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# Influence of red algal polysaccharides on neutrophils activation, cytokine synthesis and intestinal epithelial cells *in vitro*

*The study aim was to investigate in vitro immunomodulatory properties of carrageenans with various structural types. The data revealed that carrageenans alone did not affect the neutrophils' size and granularity but stimulated the synthesis of cytokines in blood in vitro. Carrageenans activated neutrophils with much less potency than LPS. Carrageenans especially with low contents of sulphate groups were able to interfere with LPS in vitro resulting in reducing neutrophils activation.*

*Key words: carrageenans, neutrophils, cytokines, echinochromes*

Red algal sulphated polysaccharides - carrageenans - are widely used in food industry due to their physical and chemical properties; however they also have numerous biological activities. The following carrageenan structural types isolated from red algae collected from the Pacific coast were used in the study: κ-, κ/β-, ι/κ-, and λ-types. The study aim was to investigate *in vitro* immunomodulatory properties of carrageenans with various structural types. These properties of algal polysaccharides alone and in combination with lipopolysaccharide (LPS) were investigated by means of changes in neutrophils size and granularity and the synthesis of cytokines in blood *in vitro*. The influence of carrageenans on neutrophils' size and granularity correlating with the activation degree of these cells were measured by flow cytometry.

The data revealed that carrageenans alone did not affect the former but increased the latter. Further, a combined action of these polysaccharides with *E.coli* LPS illustrated an inhibitory effect of carrageenans on the neutrophils' size. The level of granularity induced by LPS alone reserved unaltered by the addition of the polysaccharides. Carrageenans activated neutrophils with much less potency than LPS. Carrageenans especially with low contents of sulphate groups were able to interfere with LPS *in vitro* resulting in reducing neutrophils activation.

Carrageenans are also known as potent immunomodulators with remarked ability to cytokine synthesis [5]. IL-4 promotes T helper cell type 2 (Th2) differentiation and stability and inhibits Th1-cell differentiation. All structural types of carrageenans showed decreased IL-4 production in blood *in vitro* that is correlated with the literature data reported by Tsuji and co-authors [4]. The addition of LPS to the reaction medium did not result in significant changes.

The interaction between carrageenans and the gastrointestinal tract starts with the frontline host defence coating the gastrointestinal tract—a layer of mucus composed mostly of mucins

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secreted by intestinal goblet cells. HT-29 is a colorectal cancer cell line used as an *in vitro* model for the intestinal epithelium because it retains many biochemical and physiologic features of the lower small intestine and ascending colon and can produce higher levels of mucins than other cell lines [3]. Our purpose in the study described herewas to determine the protective action of carrageenans, alone and in combination with LPS, on survival of human epithelial monolayers of HT-29 cells treated with ethanol. All of the carrageenans investigated were inert in response to intestinal epithelial HT-29 cells under normal conditions, and the detrimental effect of ethanol on the state of these cells provided an opportunity to assay the protective properties of red algal polysaccharides. Low sulphate content and the presence of the 3,6-anhydogalactose in κ/β-carrageenan were prerequisites for restoration of human intestinal epithelial HT-29 cells by carrageenans.

Due to its high ability to adsorb water, carrageenan can improve drug dissolution and thus increase the oral bioavailability poorly water soluble drugs. Echinochrome (Ech) is the water insoluble active substance in the cardioprotective drug Histochrome®, produced in Russia. Carrageenans matrixes had been used to incorporate of Ech in order to study the effect of the polysaccharide on the Ech properties [6]. The effect of Ech alone and in carrageenans complexes on HT-29 cells treated with Ethanol was investigated. Ech also possessed an ability to restore HT-29 cells after exposure to ethanol and its activity retained in complexes with κ/β-carrageenan.

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