *in vivo*, suggesting that they could be used for the treatment of neovascular diseases, including solid malignant tumors.

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T 02

ι - AND λ - CARRAGEENAN AS NEW ANGIOGENESIS INHIBITORS

H. Vogl, R. Hoffman, D.H. Paper and G. Franz

Several sulphated carbohydrates like pentosanpolysulphate inhibit angiogenesis because they are able to interact with growth factors. However, the usage as angiogenesis inhibitors is limited due to their anticoagulant activity and pharmacokinetic problems. In order to overcome these difficulties a variety of sulphated carbohydrates has been screened in growth factor binding assays and on FBHE-cells in previous studies. [1]

Carrageenans, polysulphated galactans, show low anticoagulant activity, but are potent selective growth factor antagonists in vitro, which inhibit the bFGF-induced DNA-synthesis of FBHE-cells.

The in vivo (CAM-assay) antiangiogenic effect was demonstrated as well. κ- and θ-carrageenan show only a weak activity on FBHE-cells and in the CAM-assay, whereas ι- and λ-carrageenan revealed an excellent effect in vitro and in vivo. It is noteworthy that λ-carrageenan is a stronger angiogenesis inhibitor than ι-carrageenan however the sequence in vitro is in reverse order.

An explanation for this observation could be related to pharmacokinetic problems, the better anticoagulant properties of λ-carrageenan and/or that the mechanism of this specific effect is not only due to growth factor antagonism.

This study also confirms that ι- and λ-carrageenan are better angiogenesis inhibitors than suramin.

Literature:

1. Hoffman R (1993) Biochem J 289: 331

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T 03

PRODUCTION OF LOW MOLECULAR WEIGHT ι-CARRAGEENANS AND THEIR EFFECTS ON BFGF AND ON FBHE-CELLS

D.H. Paper, R. Hoffman, M. Marchesan, G. Franz

ι-Carrageenan, a heterogenous, sulphated red algae-polysaccharide, is a selective and potent growth factor antagonist in vitro 1) and a strong inhibitor of angiogenesis in the CAM-assay as well.

Due to its high molecular weight (about 250 kD) it bears an allergenic potential and it precipitates with blood proteins. With low molecular weight ι-carrageenans (LMWIC) it is possible to circumvent these problems.

LMWICs were obtained by lyophilisation-induced cleavage of ι-carrageenan . Fractionation of the hydrolysates by gel permeation and ion exchange chromatography resulted in fractions with a small molecular weight distribution and an average molecular weight between 1.8 kD to 78 kD.

LMWICs were tested in a bFGF-binding assay and on FBHE-cells. These derivatives don't form precipitates with blood proteins, but they show a drastic reduction of inhibition of bFGF binding and of the inhibition of bFGF-induced DNA-synthesis compared to the parent compound.

The specific activity most likely depends on the high molecular weight and/or the three-dimensional structure of ι-carrageenan. In some fractions the minimal loss of sulphate can be responsible for the reduction of activity.

As a consequence LMWICs are not useful as angiogenesis inhibitors, but they are suitable for the fine structure-analysis of ι-carrageenan.

Literature:

1. Hoffman R. (1993) Biochem J 289:331

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T 04

A NOVEL ACCESS TO SULPHATED OLIGOSACCHARIDES WITH POTENTIAL ANTIANGIOGENIC EFFECT

G. Florian, D. H. Paper, A. Garna and G. Franz

In recent years it has been recognized that some sulphated oligosaccharides can play an important role in the inhibition of angiogenesis. They show many specific physiological effects (e.g. influence on the activity / binding of growth factors) and are therefore interesting leads for the treatment of cancer.

Sulphated oligosaccharides derived from natural sources always form very complex mixtures where the components only differ slightly in their structural features. It therefore takes much time to isolate and purify a well-defined sulphated oligosaccharide in larger quantities with the consequence that modifying well-defined oligosaccharides by sulphation seems to be an easier way.

A new and rapid method was developed for the large scale production of 2-desoxysugar containing oligosaccharides. The three steps of this method are the isolation of glycosides containing oligosaccharides, followed by the mild hydrolysis of the glycosides and the purification of the released oligosaccharides on silica gel. The efficiency of this approach is demonstrated by the isolation, purification and structure elucidation of the trisaccharide β-D-glucopyranosyl-(1→4)-3-O-methyl-6-desoxy-β-D-allopyranosyl-(1→4)-α,β-D-oleandropyranoside derived from the bark of Marsdenia condurango Reichb.f., Asclepiadaceae.

With this method it is possible to produce sufficient oligosaccharides for a subsequent sulphation.

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