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Review

Antitumor potential of carrageenans from marine red algae

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ABSTRACT

Marine algae are abundant and inexhaustive sources of the bioactive compounds with the various benefits for human health. Among these substances an attention is given to the sulfated polysaccharides presented as the complexes of polymer macromolecules with excellent biological features including antioxidant, anti-inflammatory anticoagulant, antiviral, and immunomodulatory activities. In addition to the aforementioned properties there is a growing number of research results suggesting the bioactive sulfated polysaccharides such as carrageenan, fucoidan, laminarin, and others exert anticancer and antimetastatic properties. The present review contains the main results of experimental studies of the carrageenan anticancer activity including systemic and intracellular mechanisms of the antiproliferative influence. Relationships between structure, physicochemical properties of carrageenan and their antitumor effects are described. There are data on the toxicology and pharmacokinetics of carrageenans as well as other aspects of their pharmacotherapeutic and pharmacoprophylactic influence that allow considering them as the potential anticancer agents.

1. Introduction

Marine macrophytes are known as a source of valuable and structurally variable biologically active compounds including polysaccharides, fatty acids, bioactive peptides, vitamins, minerals, antioxidants and a plenty of others. Many of these substances have unique chemical structure and properties that have been never found in the terrestrial species (Alves et al., 2018; Sharma, Koneri, & Jha, 2019). Basic polysaccharides of non-animal origin that were found in the marine algae include fucoidans, alginates, and laminarans of brown algae (Phaeophyceae), agar and carrageenans from red algae (Rhodophyta) and ulvans of green algae (Chlorophyta) (Lahaye & Robic, 2007). All of them are commonly used today in textile, food, cosmetic, and pharmaceutical industries as dietary and food supplements, emulsifiers, stabilizers, and thickeners (Bajpai, Rather, Lim, & Park, 2014). Within the recent decade, polysaccharides from marine algae had got an attention of many researchers because of the new beneficial properties that were discovered among their various biological activities allowing them to be considered as a base for creation of novel dietary supplements, nutraceutics, and functional food products as well as pharmaceutical substances purposed for the use as preventive and therapeutic agents (Torres, Flórez-Fernández, & Domínguez, 2019). Among the marine polysaccharides, carrageenans were found to exert various beneficial effects due to the variabilities of their structure and physical properties. The most studied effects exerted by the marine carrageenans include antiviral, immune stimulating, antioxidant, antiplatelet, and anticoagulant activities (Cosenza, Navarro, Pujol, Damonte, & Stortz, 2015; dos Santos-Fidencio, Goncalves, Noseda, Duarte, & Ducatti, 2019; Tang, Chen, & Li, 2013; Yermak et al., 2012). Some beneficial properties of the polysaccharides that are still less investigated relate to their capacity to effectively inhibit proliferation and formation of the cancer cell colonies under *in vitro* conditions and suppress the growth of malignancies and metastasis rate in experimental animals *in vivo*. These activities today had got more interest of the researchers as cancer is thought to be the one of the most severe pathologies posing a real threat to human health and life. Unfortunately, almost all pharmaceuticals used for the anticancer therapy are usually toxic and generally affect not only tumor cells but the normal healthy cells too. Therefore, the search for the novel effective and non-toxic compounds in natural sources is an important task of the modern pharmacology and biotechnology.

Based on the results of the numerous toxicological studies that were

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carried out in rats, guinea pigs, hamsters and monkeys, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001) have made a conclusion that carrageenans are safe for the diet ration and their daily consumption is neither regulated nor limited. Therefore, they are now commonly used in the food industry for improvement of the food texture as well as the gel-forming, stabilizing and thickening agents. Today, carrageenans may be found in soymilk, ice cream, yogurt, meat products and even in tooth paste in concentration varying from 0.05 to 1% (McKim, 2014; Weiner, 2014). At the same time, within the last two decades starting from the publication of the review article authored by Tobacman (2001) the discussion about the negative consequences of the food carrageenan consumption have not been discontinuing. This polysaccharide was shown in experiments with various animal models, first of all, in rodents to induce mucus lesions in colon. A major part of the researchers believes that degraded carrageenans exert pro-inflammatory effect and may induce neoplasia in experimental animals (Prajapati, Maheriya, Jani, & Solanki, 2014). This resulted in the Food and Drug Administration (FDA) to issue the regulating title imposing the molecular weight of the food carrageenans must be no less than 100 kDa. It should be emphasized that there is a lot of research publications showing no evidence of the oncogenic properties of carrageenans.

The present review contains the results of the deep analysis of the literature sources devoted to the investigation of the antitumor properties of natural and modified carrageenans and described mechanisms of their antiproliferative activity. In addition, it contains some aspects concerning the perspectives for creation of the new pharmaceuticals for a antitumor therapy with the use of the carrageenan polysaccharides.

2. Structure and physico-chemical properties of carrageenans

Carrageenan is a general term describing a group of sulfated polysaccharides contained by marine red algae where they present the main components of the cell walls and interstitial spaces functioning as the structuring compounds and providing intercellular adhesion and intercellular signaling. These natural polysaccharides are actually a mix of sulfated linear galactans and their structural units are presented with the disaccharides of α-(1→4)-linked D-galactopyranose (D) residue or 3,6-anhydrogalactopyranose (DA) and β-(1→3)-linked D-galactopyranose (G) residue. Sulfate groups are covalently linked *via* ether bonds to the carbohydrate atoms C-2, C-4 or C-6 of galactose. The number of $–O\text{-}SO3^-$ groups in the sulfated polysaccharides may be substantially high and varies from 0 to 41 % (w/w) resulting in dramatically negative charge of the polysaccharides. Galactans in the red algae make up to 40–50 % of their dry weight (Campo, Kawano, da Silva, & Carvalho, 2009; Yermak & Khotimchenko, 2003). Among 4000 species of the red algae inhabiting the world ocean carrageenanophytes were found in the families Solieriaceae, Rhabdoniaceae, Phyllophoraceae, Gigartinaceae, Rhodophilidaceae, and Thichocarpaceae. Only in Japanese sea (East sea) eight sources of carrageenans were discovered and five of them (*Chondrus pinnulatus*, *C. armatus*, *C. yendoi*, *Mastocarpus pacificus*, *Mazzaella hemisphaerica*) belong to the Gigartinaceae and Solieriaceae (Yermak, Kim, Titlynov, Isakov, & Solov'eva, 1999). Beside of Gigartinaceae, high carrageenan contents were found in the algae of the families Phillophoraceae and Thichocarpaceae, species of which are widely spread in all far eastern seas.

In accordance to the structural peculiarities of the repeating disaccharide units, six main types of carrageenans are determined and designated by the Greek alphabet letters as follows: kappa (κ-), lambda (λ-), iota (ι-), nu (ν-), mu (μ-), and teta (θ-) (see Fig. 1). Commercially important types of these polysaccharides are κ-, λ- and ι-carrageenans. Totally 20 idealized carrageenan types were determined differing by the presence of the 1,4-linked residues of 3,6-anhydrogalactose as well as location and number of the sulfate groups in the disaccharide unit. For the designation of the hybrid polysaccharide structures and their corresponding oligosaccharide fragments the letter code was proposed by Knutsen, Myslabodski, Larsen, and Usov (1994). Lettering designation of the main dimer structure of κ-carrageenan looks like G4S-DA, of ι-carrageenan - G4S-DA2S, and of λ-carrageenan – G2S-D2S, 6S (Table 1).

The carrageenans precursors found in the mature algae are μ- and ν-carrageenans, which are then converted into κ- and ι-carrageenans, respectively, through alkaline modification. μ-, ν- and λ-carrrageenans with the use of enzyme and alkaline modification may be converted into κ-, ι- and θ-polysaccharides through the formation of the 3,6-anhydrobridge of α-galactose-6-sulfate (Campo et al., 2009). Generally, κ-carrageenan for commercial purposes is obtained from *Kappaphycus alvarezii* using the hot extraction processing whereas λ-carrageenan is commonly isolated from red algae belonging to the genera *Gigartina* or *Chondrus* with the use of drum dryer procedure or ethanol precipitation (Vera, Castro, González, & Moenne, 2011). The commercial grade ι-carrageenan is derived from *Eucheuma denticulatum* with the use of repeating processes of either freezing-thawing or gelling (Necas & Bartosikova, 2013). The highest output of carrageenan may reach 70 % (dry base) from such species as *Betaphycus gelatinum*, *K. alvarezii*, or *Kappaphycus striatus*. Other red algal genera were found to provide carrageenan output approximately 30 %. Sulfate contents in carrageenans varies from 20 % in κ-carrageenan to 33 % in ι-carrageenan and up to 41 % in λ-carrageenan (Ghanbarzadeh, Golmoradizadeh, & Homaei, 2018).

Chemical structure of carrageenans is basically determined by the stepped biosynthesis process that depends on the ambient conditions, physiology and age of alga, its genus and stage of development. Algae of the same specie were found to produce different types of carrageenans within the different stages of their lifecycle (Falshaw & Furneaux, 1994; Neill, Nelson, Hurd, & Falshaw, 2018). Quantitative and qualitative characteristics of the polysaccharides depend on the ambient factors in the area of their habitat, environmental conditions during their harvesting. They also may be altered during the extraction process (Azevedo, Torres, Sousa-Pinto, & Hilliou, 2015).

Natural carrageenans quite rarely correspond to the regular structural patterns. Each species of carrageenanophytes may contain several types of carrageenans. Even within the length of one polysaccharide chain the repeating units of several different types are often present that may be explained by the step-like processes of the polysaccharide biosynthesis. During the biosynthetic process a combination of various idealized carrabiose units may be formed and distributed along the polymer chain resulting in formation of the complex hydrid structures. It is supposed that the first biosynthetic step is the formation of a main chain composed of the galactose residues. The second step of biosynthesis includes sulfation of the galactose residues and addition of other substitutes. The final step describes enzyme elimination of sulfates at C-6 of the 1,4-linked α-galactose with formation of the 3,6-anhydrocycle.

Biosynthesis of carrageenan may discontinue at any phase; therefore, the final product may be the polysaccharide with either regular or irregular structure. That is why this is very difficult to determine whether the isolated carrageenans present a mix of the separated carrageenan types or they are the polysaccharides which polymer chains are made of disaccharide units of carrageenans with different structural patterns and have the blocks of hybrid structure. The use of specific enzymes termed carrageenases in the structural analysis of carrageenans have demonstrated that mostly natural carrageenans typically have hydrid structures (Anastyuk et al., 2011; Falshaw et al., 1996; Kravchenko et al., 2016; Van de Velde, Peppelman, Rollema, & Tromp, 2001). Polysaccharides isolated from industrial algae *Sarcothalia crispata, Mazzaella laminarioides* and *Chondrus crispus* were shown to have a hydrid structure of κ/ι-carrageenans (Van de Velde et al., 2001). Highly sulfated polysaccharide with complex structure from *Melanema dumosum* inhabiting Australian shores was identified as a hydrid of ι/κ/β-carrageenan (Chiovitti et al., 1995). Polysaccharides from five algal species of Dicranemataceae family were investigated and the results showed that all of them have hydrid structure of κ/β-carrageenans

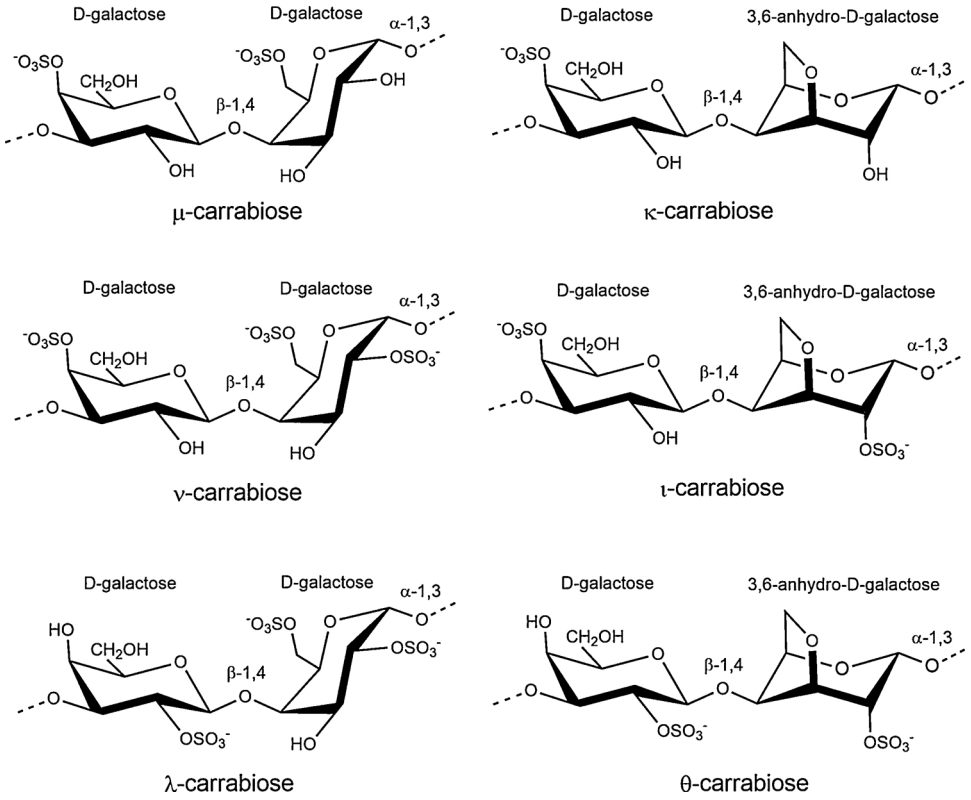

Fig. 1. Disaccharide units of the most common carrageenan types are differing by the presence of 1,4-linked residues in the form of 3,6-anhydrogalactose, location sites and the number of sulfate groups.

Table 1
Letter codes for indication of the carrageenan types.

Lettering code	Full name in accordance to the nomenclature approved by the International Union of Pure and Applied Chemistry (IUPAC)
D	4-α-D-galactopyranosyl
DA	4 – 3,6-anhydro-D- galactopyranosyl
G	3-β-D- galactopyranosyl
S	Sulfate ether (SO_3^-)
M	O-methyl
P	4,6-O (1-carboxyethyliden)

(Liao, Kraft, Munro, & Craik, 1993). Among the galactans with hydrid structure the one polysaccharide from *Mastocarpus stellatus* should be noted as it presents κ/ι-carrageenan with insignificant amounts of its biosynthetic precursors – μ- and ν-carrageenans (Hilliou et al., 2012). Marine alga *Stenogramma interruptum* with cystocarps produces gelling ι/α-carrageenan (Cáceres, Carlucci, Damonte, Matsuhiro, & Zuñiga, 2000).

Some carrageenans may contain not only sulfate groups but also some other substitutes. Small amounts of 6-*O*-methyl-D-galactose were found in the κ-carrageenan from *K. alvarezii* (Goes & Reis, 2012). In many carrageenans xylose residues are found but their location sites are still not determined (Liao et al., 1996). Based on the NMR spectroscopy results it was discovered that the location of the xylose residue in the atypical polysaccharide from *Phacelocarpus peperocarpos* is C-3 4-α-D-galactose whereas in ι-carrageenan from *Eucheuma denticulatum* this monosaccharide is attached to the C-6 3-β-D-galactose (Van de Velde et al., 2001). Carrageenans with the high pyruvate contents were isolated from the alga of the *Callophycus* (Solieriaceae) genus. Pyruvate forms cyclic acetals in the C-4 and C-6 galactose residues frequently occurring in disaccharide units of α- and θ-carrageenans (Chiovitti et al., 1998). In many algal species belonging to the families of Cryptonemiales, Rhodymeniales, and Bonnemaisoniales the sulfated galactans having D/L-hydrid structure *i.e.* containing agar were discovered. In algae of the Gigartinales family known as classical source of carrageenans similar hydrids are found just in small amounts (Miller & Blunt, 2000).

These data show the evidence of the high variability of the carrageenan structure depending on the source type, its age and area of habitat and environmental conditions as well as on the methods of isolation. These structural peculiarities determine the types and strength of biological activities exerted by carrageenans.

3. Structure–activity relationships of carrageenans

Carrageenans are found to be used in various fields of industry due to their capacity to form gels and increase viscosity of aqueous solutions. Besides, carrageenans were found to exert a wide spectrum of biological activities due to the heterogeneity of their molecular structure. Relationships between chemical structure and physical properties of sulfated galactans are complicated because of the polydispersity of the polysaccharides and their tendency to self-association. Similar to other polysaccharides, carrageenans have typical molecular-mass distribution. Majority of them have molecular weight varying from 500 to 1000 kDa, however they may contain up to 25 % of the polysaccharides with molecular weight less than 100 kDa (Lascombes et al., 2017). Commercially available food grade samples of carrageenans have average molecular weight within the limits between 200 and 400 kDa (Prajapati et al., 2014), sometimes reaching 800 kDa (Pereira, Critchley, Amado, & Ribeiro-Claro, 2009; Torres et al., 2019).

Carrageenans are generally extracted directly from algae into an aqueous solution with a hot water or a mild alkaline solution, such as potassium or sodium hydroxide. In some cases alkaline aqueous solution or aqueous salt solutions of barium or potassium chloride can be applied as extracting agents under various conditions, temperature and extraction time (Manuhara, Praseptiangga, & Riyanto, 2016). There are also new evidences that the use of subcritical water extraction methods

under various conditions adding different ionic liquids as catalyst to isolate carrageenan polysaccharides is quite favorable. The yields of carrageenans were comparable or even higher than those of the classic aqueous extraction; however, the polysaccharides obtained were characterized by lower values of gel strength and viscosity. (Gereniu, Saravana, & Chun, 2018). In addition to water and alkaline extraction techniques, physicochemical methods of extraction using microwave technology or ultrasound have become increasingly important in recent years. (Sari, Barleany, Lestari, & Mustikawati, 2019).

All carrageenans obtained through these methods are contingently divided into the gel-forming polysaccharides (κ-, θ- and ι-carrageenans) making the gels in the presence of specific cations, and non-gelling ones (ν-, μ- and λ-carrageenans) (Stortz & Cerezo, 1992). Gel-forming capacity of carrageenans is determined by the structure of their main disaccharide unit, in particular, by the presence of 4-linked D-galactose residue in the form of 3,6-anhydroderivatives, number of the sulfate groups per one carrabiose unit as well as their location sites in the molecule. Moreover, gelling properties of the polysaccharides are increasing as the higher is the content of the 3,6-anhydrogalactose in them which residues give stiffness to the polymer chain, provide spiral conformation and specific distribution of sulfate groups with the presence of specific cations (Robal et al., 2017; Torres, Chenlo, & Moreira, 2017). Carrageenans that are able to form gels were found to exert detoxifiying and lipid lowering effects due to their high capacity to trap ions into their molecular structure. As gel-forming capacities of the carrageenans depend not only on their structure but on the concentration, temperature of solution, and the nature of the cations added, investigation of carrageenans in different studies had given controversial results.

Carrageenans may be divided into high molecular or “non-degraded” ones and low molecular or “degraded” carrageenans with an average molecular weight 10 − 20 kDa. Degraded carrageenans may be obtained *via* oxidative degradation (Chen, Yan, Wang, Xu, & Zhang, 2010), irradiation (Relleve et al., 2005), enzyme hydrolysis (Wu, 2012), and mild acid hydrolysis (Yang et al., 2009). Biological properties of carrageenans including their pharmacological activity may depend on their primary structure (structure of the basic disaccharide unit), number and location of the sulfated groups, and degree of the molecular polymerization as well as on conformation of macromolecules in solution and their macromolecular organization.

Numerous structural features were found to contribute to the various bioactivities of carrageenans such as antioxidant, antitumor, immunomodulatory, anti-inflammatory, anticoagulant, antiviral, antibacterial, antifungal, and antihyperlipemic (Diogo, Galdo Novo, González, Ciancia, & Bratanich, 2015; Dhanalakshmi & Jayakumari, 2019; Pangestuti & Kim, 2014; Soares, Fernandes, Silva, Pereira, & Gonçalves, 2016; Yermak & Khotimchenko, 2003). There are no doubts that biological activities of carrageenans as well as of other sulfated polysaccharides are a function of structural features such as the molecular weight, amount and positioning of the sulfate groups, type of neutral sugars attached, and glycosidic branching. And as it was mentioned before it depends of the native carrageenan extraction methods (Jiao, Yu, Zhang, & Ewart, 2011; Kalitnik et al., 2013; Khotimchenko, 2010). Therefore, the chemical modifications of carbohydrates lead to variations in their biological activities (dos Santos-Fidencio et al., 2019; Yuan, Zhang, Li, Lu, & Li, 2005; Yuan, Song, Li, Li, & Liu, 2011).

Comparative investigation of the native dominant ι-carrageenan extracted from *Solieria chordalis* with the relative molecular weight 913 kDa and the low molecular weight fractions (below 20 kDa) had shown, that ι-oligocarrageenans provide the great immune stimulating properties enhancing neutrophil phagocytosis, cytotoxicity by natural killer cells, antibody-dependent cell cytotoxicity, and stimulate the lymphocyte proliferation. The significant impact of the molecular weight of degraded carrageenans on the immunological responses leads to the highest activities usually induced by the lowest molecular weight fractions (Stephanie, Eric, Sophie, Christian, & Yu, 2010). The low sulfate containing κ-carrageenan oligosaccharides expressed no effect on angiogenesis while λ-carrageenan with higher sulfate content showed the highest antiangiogenic activity suggesting the degree of sulfation is a critical structural parameter for the ability of carrageenan oligosaccharides to inhibit angiogenesis (Chen, Yan, Lin, Wang, & Xu, 2007). Besides the degree of sulfation, the position of the sulfate group is essential for the inhibition of angiogenesis. It seems likely that a sulfate group at position 4 of the galactose is important (Paper, Vogl, & Franz, 1995). The antioxidant activities of kappa-carrageenan oligosaccharides could be related to the degree of polymerization, the content of the reduced sugars and sulfate groups, and the structure of reducing terminus. The κ-carrageenan oligosaccharides degraded by different methods were structurally very different and exerted different antioxidant effects. Among all of the hydrolysates, H_2O_2 hydrolysates exhibited much higher antioxidant abilities in all assay systems (Sun et al., 2015). Commercially available λ-carrageenan possesses high anticoagulant activity in comparison to ι- and κ-carrageenans (Silva et al., 2010). This polysaccharide was also shown to be the strongest inhibitor of the collagen-induced platelet aggregation in platelet rich plasma (Sokolova et al., 2014). Reduction of the average molecular weights (Mn) results in reduced activity which is probably caused by the decrease of the degree of sulfation. This activity becomes much weaker if the degree of sulfation is lower than 20 % referring to the loss of an average sulfate group per carrageenan disaccharide unit. The anti-Xa activity of λ-oligocarrageenans increases in accordance to the initial reduction of their chain length, but then, starting from 20 kDa, this activity decreases. While most of the low MW λ-carrageenan derivatives inhibits factors Xa and IIa through their interaction with AT-III, the smallest desulfated λ-oligocarrageenan with molecular weight of 2.77 kDa was identified as a direct thrombin inhibitor (Groult et al., 2019). The antiviral activity of carrageenans is substantially dependent on their molecular weight, and degradation of the carrageenans leads to decreased antiviral activity (Kalitnik et al., 2013). The sulfate content as well as the positioning of the sulfate groups were found to be important for the anti-influenza A virus effects of the carrageenan oligosaccharides (Wang et al., 2012). It was shown that O-acylated carrageenan oligosaccharides with different MW had increased anti-HIV activity by depolymerization and sulfation (Yamada, Ogamo, Saito, Uchiyama, & Nakagawa, 2000). High molecular weight kappa-carrageenans have higher activity against tobacco mosaic virus than that of their low molecular weight derivatives with molecular weight from 1.2–4.3 kDa regardless of the depolymerization method. But, nevertheless, the method of depolymerization had some influence on the antiviral activity. Low molecular weight derivatives of κ- and κ/β carrageenans obtained through mild acid hydrolysis showed higher antiviral activity than the products of the free radical depolymerization. The oligosaccharides prepared by enzymatic degradation of the κ-carrageenase possess the lowest activity (Kalitnik et al., 2013).

These data show the huge impact of the chemical structure of carrageenans on their biological activity. Recently it was shown that different carrageenans demonstrate antitumor effects. Mechanisms of the antitumor and antimetastatic activities of carrageenans are much more complex and thus, they vary depending on the various structural features. Therefore, such carrageenans pose an interest for researchers. Relationships between structure and physico-chemical properties of carrageenans with antitumor activity are discussed in the next chapters of this review.

4. Antiproliferative activities of carrageenans

During analytical investigation of the experimental studies devoted to the anticancer activity of carrageenans, attention was given not only to their antiproliferative, antitumor, and antimetastatic effects, but also to the sources these polysaccharides were isolated from, carrageenan types, their molecular weight, degree of sulfurization, and experimental conditions.

Table 2
Degree of inhibition of the cancer cell proliferation and half-inhibiting carrageenan concentration (IC_{50}) *in vitro*.

Sample	Source	Molecular weight	Cell line	Concentration range	Degree of inhibition/ IC_{50}	Reference
κ-carrageenan	(Yantai Seaweed Industry Co.)	h/m*	tsFT210	not given	no effect	Mou et al. (2003)
κ-oligocarrageenan		1726 Da				
κ-oligocarrageenan						
κ-oligocarrageenan						
λ-carrageenan	(Sigma-Aldrich)	h/m	B16-F10, 4T1	250.0–1000.0 μg/mL	no effect	Luo et al. (2015)
ι-carrageenan	(Sigma-Aldrich)	h/m	HCT116	100.0 – 1000.0 μg/mL	no effect	Raman (2015)
κ-carrageenan	(Sigma-Aldrich)	h/m	HeLa	25.0 – 2500.0 μg/mL	IC_{50} 550.8 ± 7.6 μg/mL	Prasedya et al. (2016)
λ-carrageenan		h/m			IC_{50} 475 ± 12 μg/mL	
κ-carrageenan	*Kappaphycus striatus*	37.7 kDa	BGC	125 μg/mL	Degree of inhibition: 11.3 %	Yuan and Song (2005)
				250 μg/mL	21.6 %	
				500 μg/mL	20.4 %	
			HeLa	125 μg/mL	3.7 %	
				250 μg/mL	6.8 %	
				500 μg/mL	17.5 %	
κ-oligocarrageenan		1.2 kDa	BGC	125 μg/mL	8.1 %	
				250 μg/mL	24.1 %	
				500 μg/mL	54.4 %	
			HeLa	125 μg/mL	17.4 %	
				250 μg/mL	32.2 %	
				500 μg/mL	50.1 %	
κ-carrageenan	*Hypnea musciformis*	PMM** 519.1 kDa	SH-SY5Y	600 μg/mL	Degree of inhibition: ~20 %	Souza et al. (2018)
				1000 μg/mL	~40 %	
κ-carrageenan	*Palisada perforata*	PMM 320 kDa	MCF-7	25 – 1000 μM	IC_{50} ~200 μM	Ghannam et al. (2018)
ι-carrageenan	(formerly *Laurecia*	PMM 560 kDa			IC_{50} ~50 μM	
λ-carrageenan	*papillosa)*	PMM 258 kDa			IC_{50} ~25 μM	
ι-carrageenan	*Kappaphycus alvarezii*	h/m	Caco-2,	62.5 – 2000 μg/mL	IC_{50} > 1000 μg/mL	Ariffin, Yeen, Abidin, Wahab, Ariffin, & Senafi, (2014)
κ-carrageenan	(Sigma)	h/m	HepG2			
ι-carrageenan		h/m				
κ-oligocarrageenan	*Kappaphycus alvarezii*	not given	Caco-2	62.5 – 2000 μg/mL	IC_{50} 500 μg/mL	Ariffin, Yeen, Abidin, Wahab, Ariffin, & Senafi, (2014)
			HepG2		IC_{50} 800 μg/mL	
κ-oligocarrageenan	(Sigma)	not given	Caco-2		IC_{50} 640 μg/mL	
			HepG2		IC_{50} 800 μg/mL	
ι-oligocarrageenan	(Sigma)	not given	Caco-2		no effect	
			HepG2		no effect	
Tamoxifen			Caco-2		IC_{50} 9.2 μg/mL	
			HepG2		IC_{50} 9.0 μg/mL	
κ-carrageenan	*Hypnea muscifomis*	215 kDa	LM2		IC_{50} 0.23 ± 0,02 mg/mL	Calvo et al. (2019)
ι-carrageenan	*Euchema denticulatum* (Sigma-Aldrich)	460 kDa			IC_{50} 0.20 ± 0,04 mg/mL	
λ-carrageenan	*Iridaea undulosa*	619 kDa			> 1 mg/mL	
κ-oligocarrageenan	*Hypnea muscifomis*	Average number of disaccharide units 6.5			IC_{50} 0.38 ± 0,04 mg/mL	
κ-oligocarrageenan	*Hypnea muscifomis*	10.4 kDa			IC_{50} 0.39 ± 0,05 mg/mL	
λ- oligocarrageenan	*Iridaea undulosa*	27.2 kDa			IC_{50} 0,95 ± 0,11 mg/mL	
ι-carrageenan	(Sigma)		HOS	0, 0.5, 1, 1.5, 2 mg/mL	no effect	Jin et al. (2013)
ι- oligocarrageenan	ι-carrageenan (Sigma)	40 kDa			IC_{50} 1.41 ± 0.07 mg/mL (48 h)	
κ-carrageenan	*Kappaphycus alvarezii*	not given	MCF-7	10, 25, 50, 100, 250 μg/mL	IC_{50} 103.2 μg/mL	Suganya, Sanjivkumar, Chandra, Palavesa, & Immanuel, (2016)
			HT-29		IC_{50} 73.87 μg/mL	
			HepG2		IC_{50} 56.71 μg/mL	
			MG-63		IC_{50} 47.85 μg/mL	

Footnote: *h/m – high molecular; **PMM – the peak molar mass.

It was shown experimentally that the high molecular commercially available κ-carrageenan (Yantai Seaweed Industry Co., China) and κ-oligosaccharides with molecular weight 1726 Da and sulfate contents 1.0, 2.1, and 3.8 mol/mol in a disaccharide unit do not exert any antiproliferative activity regarding cancer cells tsFT210 under *in vitro* conditions (Table 2). However, oral administration of all samples of the degraded κ-carrageenans in a dose 100 mg/kg bw within the 15-days period resulted in significantly slowed growth of the Sarcoma 180 tumor with the degree of inhibition 64.6 %, 78.2 %, and 26.9 %, respectively. Degree of inhibition noted with antitumor drug cyclophosphane administered intramuscularly was 85.3 %. Besides, κ-oligocarrageenan with the 2.1 mol/mol sulfate content were found to increase activity of the two antioxidant enzymes catalase and superoxide dismutase. Their simultaneous administration with cyclophosphane prevented reduction of the macrophage phagocytic activity (Mou, Jiang, & Guan, 2003). κ-Oligocarrageenans with an average

molecular weight 681 and 798 Da and a sulfation degree 17.2 % and 21.8 % inhibited the growth of the xenograft S180 tumor in mice in a dose-dependent manner. Given orally in a dose 100 mg/kg bw these oligosaccharides provoked the degree of inhibition 41.5 % and 70.8 %, respectively. Degree of inhibition caused by administration of 50 mg/kg bw of cyclophosphane in that model was 74.5 %. In contrast to the cyclophosphane, the effects of κ-oligocarrageenans were associated with increased liver catalase activity. As it is seen in the experimental results presented, polysaccharides with the higher sulfate contents had more pronounced antitumor activity (Hu, Jiang, Aubree, Boulenguer, & Critchley, 2006). Commercially available λ-carrageenan (Sigma-Aldrich) in a concentration range from 250.0 to 1000.0 μg/mL applied for 24 h did not affect cell viability in mice melanoma line B16-F10 and breast cancer 4T1 in the *in vitro* experiments and slightly decreased it after the longer use (Luo et al., 2015). Thus, it showed light cytotoxicity regarding cancer cells, ι-carrageenan in the concentrations up to 1.0 mg/mL similarly did not demonstrate cytotoxic effects in the colon carcinoma HCT116 cells (Raman, 2015).

High molecular κ- and λ-carrageenans (Sigma-Aldrich) were tested in another *in vitro* study on the cervical cancer cell lines HeLa and human umbilical vein endothelial cells (HUVEC) in concentrations 250, 500, 1000, and 2500 μg/mL. Both carrageenan samples did not affect cell vitality in HUVEC line but inhibited the HeLa cell growth in a dose-dependent manner with the IC_{50} values 550.8 ± 7.6 μg/mL for κ-carrageenan and 475 ± 12 μg/mL for λ-carrageenan within 72 h period. Analysis of the cell cycle profiles had shown that κ-carrageenan induced a slowing of growth of the HeLa cells in the G2/M phase whereas λ-carrageenan did not exert such effect. However, single-frame shooting with the 10 min intervals of the HeLa cells within 72 h have shown that unaffected tumor cells have passed the whole cell cycle in 26 ± 0.67 h whereas the same cells in the λ-carrageenan rich media (1000 μg/mL) completed their cell cycle as an average in 59 ± 4.6 h due to the prolonged G1 phase and G2/M phase. This resulted in the slowed progress of the whole cell cycle. Duration of the cell cycle in the κ-carrageenan rich media was 50.2 ± 2.9 h as it was mentioned mostly due to the prolonged G2/M phase. Moreover, the cells treated with κ-carrageenan were able to divide at least onetime before their death whereas major part of the cells treated with λ-carrageenan could not perform a cell division (Prasedya, Miyake, Kobayashi, & Akihiro Hazama, 2016). It looks like λ-carrageenan potentially possesses stronger inhibiting influence toward the tumor HeLa cells in comparison to κ-carrageenan. The difference of the effects exerted by two carrageenans are probably caused by the difference of their degree of sulfation which is higher in the λ-carrageenan.

Cytotoxic effects of the chemical compounds may be classified on the base of the half-inhibiting concentration (IC_{50}) values. IC_{50} values lower than 100 μg/mL indicate potentially cytotoxic compounds. Substances with the IC_{50} values within the range 100 − 1000 μg/mL is considered as the ones with moderate cytotoxicity whereas compounds with IC_{50} values exceeding 1000 μg/mL are thought to be non-toxic regarding any cells (Ariffin, Yeen, Abidin, Wahab, Ariffin, & Senafi, 2014; Jurisic & Bumbasirevic, 2008). From this point of view both carrageenans (κ and λ) exerted moderate toxic influence on the HeLa cells.

Comparative *in vitro* investigation of the antiproliferative activity of native κ-carrageenan from *Kappaphycus striatus* (37,7 kDa) and κ-oligocarrageenan (1.2 kDa) prepared using the mild hydrochloride hydrolysis of the natural κ-carrageenan in the cell lines of the human nasopharynx carcinoma, human gastric carcinoma, and cervical cancer HeLa have demonstrated that within the whole concentration range (125, 250 and 500 μg/mL) the degree of inhibition caused by interaction with κ-oligocarageenan exceeded that of the high molecular κ-carrageenan approximately in 2.7–6.3 times (Yuan & Song, 2005). A summarized κ-oligocarrageenan fraction given orally in the doses 100 and 200 μg/mL significantly suppressed the growth of xenograft S180 sarcoma tumor with the degree of inhibition 36.3 % and 39.82 %, respectively. Chemotherapeutic drug ftorafur administered in a dose 120 mg/kg bw inhibited the tumor growth by 46.4 (Yuan, Song, Li, Li, & Dai, 2006). Chemically modified derivatives obtained by the methods of sulfation (from 8.98 % to 47.90 %), acetylation (1.13 %) and phosphorylation (2.98 %) significantly increased antitumor activity of the κ-oligocarrageenans. A sulfated derivative administered in a daily dose 200 mg/kg exerted the highest antitumor activity with the tumor growth inhibited by 54.12 % in mice with the xenograft S180 tumor. Antitumor effects of the sulfated and phosphorylated derivatives of the κ-oligocarrageenans were similar to the effects of ftorafur given in the appropriate doses (Yuan et al., 2011).

Comparative assessment of the antitumor effects in the xenograft tumor models exerted by native λ-carrageenan with molecular weight 650 kDa isolated from *Chondrus ocellatus* and several oligosaccharide samples with different molecular weight 240, 140, 15 and 9.3 kDa obtained through the method on microwave degradation have shown that inhibiting activity of λ-carrageenans increases with the direct correlation to the reduction of their molecule size. Degree of the growth inhibition in the xenograft tumors S180 and H22 was found to be the highest for λ-oligocarrageenan with an average molecular weight 15 kDa and sulfate content 27.8 % and was about 50.52 % and 68.97 % given in a dose 200 mg/kg/day, respectively (Zhou et al., 2004; Zhou et al., 2005). Joint administration of the λ-oligocarrageenan with molecular weight 15 kDa and 5-FU led to stronger antitumor effect exerted by the drug. Thus, degree of inhibition of the H22 tumor cells after administration of 5-FU (25 mg/kg) was 30.76 %, after administration of λ-oligocarrageenan (100 mg/kg) – 43.97 %, and after simultaneous joint administration of both compounds – 51.73 %. ($P < 0.001$).

Approximately the same results were obtained through the testing of the antitumor activity of λ-oligocarrageenan with an average molecular weight 9.3 kDa (Zhou et al., 2005). After chemotherapy with 5-FU some undesirable effects such as slowed weight gain, reduced spleen weight, suppressed lymphocyte proliferation, and lowered serum TNF-α level were noticed. At the same time λ-oligocarrageenans that were being tested significantly weakened immune suppressive influence of the 5-FU (Zhou, Sheng, Yao, & Wang, 2006).

κ-Carrageenan oligosaccharides (κ-neocarrabiose sulfate, κ-neocarrahexose sulfate and κ-neocarraoctaose sulfate – KOS) obtained through the enzyme hydrolysis treatment were tested for their antitumor and antiangiogenic activities. This composition was made of three listed κ-oligocarrageenans with molecular weight 425.27 Da, 1242.95 Da, and 1681.04 Da, respectively, and molar ration of each polysaccharide in the mixture was about 4:2:1. KOS was found to demonstrate strong antitumor activity on the models of xenograft mice S180 sarcoma and human breast cancer MCF-7 cells. Degree of the growth inhibition of the sarcoma S180 in Kunming mice after intragastric administration of KOS within 15 days in a dose 50 mg/kg/day was 14.94 % ($P < 0.05$), in a dose 100 mg/kg–39.45 % ($P < 0.01$), and in a dose 200 mg/kg–56.77 % ($P < 0.01$). Inhibiting activity exerted by KOS given in a dose 50 mg/kg was close to the activity of cyclophosphamide which degree of inhibition in a dose 20 mg/kg was 69.50 %. Statistically significant inhibition of the xenotransplant MCF-7 growth in the naked mice after KOS administration started to be noted on day 10th of the experiment whereas after administration of thalidomide (positive control) in a dose 76 mg/kg – on day 7th (Yao, Wu, Zhang, & Du, 2014).

κ-Carrageenan isolated from *Hypnea musciformis* was characterized by peak molar weight 519.1 kDa and free sulfate contents 17.3 %. This isolated carrageenan did not exert cytotoxic effect on the human breast cancer MCF-7 cell line and human neuroblastoma SH-SY5Y cells. But it significantly reduced proliferation of the SH-SY5Y cells when they were applied in a dose 0.6 and 1.0 mg/mLSimilarly, native κ-, ι-, λ-carrageenans isolated from *Laurencia papillosa* were characterized by the peaks of their molecular weight about 320 kDa, 560 kDa, and 258 kDa, respectively. These samples suppressed proliferation of the human breast cancer MCF-7 cells in a dose-dependent and time-dependent

Table 3
Cytotoxicity of κ-carrabiose expressed in the values of half-inhibiting concentration regarding tumor cells exposed for 48 h (Mean ± SD) (Calvo et al., 2019).

Cell line	IC_{50}, mg/mL
Mouse breast adenocarcinoma LM2	0.043 ± 0.009
Human ovarian cancer IGROV-1	0.099 ± 0.004
Myelogenous leukemia of erythroleukemic type K562	0.049 ± 0.002
Mouse melanoma B16-F10	0.039 ± 0.005
Mouse bladder cancer MB49	0.045 ± 0.008
Human bronchoalveolar cancer A549	0.066 ± 0.005
Mouse squamous cell carcinoma Pam212	0.051 ± 0.006

manner with IC_{50} ~200 μM, ~50 μM, and ~25 μM, respectively, within the first 24 h (Ghannam, Murad, Jazzara, Odeh, & Allaf, 2018).

Effects of native and degraded carrageenans on the growth of the human epithelial colorectal carcinoma Caco-2 cell line and human hepatocellular carcinoma HepG2 cells were tested under *in vitro* conditions in the concentration range from 62.5–2000 μg/mL. Carrageenans extracted from the *K. alvarezii* and commercial available carrageenans (Sigma) were investigated. The results showed that degraded commercial and food grade κ-carrageenans significantly reduced cell vitality of the Caco-2 line in 24, 48, and 72 h of agitation period. In contrast non-degraded food grade ι-carrageenan showed cytotoxic effects when applied in IC_{50} exceeding 1000 μg/mL whereas IC_{50} values for the commercial ι-carrageenan were not determined. Tamoxifen that was used as a positive control in this model suppressed the cell proliferation with IC_{50} 9.2 μg/mL, 7.4 μg/mL, and 7 μg/mL in 24, 48, and 72 h, respectively. Degraded κ-carrageenans but not ι-carrageenans also significantly inhibited proliferation of the normal human small intestine cells of the line Fhs 74 Int. Degraded κ-carrageenans reduced cell vitality in the HepG2 line in dependence on the concentration applied. In contrast ι-carrageenans did not exert cytotoxic effect regarding HepG2 cells; IC_{50} values were higher than 1000 μg/mL. Degraded κ- and ι-carrageenans also induced toxic influence on the human hepatocyte cells Fa2N-4. None of all non-degraded κ- and ι-carrageenans did exert toxic influence neither on tumor cell nor on the normal cells of human intestine and liver. Values of the half-inhibiting concentrations of degraded κ- and ι-carrageenans and antitumor drug tamoxifen are given in the Table 2. The data given in this table show that tamoxifen is averagely 54.3 and 69.6 times more toxic than degraded κ-carrageenans regarding the Caco-2 cell line and 88.9 times – regarding cells of the HepG2 line (Ariffin, Yeen, Abidin, Wahab, Ariffin, & Senafi, 2014). Effects of the degraded carrageenans on the tumor cells of the Caco-2 and HepG2 lines and on the normal cells of intestine FHs 74 Int and liver Fa2N-4 that were tested in the quoted study may be considered as a moderate toxic influence.

Large and substantial work devoted to the comparative estimation of the antiproliferative activity of the high and low molecular carrageenans and their different types was carried out by the researchers from Argentina (Calvo et al., 2019). Native κ-carrageenan with molecular weight 215 kDa obtained from *Hypnea muscifomis,* commercially available ι-carrageenan (460 kDa) from *Euchema spinosum* (Sigma-Aldrich), and λ-carrageenan (619 kDa) extracted from *Iridaea undulosa* were used for the preparation of the degraded κ-carrageenans with an average number of disaccharide units (n) 6.5 and 25.5 (10.4 kDa) and degraded λ-carrageenan (n = 43.2, 27.2 kDa). In addition to the abovementioned oligosaccharides there were low-molecular compounds obtained including unpurified and purified κ- and ι-carrabiose, κ/ι-carrabiose mixtures with the ratio 3:1 and 9:1 as well as κ-carrabitiol and its triethylammonium salt, κ/ι- carrabiitol mixture, and α-carrabiitol. All compounds were tested on the cell lines of the mice breast adenocarcinoma LM2, human ovarian cancer IGROV-1, myelogenous leukemia of erythroleukemic type K562, mice melanoma B16-F10, human bladder cancer MB49, bronchoalveolar human lung cancer A549, and mouse squamous cell carcinoma PAM212. Native κ- and ι-carrageenans had shown similar cytotoxic activity in the LM2 cells with IC_{50} values 0.23 ± 0.02 and 0.20 ± 0.04 mg/mL, respectively. λ-Carrageenan showed signs of toxicity only at concentration 1 mg/mL. Degraded κ-carrageenans with n = 25.5 and 6.5 have exerted more pronounced toxicity regarding LM2 cells in comparison to the native κ-carrageenan with the IC_{50} values 0.39 ± 0.05 and 0.38 ± 0.04 mg/mL, respectively. Moreover, the degraded λ-carrageenan (n = 43.2) showed more pronounced cytotoxicity with IC_{50} 0.95 ± 0.11 mg/mL in comparison to the original high molecular polysaccharide ($P < 0.01$) (Calvo et al., 2019).

Among the disaccharides studied, the pure κ-carrabiose and mixture (9:1) of κ- and ι-carrabiose were found to be more cytotoxic compounds with the IC_{50} values 0.043 ± 0.009 mg/mL and 0.052 ± 0.005 mg/mL, respectively, regarding the LM2 cells. Among the κ-carrabioses, the pure compounds were more cytotoxic than the unpurified ones (0.074 ± 0.009 mg/mL) whereas among the ι-carrabioses the results were opposite (0.338 ± 0.035 mg/mL and 0.183 ± 0.015 mg/mL, respectively). These findings suggest that deep depolymerization up to the disaccharide units increases carrageenan cytotoxicity, at least, for κ-carrageenan. κ-Carrabiose exerts higher cytotoxicity than ι-carrabiose. It looks like that sulfation at the C-2 of 3,6-anhydrosugar does not contribute this activity. Mixture of the κ- and ι-carrabioses had the intermediate parameters of IC_{50} indicating the lack of the synergistic activity of the disaccharides. The presence of the aldehyde group (being masked as hydrate) in the carrabioses generate biological activity, which may disappear if these disaccharides are reduced to their aldites (carrabiitols) despite the sulfate contents are the same in both compounds. Purified κ-carrabiose was discovered to be a compound showing the highest cytotoxicity regarding LM2 tumor cells. For other cell lines cytotoxicity of the κ-carrabiose expressed in the IC_{50} values for 48-h period varied approximately within the limits between 0.0389 and 0.099 μg/mL (Table 3).

We would like to quote the paper authored by Simon et al., 2001 for the correct and obvious comparison. The authors of that have found IC_{50} values for the chemotherapeutic drugs after incubation for 48 h such as 0.197 μg/mL (0.34 μM) for doxorubicin, 11.0 μg/mL (27.3 μM) for cisplatin, and 19.8 μg/mL (53.4 μM) for carboplatin (Simon, Knebel, Baumgartner, Aufderheide, Meyer-Lindenberg, & Nolte, 2001). Analysis of the cell cycle have showed that purified κ-carrabiose in concentration 0.06 mg/mL induced an increase of the LM2 cell population at the phase sub-G1 up to 21.3 % against 5.3 % in the control demonstrating the increased apoptotic cell death rate. In addition, κ-carrabiose contributed to enhanced blockage at S-phase with corresponding reduction of the relative contents of the cells being at phases G1 and G2-M. Authors supposed that it is possible to use such disaccharides as carrabioses in combination with antitumor drugs for enhancement of their cytotoxicity and strengthening of the antimetastatic properties as well as their application as adjuvants or carriers of antitumor agents (Calvo et al., 2019).

Results of the *in vitro* experiments showed that degraded ι-carrageenan with the molecular weight 40 kDa obtained through the acid hydrolysis of the commercial ι-carrageenan (Sigma) have inhibited cell growth in the human osteosarcoma HOS culture with IC_{50} 1.41 ± 0.07 mg/mL in 48 h. This inhibition was as concentration dependent as time dependent. Original high molecular ι-carrageenan was not found to exert such influence. Degraded ι-carrageenan decelerated cell cycle at G1 phase; percent amount of the HOS cells being at G1 phase in the control samples was 34.8 ± 9.2 %. Treatment of these samples with original native ι-carrageenan slightly raised this portion to 36.6 ± 5.4 % whereas after treatment with degraded ι-carrageenan this portion increased to 64.3 ± 6.8 %. Relative share of the apoptotic NOS cells induced by degraded ι-carrageenan within 48 h was 26.3 ± 2.7 % whereas after treatment with original ι-carrageenan only 3.1 ± 0.4 % cells were considered apoptotic. Their portion in the control samples was only 1.4 ± 0.2 %. Antitumor influence of the same degraded ι-

carrageenan was estimated in the *in vivo* studies with the use of the xenograft tumor model. On the day 25th after the injection of the tumor cells the volume of the node was 521 ± 35mm^3 and its weight was 384 ± 18 mg. In mice with xenograft tumors, which were administered high molecular carrageenan directly into the tumor node (140 μg in 100 μl), the volume of the tumor was 507 ± 14 mm^3 and its weight was 356 ± 21 mg. After administration of the same dose of degraded carrageenan these values were 275 ± 2 mm^3 and 172 ± 15 mg, respectively ($P < 0.05$). The data showing the mice survival rate were very picturesque: control mice (with xenograft tumors) and mice with tumors that were administered the original native carrageenan started to die since 7th day of the experiment. After administration of the degraded carrageenan the first mice perished were noted since 13th day. Moreover, at the end of the experiment (30 days) 16 animals from the 20 mice in the group given degraded carrageenan were still alive whereas all mice in other group were perished ($p = 0.013$) (Jin, Han, & Han, 2013).

The study performed by Suganya, Sanjivkumar, Chandran, Palavesam, and Immanuel (2016) stands out from the series of works with the similar conclusions about correlations between molecular weight of carrageenans and their antiproliferative activity. The natural κ-carrageenan isolated from *K. alvarezii* was rested under *in vitro* conditions on the several tumor cell lines including human breast cancer MCF7, colon adenocarcinoma HT-29, carcinoma HepG2, and human osteosarcoma MG-63. Within the concentration range from 10 to 150 μg/mL κ-carrageenan affected survival and growth of the tumor cells in a dose-dependent manner. Significant reduction of the MCF7 cell vitality was noted at the concentrations used 100 and 150 μg/mL (cell survival was 50.93 ± 0.174 % and 43.63 ± 0.257 %, respectively). Growth inhibition at these concentrations was 49.07 ± 0.174 % and 56.37 ± 0.257 %, respectively, with IC_{50} 103.2 μg/mL. Under the same conditions the IC_{50} for cyclophosphamide (CP) was 47.65 μg/mL. Degree of the HT-29 cell proliferation inhibition was increased to 5.30 ± 0.215 % with the carrageenan concentration applied 10 μg/mL and up to 67.67 ± 0.168 % at concentration 150 μg/mL. IC_{50} values were 73.87 μg/mL for carrageenan and 44.33 μg/mL for CP. The lowest carrageenan concentration (10 μg/mL) inhibited the growth of the HepG2 cells by 31.5 ± 0.66 % whereas concentration of carrageenan 150 μg/mL provoked inhibition by 64.81 ± 0.87 %. IC_{50} values for carrageenan were 56.71 μg/mL and for CP – 125 μg/mL. Degree of MG-63 cell inhibition was 23.4 ± 0.92 % for carrageenan concentration 10 μg/mL and 65.70 ± 1.62 % for the concentration 150 μg/mL. IC_{50} values for CP and κ-carrageenan were 26.48 and 47.85 μg/mL, respectively. Virtually the same effects were noted in experiments with commercially available carrageenan (Suganya et al., 2016). Authors supposed that inhibition of the MCF-7 and HT-29 cell proliferation is caused by the induction of apoptosis as through the mitochondria-related pathway as through the way linked to the cell death receptor *via* direct and indirect caspase-3 activation.

Commercial κ-, λ- and ι-carrageenans (Sigma-Aldrich) applied in concentrations 100, 500, and 1000 μg/mL in the *in vitro* experiments did not exert cytotoxic effects on the Caco-2 cells. They did not affect cell vitality of HT-29, HCT-8, and HepG2 cells applied in concentrations 0.1, 1.0, and 10 μg/mL for 24 h. Based on these results the authors have made a conclusion about the absence of toxicity of the natural (high molecular) carrageenans regarding tumor cells (McKim et al., 2016). Sulfated polysaccharide with the molecular weight 420 kDa isolated from *Acanthophora spicifera* containing 73.5 % of galactose, 9.2 % of xylose, 1.9 % of mannose, and 10.9 % of arabinose with the total sulfate contents about 21.9 % did exert dose-dependent cytotoxic effect on the A549 cell line within the concentration range 100 − 1000 μg/mL with the IC_{50} 400 μg/mL in 48 h (Anand et al., 2018).

Sulfated polysaccharide isolated from *Champia feldmannii* was shown to have IC_{50} values exceeding 25 μg/mL regarding the cell lines of myeloid leucosis HL-60, melanoma MDA-MB-435, glioblastoma SF-295, and carcinoma HCT-8. IC_{50} for 5-FU in those models were within the range between 0.36 and 12.59 μg/mL (Lins et al., 2009). Therefore, author have estimated the effects of this polysaccharide as non-toxic regarding the tumor cells tested. But in the experiments with the xenograft sarcoma 180 this carrageenan suppressed tumor growth with the degree of inhibition 48.62 % and 48.16 % given in doses 10 and 25 mg/kg, respectively. When administered simultaneously it enhanced the inhibiting effect of 5-FU from 48.66%–68.32 % (Lins et al., 2009).

A series of studies devoted to the assessment of the antitumor, antimetabolic, and immunotropic activities of carrageenans isolated from *Chondrus armatus* and their low molecular derivatives on such cell lines as human esophagus adenocarcinoma FLO-1 and esophageal squamous cell carcinoma KYSE-30 were carried out in our laboratory (Cicinskas, Begun, Tiasto, Belousov, & Vikhareva, 2020; Tiasto, Mikhailova, Gulaia, Vikhareva, & Zorin, 2018). Average molecular weights of the native κ- and λ-carrageenans were 174.5 and 260 − 300 kDa, respectively, whereas average molecular weights of the κ- and λ-oligocarrageenans obtained through the mild acid hydrolysis was 0.7–20 and 10 − 170 kDa, respectively. All the samples of carrageenans tested in *in vitro* experiments within 2 h of the incubation period did not substantially affect survival and proliferation rate of the KYSE-30 and FLO-1 cells in all concentrations applied. In 48 h, the number of KYSE-30 cells incubated with native κ- and λ-carrageenans and degraded κ- and λ-carrageenans at the carrageenan concentration 400 μg/mL was reduced in comparison to the control group on the average in 2.02, 2.71, 3.01, and 2.06 times, respectively. The number of the FLO-1 cells within the same period of time was reduced in 1.59, 1.56, 1.41, and 1.33 times, respectively, in comparison to the control group. At the same time the number of the killed tumor cells after 48 h of incubation with all carrageenan samples was not higher than 10 %. Therefore, the results suggest that carrageenans do not exert pronounced cytotoxic influence on such cell lines as KYSE-30 and FLO-1. All carrageenans have demonstrated dose-dependent (except κ-carrageenan) antimetabolic activity. In 24 h of incubation the κ- and λ-carrageenans applied in concentration 400 μg/mL was found to reduce metabolic activity of the FLO-1 cells as an average by 52.0 and 30.1 %, respectively. In the same model paclitaxel induced the reduction of metabolic activity approximately by 55.6 % when it was applied in concentration 10 mM. In the KYSE-30 cells the native κ- and λ-carrageenans caused reduction of the metabolic activity as an average by 52.5 % and 44.9 %, respectively, whereas these values for the degraded κ- and λ-carrageenans were by 36.7 % and 34.3 % lower, respectively, in concentration 400 μg/mL. Paclitaxel suppressed metabolic process in these cells by 61.0 % at average in concentration 1 mM. The results suggest that probably KYSE-30 tumor cells are more sensitive to the antimetabolic influence of carrageenan than FLO-1 cells. Native carrageenans are more efficient regarding the KYSE-30 cells whereas degraded carrageenans show stronger effects in the FLO-1 cells (Cicinskas et al., 2020).

4.1. Relationships between structure and physico-chemical properties of carrageenans and their anticancer activity

Results of the analysis of experimental papers make a ground to claim that the antitumor activity of carrageenans is definitely dependent on their molecular weight, degree of sulfation as well as experimental conditions. Under *in vitro* conditions high molecular, in particular, natural carrageenans generally do not exert cytotoxic effects regarding all types of cancer cell lines (Jin et al., 2013). This effect was noted only in the studies, in which very high concentrations of the polysaccharides exceeding 1000 μg/mL were used (Ariffin et al., 2014; Calvo et al., 2019).

In experiments under *in vivo* conditions antitumor effects were found to be exerted by as high molecular as low molecular carrageenans obtained through the various methods of degradation of a native polysaccharide. But stronger effects were typical of the low molecular carrageenans (Calvo et al., 2019; Zhou et al., 2004; Zhou et al., 2005). That effect of the natural carrageenans is not caused by the direct

influence on the tumor cells but probably is a result of the immune mechanism activation (Calvo et al., 2019; Luo et al., 2015; Mou et al., 2003).

Experiments carried out under *in vitro* condition clearly showed the correlation that antiproliferative activity of oligocarrageenans increases in accordance to the reduction of their molecular weight (Calvo et al., 2019; Yuan & Song, 2005; Zhou et al., 2004) up to the disaccharide size. Thus, disaccharide κ-carrabiose was shown to exert the highest cytotoxicity in major part of the experimentally tested tumor cells (Calvo et al., 2019). Therefore, low molecular carrageenans and carrageenan oligosaccharides are thought to be more promising anticancer agents than the high molecular natural products belonging to the same class of the polysaccharides (Fedorov, Ermakova, Zvyagintseva, & Stonik, 2013).

Form the point of view of the carrageenan types there is no definite correlation determined because of the lack of sufficient number of the comparative studies. In some studies, the results showed stronger antitumor effect exerted by λ-carrageenans (Prasedya et al., 2016; Li et al., 2017), another studies displayed higher activity of the κ-carrageenans or ι-carrageenans (Jin et al., 2013). It should be mentioned that ι-carrageenans either have shown slight antitumor activity or were not active at all in the majority of the tumor cell lines (Ariffin et al., 2014). Thus, these aspects require more additional studies to be carried out.

Regarding the role of the degree of sulfation, its influence on the carrageenan anticancer properties is more or less clear. A prevailing number of the research results have confirmed that sulfate content in the sulfated polysaccharide is an ideal structural feature providing substantial biological activity including anticancer effects (Anand et al., 2018; Hu et al., 2006; Jiao et al., 2011; Liang, Mao, Peng, & Tang, 2014; Yuan et al., 2011). The results of only one experimental study showed that slightly sulfated κ-oligocarrageenan exert stronger antitumor effect than the highly sulfated κ-oligosaccharide (Mou et al., 2003). Perhaps, not only the degree of sulfation but also location of the sulfate groups plays a role in the tumor growth inhibition as it was found to be typical of the angiogenesis inhibition. For example, the location sites of sulfated groups attached to the C4 of galactose were found particularly important (Paper et al., 1995).

The results given in this chapter of the review suggest that carrageenans possess antiproliferative and antitumor properties. Next chapters will be devoted to the details of the mechanisms of the abovementioned effects.

5. Cellular and system mechanisms of the antitumor activity of carrageenans

5.1. Apoptosis

Discontinued cell cycle and cell death are the main events the antitumor influence of the drugs is focused on. Uncontrolled cell proliferation is a typical characteristic sign of all cancer cells. Thus, the cell cycle blockage is considered effective strategy for eradication of the tumor cells. Many chemotherapeutics agents exert antiproliferative influence. Conception of the cell death caused by the cell cycle block attracts attention because this approach may provide an opportunity to avoid pharmaceutical resistance, decrease the rate of mutagenesis, and reduce toxicity. This should be emphasized that the cell death is usually seen dichotomously as apoptosis or necrosis. Apoptosis is basically described as an active programmed process of the autonomous cell dismantlement allowing prevention of inflammation. Necrosis is usually described as a passive accidental cell death due to the harmful influence of the ambient factors with uncontrolled expulsion of the inflamed cell contents (Ouyang et al., 2012). As apoptosis is thought to be a regulated and controlled process, it is preferable if a new agent can induce death of the malignant cells *via* the mechanisms of apoptosis (Kandeel, Kamal, Naguib, & Hassan, 2018).

Morphological features of apoptosis such as plasma membrane blebbing, cell detachment, externalization of phosphatidylserine, nuclear condensation and formation of the apoptotic bodies were registered in the tumor Caco-2 and HepG2 cells as well as in normal cells of human intestine FHs 74 Int and human hepatocyte Fa2N-4 cell line after incubation with degraded κ-carrageenans at the concentrations that correspond to the appropriate IC50. Besides, degraded κ-carrageenans suppressed gene expression of the cell proliferation markers PCNA, MKI67 and survivin in HepG2 cells (Ariffin et al., 2014). These findings confirm the death of tumor cells induced by the κ-oligocarrageenan influence are supposed to be related to the apoptosis. Toxicity of the degraded carrageenans realized through the mechanisms of apoptosis and inflammation is supposed to be caused by the production of the reactive oxygen species (ROS) (Chen et al., 2010).

Antiproliferative effect of the κ-carrageenan on the carcinoma MCF-7 and HT-29 cells is also considered to be linked to the induction of apoptosis as through mitochondria-related pathway as *via* the receptor-dependent cell death caused by the direct and indirect caspase-3 activation (Suganya et al., 2016). Apoptotic effects of the κ-carrabiose samples on the LM2 tumor cells (Calvo et al., 2019) and degraded ι-carrageenan on the human osteosarcoma HOS cells were determined (Jin et al., 2013). Antiproliferative influence of the native ι-, λ-carrageenans isolated from *Palisada perforata (*formerly *Laurencia papillosa)* on the MCF-7 cells results in significant induction of apoptosis with the characteristic signs such as increased activity of caspase-3 (in 2.0 and 2.4 times), PARP (in 44.8 and 70.6 times) and p53 (in 22 and 38 times) with the bax expression shift of bcl-2 (Ghannam et al., 2018). Signs of apoptosis were also noted in the tumor A549 cells after the treatment with sulfated polysaccharide (420 kDa) isolated from *A. spicifera* (Anand et al., 2018). κ-Carrabiose exertng the highest cytotoxicity regarding several tumor lines had caused a cell death due to the induction of the phase G2/M block and apoptosis (Calvo et al., 2019).

At the same time, the high molecular commercial κ-carrageenan and the κ-oligocarrageenans obtained from the first one did not change the proliferative activity of the tsFT210 tumor cells under *in vitro* conditions and did not demonstrate any signs of apoptosis (Mou et al., 2003). Therefore, carrageenans, in particular, oligocarageenans directly affect tumor cells under *in vitro* conditions, and their influence is generally associated with the apoptotic cell death signs registered.

5.2. Wnt-cascade role in the antiproliferative influence of carrageenans

One of the most important cellular signaling pathways is the Wnt cascade playing a key role in the embryonal development, stem cell phenotype sustaining, determination of the cell polarity and their migration (Goldsberry, Londono, Randall, Norian, & Arend, 2019). There is an important fact known that mutations occurring in that cascade are linked to the development of malignant tumors. Thus, the Wnt signaling itself is considered a target in the screening of compounds with antitumor properties (Tabatabai, Linhares, Bolos, Mita, & Mita, 2017; Tatarskiy, 2016). Wnt is a family of the highly conservative growth factors presenting a group of secreted glycolipoproteins that induce the signaling pathway for the direction of cell proliferation and their differentiation during each stage of ontogenesis. Wnt-cascade is decided to be composed of at least three pathways: Wnt/β-catenin (or Wnt canonic) signaling pathway, the non-canonic calcium cascade, and the cell-polarity cascade (Katoh, 2017). Components of the first signaling systems are the best studied ones. If Wnt is not active or substantially inhibited, the cytoplasm β-catenin forms complexes with Axin and APC (adenomatous polyposis coli) proteins, casein kinase 1 (CK 1), and glycogensynthase 3β kinase (GSK3β). As this complex is formed both CK1 and GSK3β acting together phosphorylate β-catenin, which is then recognized and subjected to ubiquitination by the Trcp protein with ubiquitin ligase subunit E3 forcing β-catein to the following proteosome degradation. Due to the cascade activation Wnt links to the target receptors including Frizzleds (Fr) receptors and coreceptor proteins 5 and 6 associated with the low density lipoprotein receptors (LPR 5/6)

resulting in activation of the Dishevelled (Dvl) protein stabilizing a "destructing complex" Wnt/Fr/LRP/Dvl/Axin and inhibiting the GSK3β. The dephosphorylated β-catenin usually avoids degradation, accumulates in cytoplasm, and being translocated into nucleus. It forms a complex with transcription factors TCF (T-cellular transcription factor) and LEF (lymphoid enhancer-linking factor) as well as with co-activators of transcription inside the nucleus inducing transcription of the dependent genes including the target oncogens such as c-myc, cyclin-D1, survivin, MMP7, MDR1, CD44, and Axin2. Therefore, it increases a cell proliferation rate. Other secreted factors such as WIF-1 and Frzb/sFRP3 inhibit Wnt linking with Frizzled receptors whereas Dickkopf (Dkk) proteins counteract the Wnt/LPR interactions (Dijksterhuis, Petersen, & Schulte, 2014; Tabatabai et al., 2017).

Investigation of the mechanisms of the earlier registered antitumor influence exerted by the degraded ι-carrageenan on the osteosarcoma HOS line model in mice showed that ι-oligocarrageenan blocks Wnt/β-catenin signaling pathway in the HOS cells *via* suppression of expression and phosphorylation of the co-receptor LPR6, which is the only one Wnt receptor found in the HOS cells. Plasma β-catenin level was found to be substantially decreased due to the influence of the degraded ι-carrageenan in comparison to control and, thus, degraded ι-carrageenan affects accumulation rate of the β-catenin inside the tumor HOS cell nuclei. Besides, concomitant increase of the Axin2 and GSK-3β levels in comparison to the control was registered in the cellular extracts of the HOS cells treated with degraded ι-carrageenan (Jin et al., 2013). Receptor degradation induced by the ligand is an important cellular process suppressing activity of the various signaling pathways. Degraded ι-carrageenan is likely to bind to the extracellular Wnt5 receptor in the HOS cells, thus, reducing stability of this receptor and suppressing an overall Wnt signaling mechanism.

The results of the study focused on investigation of the effects exerted by commercial λ-carrageenan (Sigma-Aldrich) on the normal human epithelial colon cells NCM 460 and malignant human colon cell of the HT-29 line merit to be mentioned. Influence of the carrageenan (1 μg/mL, 24 h) increased the levels of cellular β-catenin and nuclear β-catenin in both lines. In the NCM460 cells the nuclear β-catenin level was increased by ~212 % and the its cellular level – by ~68 % (Bhattacharyya, Borthakur, Dudeja, & Tobacman, 2007). In contrast, in the malignant HT-29 cells nuclear β-catenin was increased only by 38 % and total cellular β-catenin was only 29 % lower. Initial values and the ones after stimulation were much higher in the HT-29 cells than those in the NCM460 cells. The increase of the total cellular β-catenin to a major part was caused by increased nuclear β-catenin level. These effects were accompanied with the TCF/LEF activation, increase of the cyclin D1 expression, inhibition of the thioredoxin reductase and, therefore, it resulted in enhanced nucleoredoxine oxidation (Bhattacharyya, Feferman, Borthakur, & Tobacman, 2014).

If NRX is oxidized, free Dvl may link to axin and thus destroy β-catenin nuclear destructing complex, and that results in the release of β-catenin and nuclear translocation. Apart of that, after the influence of carrageenan an increase of the Wnt9A expression was noted that helped to continuing stimulation of the Wnt/β-catenin pathway. The effects described before were completely disappeared due to the action of ROS absorber tempol (Bhattacharyya et al., 2014), confirming that carrageenan in the NCM460 cells activates Wnt/β-catenin pathway *via* ROS-associated effects. These data suggest that carrageenans possess pro-inflammatory and oncogenic potential, at least, regarding colon epithelial cells. Finally, we can conclude that today we understand two results of the carrageenan influence on the Wnt cascade in the tumor cells. Firstly, commercial λ-carrageenan enhances Wnt/β-catenin pathway stimulation in the human colon adenocarcinoma HT-29 cells. Degraded carrageenan is very likely would be shown to exert the same effects. On the other hand, degraded ι-carrageenan inhibits Wnt/β-catenin pathway in the human osteosarcoma cells with induction of apoptosis and inhibition of the tumor cell proliferation. These data are not supposed to be considered controversial. We may suggest that this is just a beginning of investigation of the intracellular mechanisms of the effects exerted by carrageenans toward tumor cells. The more detailed understanding of these mechanisms and their peculiarities requires expanded spectrum of the test lines tested and an increased set of the compounds being investigated.

5.3. Immune mechanisms of the antitumor influence of carrageenans

Tumor microenvironment is thought to a greater extent to contribute the development of immune resistance and plays a crucial role in the mechanisms of survival, proliferation, and migration of the tumor cells. Therefore, one of the approaches for the antitumor therapy may be the immune system modulation purposed for stimulating antitumor immune response and overcoming immune suppressing barriers in the therapy of cancer (Coulie, Van den Eynde, van der Bruggen, & Boon, 2014; Schreiber, Old, & Smyth, 2011).

Even the first studies of the antitumor and immune modulating activity of κ- and λ-carrageenans under *in vivo* conditions suggested that antitumor effects of the sulfated polysaccharides are enhanced by the immune system's activity. Tumor growth inhibition was shown to be associated with an increase of the humoral and cell-mediated immunity in experimental animals (Yuan et al., 2006; Zhou et al., 2004). The carrageenan oligosaccharides from *K. striatus* with the S180 cell suspension inoculated were orally administered to mice for 14 days. That led to the inhibited growth of the xenograft sarcoma cells, increased macrophage phagocytosis, increased antibody synthesis and lymphocyte proliferation, enhanced natural killer (NK-cells) activity and serum levels of cytokines, interleukin 2 (IL-2) and tumor necrosis factor-alfa (TNF-α) (Hu et al., 2006). NK-cell activity even exceeded that of normal mice (Yuan et al., 2011), which may be a key factor in the mechanisms of antitumor activity of the oligocarrageenans.

Immature dendritic cells (DCs) isolated from the mice C47BL/6 bone marrow were treated with λ-carrageenan (Sigma-Aldrich) with concentrations 10, 50, and 100 μg/mL. The results showed that this polysaccharide did not exert any influence on cell vitality. But at the same time, λ-carrageenan accelerated maturing of DCs, thus, in a dose-dependent manner stimulating expression of CD40, CD80, CD86 and II class molecules of the major histocompatibility complex (MHC II) on DCs and increasing synthesis of cytokines IL-12, IL-1β and TNF-α. DCs treated with λ-carrageenan exerted stronger influence on the splenocyte proliferation in comparison to the regular DCs. More pronounced effects were noted in experiments with concentration 100 μg/mL; that effect of carrageenan was higher than the effect of LPS applied in a dose 20 ng/mL. It was found out with the use of Toll-like receptors 4 (TLR4) inhibitor TAK-242 and with the determination of the protein levels and phosphorylation state of the molecules in the MAPK (p38 and IκB) and NF-κB (ERK and NF-κBp65) signaling pathways that λ-carrageenan helps DCs maturing and cytokine production through the TLR4 signaling pathway (Li et al., 2017). Preliminary treatment of the dendritic cells with TAK-242 substantially inhibited CD40 and CD86 expression on DCs and suppressed IL-2 and TNF-α secretion induced by λ-carrageenan or LPS. In addition, antitumor effects of λ-carrageenan were shown to be associated with inhibition of the regulatory T-cells (nTreg) and myeloid suppressor cells than are known to suppress antitumor immune response and thus contribute tumor aggression. These cells may suppress activation, proliferation and effector functions of the immune competent cells including $CD4^+$ and $CD8^+$ T-cells, NK- and NKT-cells, B-cells and antigen representing cells (Li et al., 2017).

One of the stromal cell types in the tumor microenvironment namely macrophages associated with a tumor contribute to the tumor progression due to the suppressed cytotoxic activity of the $CD8^+$ T-lymphocytes (Castells, Thibault, Delord, & Couderc, 2012). There are two phenotypes of activated macrophages known: M1 (antitumor) stained as $F4/80^{low}$ cells and M2 (alternative pro-tumor) stained as $F4/80^{high}$ cells. Resident tumor macrophages show high immune suppressing M2 profile whereas M1 macrophages going inside tumor generally

are immune stimulating and cytotoxic for the tumor cells (Wynn, Chawla, & Pollard, 2013). In experimental studies the laboratory mice were subcutaneously injected cells of the mice melanoma B16-F10 and breast cancer 4T1. Then λ-carrageenan (Sigma-Aldrich) was injected directly into a tumor in a dose 50 mg/kg every other day significantly preventing an increase of the tumor volume and weight with the degree of inhibition 39.0–56.8 %. That effect of the λ-carrageenan was close to the effect exerted by adriamycin in the 4T1 model. Inhibition of the B16-F10 tumor growth was associated with the 10-fold increased portion of the $F4/80^{low}$ macrophages and 4-fold increased portion of the dendritic cells $CD11c^{+}$ in the tumor tissue as well as enhanced proliferation and activation of the $CD4^{+}$ and $CD8^{+}$ T-lymphocytes. Intratumor injection of the λ-carrageenan resulted in increased number of the T-helper cells (Th17) and increased IL-17A secretion in the spleen lymphocytes contributing immune response stimulation in mice with tumors. Besides, expression of TNF-α secreted by the $CD11b^{+}F4/80^{+}$ macrophages in the B16-F10 tumor tissue was 5-fold increased whereas mRNA TNF-α expression was 5.5 times higher (Luo et al., 2015).

Immune modulating activity of the native κ- and λ-carrageenans isolated from *Chondrus armatus* and their low molecular derivatives was estimated basing on the levels of pro-inflammatory (IL-1β, IL-6, IL-8, IL-18 and TNFα) and anti-inflammatory (IL-10) cytokines after 24 h long incubation of monocytes with the carrageenan samples applied in concentrations 1, 10 and 100 μg/mL. All tested carrageenans stimulated IL-1β production in a dose-dependent manner (except κ-oligocarrageenan). Low molecular λ-carrageenan was found to be the most active sample and it significantly increased IL-1β secretion in concentration 1 μg/mL whereas all other samples exerted the same effect only when they were applied in concentration 10 μg/mL. All carrageenans stimulated IL-6 secretion and low molecular polysaccharides were more effective in comparison to the native carrageenans: the first ones showed activity at concentration 20 μg/mL, the latters – at concentration 100 μg/mL. Both λ-carrageenan samples and high molecular κ-carrageenan contributed to a significant increase of the IL-8 secretion when applied in concentration 100 μg/mL. Native λ-carrageenan and λ-oligocarrageenans more effectively stimulated TNFα secretion at concentration 1 and 10 μg/mL, respectively, than both κ-carraggenans samples applied in concentration 100 μg/mL. λ-Oligocarrageenan was found the most effective inducer of the TNFα secretion leading to its 6-fold increased level at 100 μg/mL whereas all other polysaccharides increased TNFα level only in 4 times. Degraded λ-carrageenan was also the most effective inducer of the IL-10 secretion (Cicinskas et al., 2020).

Therefore, there is an experimental evidence obtained up to date that the carrageenan antitumor activity to a some degree may be provided by their influence on the immune regulating mechanisms including stimulation of the immune cell proliferation and transformation, synthesis of the cell immune factors, and activation of the complement system. In particular, carrageenans may affect the T-lymphocyte effective mitogens, macrophage phagocytosis activators, stimulants of the antibody synthesis and lymphocyte proliferation, natural killer and NKT cell inducers as well as proinflammatory cytokine secretion stimulants. Natural carrageenans and oligocarrageenans may enhance efficiency of the functions of such immune organs as thymus and spleen. At least, λ-carrageenan contributes the dendritic cell maturing and cytokine production in the dendritic cells *via* TLR4 signaling pathway. The practical point of view should be emphasized as the carrageenan oligosaccharides are capable to weaken immune suppressive influence of the antitumor drugs if used as the combination therapy components.

5.4. Antioxidant effects of carrageenans

According to the up-to-date knowledge an oxidative stress and consecutive inflammation are the crucial components of initiation, promotion, and progression of the tumor development (Vallejo, Salazar, & Grijalva, 2017). Generally, oxygen superoxide is formed in mitochondria including the ones inside the tumor cells and then it is reduced to an oxygen peroxide and hydroxyl radical that may cause DNA damage, genome instability, cell proliferation, formation of tumors, and their steady progression. Hypoxia conditions in the mitochondria breathing chain inside the tumor cells leads to increased production of the nitrogen oxide (NO), and further – of the reactive nitrogen species (RNS). Nitrites, nitrates, and peroxynitrites as RNS are the by-products of the NO metabolism and they also may generate other reactive species such as malone dialdehyde and 4-hydroxynonenal. Alterations in the protein metabolism caused by ROS and RNS misbalance increase inflow of the abnormal proteins and compounds inducing oncogenic processes (Lee, Cai, Shu, & Nechuta, 2017; Rezatabar et al., 2019). Immune cell damage caused by the reactive oxygen is thought to be the one of the mechanisms inducing cancer development (Wang, Gao, Jiao, Zhao, & Yang, 2018).

The data showing correlations between oxidative stress, carcinogenesis, and antioxidant reactions substantiate the possibility of the use of antioxidant pharmaceuticals to slow down or prevent carcinogenesis (Gothai et al., 2018; Parohan, Sadeghi, Khatibi, Nasiri, & Milajerdi, 2019). However, the aspects concerning the role of antioxidant therapy in prevention and, particularly, in the treatment of the cancer needs deep evaluation as many antitumor drugs exert cytotoxicity toward tumor cells due to the free radical formation (Yasueda, Urushima, & Ito, 2016). Without a discussion on this problem we would like to note that there is a large number of literature sources providing a ground that allows to consider the antioxidants of plant origin as the promising agents for the antitumor therapy with fewer disadvantages typical of chemotherapeutic pharmaceuticals (Gholamian-Dehkordi, Luther, Asadi-Samani, & Mahmoudian-Sani, 2017). Natural compounds like flavonoids and antocyans, isoflavone glycosides, sesquiterpene pyridine alkaloids and others not only possess significant activity regarding ROS eradication but also display cytotoxicity toward some human tumor cell lines (KB, A-549, HCT-8, RPMI-795 and TE-67). They were found to induce apoptosis and the cell cycle block in G_2/M phase (Husein et al., 2014; Hsu et al., 2015; Lesiak et al., 2010).

Marine green, brown, and red algae were shown to be a rich source of the natural antioxidants possessing various beneficial effects in humans such as anti-inflammatory, anti-diabetic, anti-ulcer, and glucose lowering ones (Alharbi, 2019; Gunathilaka, Samarakoon, Ranasinghe, & Peiris, 2019; Hanif, Ghazala, Farid, & Farooqi, 2016) as well as very important antiproliferative and pro-apoptosis effects (Kumosani et al., 2017). Yuan et al. were among the first researchers who have shown that κ-carrageenan oligosaccharides from *K. striatus* and their oversulfated, acetylated, and phosphorylated derivatives tested in the *in vitro* systems possess antioxidant activity (Yuan et al., 2005). de Souza et al. also noted that λ-carrageenan from *Chondracanthus acicularis* and *G. pistillata*, κ-carrageenan from *K. alvarezii* and ι-carrageenan from *Eucheuma denticulatum* (Sigma Aldrich) demonstrate a capacity of capturing hydroxyl radicals. ι-Carrageenan exerted stronger inhibiting effects on the hydroxyl radials with the IC_{50} value 0.281 ± 0.072 μg/mL in comparison to λ-carrageenan (EC_{50} = 0.357 ± 0.120 μg/mL) and κ-carrageenan (EC_{50} = 0.335 ± 0.016 μg/mL). Authors emphasized that there is a positive correlation between the carrageenan sulfate contents and their antioxidant capacity (de Souza et al., 2007). Oligocarrageenans obtained through a γ-irradiation of the high molecular carrageenans have shown antioxidant properties in various tests such as hydroxyl radical scavenging, reducing capacity, and capacity to scavenge DPPH radicals (Mou et al., 2003). The high molecular weight sulfated polysaccharide (420 kDa) from *A. spicifera* was also capable of absorbing DPPH radicals (Anand, Sathuvan, Babu, Sakthivel, Palani, & Nagaraj, 2018). It was discovered that different types of carrageenans may be arranged with the following sequence according to their activity as κ > ι > λ (Abad, Relleve, Racasouzadio, Aranilla, & De la Rosa, 2013). ι-Carrageenan from *Solieria filiformis* with molecular weight 210.9 kDa and high sulfation degree (1.08) scavenged DPPH radicals with the IC_{50} value 1.77 μg/mL and possessed iron-chelating capacity as

38.39 % (Sousa et al., 2016). In contrast to the κ-oligocarrageenan (682 Da) with the low sulfate content (17.2 %) antitumor effect of the κ-oligocarrageenan (798 Da) with the high sulfate content (21.8 %) in mice with the xenograft sarcoma 180 tumor was associated with the significant dose-dependent increase of the thymus weight and enhanced liver catalase activity [Hu et al., 2006]. Antioxidant activity of the κ-carrageenans with an average molecular weights 209.0, 15.08, 5.82, and 3.25 Da expressed in EC_{50} values regarding a superoxide anion was 8.13, 6.66, 3.22, and 2.65 mg/mL, and regarding the hydroxyl radicals – 0.110, 0.062, 0.049, and 0.014 mg/mL, respectively (Sun, Tao, Xie, Zhang, & Xu, 2010). These results demonstrate that κ-carrageenans with lower molecular weight possess higher antioxidant activity.

Sulfated κ-oligocarrageenans with molecular weights 425.27 Da (κ-neocarrabiose-sulfate), 1242.95 (κ-neocarrahexose-sulfate), and 1681.04 Da (acetylated κ-neocarrooctaose) obtained *via* enzyme hydrolysis inhibited the nitrogen oxide (NO) release as well as outflow of the TNF-α and IL-10 in the microglia cells activated by the bacterial lipopolysaccharide. And their de-sulfation resulted in reduction of these effects (Xu, Yao, Wu, Wang, & Zhang, 2012).

Native carrageenan from *K. alvarezii* and commercial carrageenan (Sigma-Aldrich) also possess high total antioxidant activity and hydroxyl radicals scavenging capacity as well as the one regarding NO and DPPH radicals. At the same time, they exerted simultaneous strong antiproliferative effect regarding MCF7, HT-29, HeoG2, and MG-63 cells (Suganya et al., 2016). The numerous research results demonstrate increased activity of the antioxidant enzymes such as catalase and superoxide dismutase accompanying antitumor effects of carrageenans (Mou et al., 2003) and confirm direct correlation between antitumor activity of the sulfated polysaccharides and their antioxidant capacity (de Souza et al., 2007). Similarly, with the antiproliferative activity, the higher degree of the carrageenan sulfation leads to the more pronounced antioxidant effects. Antioxidant capacity in turn may provide proliferative activity of the polysaccharides (Suganya et al., 2016; Wang et al., 2018).

Therefore, carrageenans, and, first of all, κ-carrageenans possess moderate antioxidant activity that rises with the reducing molecular weight and increasing degree of sulfation. They may be considered as the perspective agents for the cancer prevention.

5.5. Anti-angiogenic mechanisms of the carrageenan activity

Angiogenesis is a formation of the new blood vessel network on the base of already existing vessels. It plays a key role in the growth and development of solid tumors. Cancer is generally characterized by the very disorganized vessel network leading to the worsened blood flow and increased vessel permeability that may result in the higher metastatic potential. Several decades back Folkman (1972) supposed that suppression of the tumor blood vessel growth may provide an opportunity for the cancer treatment. And some researchers have obtained an evidence that disruption of the angiogenetic process is an effective method for the antitumor pharmacotherapy (Fischer, Mazzone, Jonckx, & Carmeliet, 2008; Grothey & Allegra, 2012). Within the last decades a number of strong inhibitors of the endogenous angiogenesis was investigated and tested in clinical trials (Bridges & Harris, 2011; Ribatti, 2011). But the major part of these inhibitors are proteins. Therefore, they typically are more expensive and pose a risk of the xenobiotic toxin transfer as well as a number of difficulties in manufacturing. Small molecules with anti-angiogenic activity obtained from the natural sources may not have such limitations. κ-Oligocarrageenans (κ-neocarrabiose sulfate, κ-neocarrahexaose sulfate, and κ-neocarraoctaose sulfate – KOS) did not affect proliferation of the human pubic vein endothelial cells ECV304. But in concentration 100 and 200 μg/mL in a dose-dependent manner they inhibited proliferation of these cells induced by the cultural fluid of the human breast MCF-7 cancer cells. Total fraction of κ-oligocarrageenans also suppressed migration of the ECV304 cells induced by MCF-7 cells by 71.5 ± 8.0 % and completely inhibited formation of the vessel-like structures in the collagen gel under *in vitro* conditions in concentration 200 μg/mL (Yao et al., 2014). Antiangiogenic activity of κ-oligocarrageenans was close to the activity of thalidomide. Mechanism of the antiangiogenic effects of the oligocarrageenans was shown to be related to inhibition of the mRNA expression of cytokines and such receptors as vessel endothelium growth factor 1 (VEGF1), VEGF1 receptor (KDR), basic fibroblast growth factor (bFGF), bFGFR receptor and CD105. The effects of the oligocarrageenans inhibiting expression mRNA bFGF, bFGFR, and CD105 administered in the doses 50 and 100 μg/kg was higher than those of thalidomide. Carrageenans were also found to inhibit bFGF binding transforming growth factor β1 (TGF β1) and platelet growth factor. At the same time, carrageenan oligosaccharides inhibit the growth of the new blood vessels developed from the tumor cells (Yao et al., 2014). λ-Carrageenan effect of inhibiting invasion and migration of the endothelial cells is caused by suppressed intracellular matrix metalloproteinase (MMP-2) expression inside the endothelial cells.

Degraded ι-carrageenan in the xenograft human osteosarcoma HOS line model in mice exerted antitumor influence and at the same time significantly reduced blood vessel density in the tumor (Jin et al., 2013) suggesting inhibition of angiogenesis. λ-Oligocarrageenan with the higher sulfate contents had demonstrated the highest antiangiogenic activity (Chen et al., 2007) confirming suggestion that the degree of sulfation is the crucial structural parameter providing capacity of the carrageenan oligosaccharides to inhibit angiogenesis in the growing and metastasizing tumors.

Carrageenans are supposed to have biological properties, which to some degree are caused by their similarity to the glycosaminoglycans that are present in the mammal cellular membranes. Antitumor activity of carrageenans is presumed to be related to the destabilizing interactions of the glycosaminoglycan part of proteoglycans with intracellular matrix proteins, thus preventing tumor cell adhesion to a matrix that is required for the metastasizing processes (Calvo et al., 2019). It was shown in addition that oligosaccharides sialyl LewisX and sialyl LewisA known as tumor associated antigens due to their high expression in the tumor cells are involved into the processes of the tumor cell adhesion to vessel epithelium and tumor progression (Takada et al., 1993). There was a suggestion proposed that carrageenans possess properties of recognizing these oligosaccharides and affecting them on the tumor cell surface *via* carbohydrate-carbohydrate interactions (Yuan & Song, 2005). Hence, they block interactions between tumor cells and basal membranes preventing their proliferation and avoiding their adhesion to the various substrates. κ-Carrabiose was shown to affect expression and intracellular distribution of the cell adhesion proteins E-cadherin and β-catenin, and cell-substrate adhesion protein vinculin. These proteins are known to participate in the tumor malignization suggesting their role in oncogenesis (Goldmann, Auernheimer, Thievessen, & Fabry, 2013). In the tumor LM2 cells treated with κ-carrabiose, an aberrant distribution of E-cadherin, β-catenin, and vinculin was noted (Calvo et al., 2019). All these findings suggest that κ-carrabiose affect intercellular and substrate adhesion of the tumor LM2 cells *via* the topography reorganization of the cytoskeleton proteins and adhesion proteins.

Another important mechanism of the carrageenan antiangiogenic influence may relate to the inhibition of heparanase (endo-β-*D*-glucuronidase), which is known to be overexpressed by the tumor cells and destruct intracellular matrix proteoglycans due the heparansulfate disintegration and thus contributing the pro-angiogenic factor release. Degraded λ-carrageenan with Mn 11,396 Da and sulfation degree 1.1 mol sulfate/mol disaccharide under *in vitro* conditions exerted strong heparinase inhibiting activity with an IC_{50} of 7.32 ng/mL compared to 10.7 ng/mL for the 16,412 Da unfractionated heparin (sulfation degree 2.0), 147 ng/mL for the 2716 Da heparin (sulfation degree 1.4), and 61.5 ng/mL for the 4710 Da degraded dextran sulfate (sulfation degree 0.9). In addition, it also displayed a capacity to slow the angiogenesis process by reducing the formation of pseudo vessels in an *in vitro*

Matrigel® test by 32 % for the first seven hours of observation (Poupard et al., 2017). Comparative investigation of the anti-heparanase activity of the purified λ-carrageenan oligosaccharides with the degree of polymerization from 2 to 8 have demonstrated that λ-carraheptaose show highest inhibition capability. Based on the fact that endothelial cell invasion is a critical and initiating event in the angiogenesis and tumor cell metastasis, authors have evaluated the effects of λ-carraheptaose on the invasion ability of the human umbilical vein endothelial cells using the Matrigel invasion assay. Exposure to 0.2–20 μM λ-carraheptaose markedly reduced the number of migrated cells suggesting that λ-carraheptaose suppressed heparanase-associated cell invasion. λ-Carrageenan oligosaccharides inactivated a basic fibroblast growth factor-induced cell proliferation, and among them, λ-carraheptaose showed the highest capability. λ-Carratriose also displayed high inhibition activity, whereas the activity of λ-carrabiose was the weakest (Niu, Zhang, Chen, & Yan, 2015). Thus, oligocarrageenans, in particular, λ-carrageenan oligosaccharides may provide a tool for the development of the new carbohydrate-based therapeutics against cancer and angiogenesis.

6. Carrageenans for the vaccine therapy

Conception of the cancer immune therapy based on the activation of the immunity system affecting tumor cells with the use of the cancer antigens as the targets supposes application of the monoclonal antibodies, adoptive cell transfer, and anticancer vaccines (Topalian, Weiner, & Pardoll, 2011). The main goal of such approach is an activation of immune response that is capable to destroy cancer cells and develop long-lasting immunity. For anticancer vaccines the DC precursors loaded with the tumor antigens differentiating into the mature DCs are generally used. These may be monocytes, $CD34^+$ hemopoietic precursors, or circulating DCs. The most frequently used method is the differentiation of the DCs from peripheral blood mononuclear cells taken from a patient (Constantino, Gomes, Falcão, Neves, & Cruz, 2017).

Perspective area in the immune therapy is the development and use of adjuvants *i.e.* compounds providing or enhancing immune response induced by the vaccine antigens *via* stimulation or modulation of the humoral or cellular immune response (Yi, He, Xia, Zhang, & Zhang, 2019). The use of TLR ligands as adjuvants is rationalized by the DCs activation strengthening the CTL function, increase of the IL-2 level, improved migration capacity in response to the CCR7 ligand, induction of the humoral immunity, and stimulation of the $CD4^+$ T cells. The effective TLR ligand for the immune response activation was happened to be imiquimod (ligand for TLR7 and TLR 8) that is commonly used in the treatment of malignant tumors (Adams et al., 2008; Di et al., 2019). Another attractive target is CD40 receptor, which expression on DCs and strong bond with its ligand CD40L on $CD4^+$ T cells results in enhanced production of the co-stimulating molecules and cytokines with the following $CD8^+$ T cell activation. Such cytokines as IL-15, IL-7, and IL-12 were shown to increase survival rate and enhance T-cell function (Sabado & Bhardwaj, 2013).

Immunologic adjuvants are basically presented with inorganic compounds, bacterial products, cytokines and some other substances. This group may be expanded by taking to account the marine algae polysaccharides (Del Guidice, Rappuoli, & Didierlaurent, 2018; Kuznetsova, Zaporozhets, Persianova, Khotimchenko, & Besednova, 2016). Focused on the estimation of the adjuvant properties of carrageenans, Li et al. have studied the antitumor effects of the DC based vaccine preliminary treated with λ-carrageenan (100 μg/mL for 12 h) and loaded with E6 and E7 proteins of the HPV-16 human papillomavirus. This vaccine was tested in mice C57BL/6 with subcutaneously implanted TC-1 cells constitutively expressing the HPV-16 E6 and E7Thus, the weight of the tumor nodes in 30 days after the tumor cell implantation was averagely 3-fold lower than the control values. The vaccine containing λ-carrageenan as an adjuvant dramatically slowed the tumor growth and, therefore, its tumor weight at the end of the experiment was more than 20-fold lower that the control values. Adjuvant effect of λ-carrageenan was comparable to the effect of the CpG-ODN 1826 adjuvant. The tumor growth inhibition shown by both vaccines was accompanied by a strong stimulation of the HPV-specific $CD8^+$ T-cell response driven by splenocytes and suppression of the regulatory T-cells (nTregs) and myeloid-derived suppressor cells (Li et al., 2017). In another experiment authors have investigated the use of carrageenan as an adjuvant regarding its capacity to generate antigen specific immune reactions and antitumor effects in mice that were vaccinated with the vaccine against HPV-16 human papillomavirus E7 protein. It was found out that carrageenan (Sigma) significantly enhances E7 specific $CD8^+$ T cell related immune responses *via* the TLR4 pathway activation and increases protective and therapeutic effects generated by E7 peptide vaccination against E7 expressing tumors (Zhang, Tsai, Monie, Hung, & Wu, 2010). As λ-carrageenan enhances the DC maturing and cytokine function it may be presumed that the carrageenan immune stimulating activity is caused by the activation of the antigen representing cells *via* TLR4 signaling pathway.

Efficacy of the λ-carrageenan used as an adjuvant in the preventing and therapeutic vaccines in mice C57BL was investigated on the tumor E.G7 line model expressing ovalbumin (cell line E.G7-OVA). In the first experiment mice were vaccinated with ovalbumin (5 μg) with or without λ-carrageenan (100 μg) trice per week. In a week since the last immunization these mice were invaded with E.G7-OVA cells and the tumor volume was registered weekly. During the second experiment mice were subcutaneously injected E.G7-OVA cells and when the tumor size reached 3 mm, they were administered the vaccine three times with weeklong intervals. The results showed that this vaccine with λ-carrageenan added as an adjuvant significantly inhibited tumor growth in comparison to the control and OVA group. At the end of the fourth week the average tumor volume was approximately seven times smaller than that in the OVA group. Treatment of mice with the therapeutic vaccine containing only ovalbumin did not show a healing effect. In contrast, injections of OVA/λ-carrageenan inhibited the tumor growth by approximately 30–40 %. When mice were immunized with the preventing vaccine containing λ-carrageenan, 60 % of mice (six from ten) were with no tumors detected for more than 40 days after tumor cell inoculation whereas all mice in the control and ovalbumin group were found to have tumors registered. In a week after final immunization the total anti-OVA immunoglobulin G serum level was significantly higher in mice immunized with OVA/λ-carrageenan and an antibody titer in this group was four times higher in comparison to the OVA group (2000 and 8,000, respectively) (Luo et al., 2015).

Therefore, λ-carrageenan may be recommended for preclinical studies and clinical trials purposed for estimation of its possible application as an adjuvant in the dendritic cell based therapeutic vaccines.

7. Carrageenan antimutagenic activity

Within the last several years the new research area in the investigation of the carrageenan biological properties became the screening of its antimutagenic properties. Compounds that potentially possess mutagenic properties are known to be characterized by their capacity to chemically interact with DNA and provoke genetic damage leading to the development of some chronic and degenerative disorders. Such pathologies, in particular cancer, are induced by DNA damages that were not effectively restored by the cellular DNA reparative enzyme systems. Ineffective restoration of the damaged DNA leads to mutations. Such alterations in the somatic cells may result in cancer. Therefore, there is a demand for the development of the prophylactic strategies using compounds that may exert chemoprotective influence on the genetic material. Among the possible sources of the anticancer chemoprophylactic agents the complex polysaccharides are considered particularly promising because of their various beneficial healthy effects. Protective properties of carrageenan type CSM-2 (Genuvisco®, CP

Kelco) against the influence of methyl-sulfonylmethane was investigated on the cultured meristematic cells *Allium cepa* that are commonly used for the screening of the natural antimutagenic compounds (Fedel-Miyasato et al., 2014). Antimutagenic tests have shown that the studied carrageenan possesses chemoprophylactic activity when applied in all concentrations tested (5, 10, and 20 μg/mL). Percent of the damage reduction varied from 62.54 % to 96.66 % suggesting a high potency of the polysaccharide used for prevention of the mutagenic damage (Nantes et al., 2014). Authors of this work have made a conclusion that carrageenan possesses de-mutagenic activity. In other words, it is capable to adsorb DNA toxic agents and inactivate them. Also, they supposed that carrageenan exerts bio-antimutagenic activity meaning it can modulate enzyme systems of the DNA reparation. It should be mentioned that the first suggestion is likely to be true because carrageenan has certain binding capacity (Khotimchenko, Khozhaenko et al., 2010;Khotimchenko, Kolenchenko et al., 2010 Khotimchenko, Khozhaenko, Khotimchenko, Kolenchenko, & Kovalev, 2010). However, taking to account the data indicating that bio-antimutagenic activity is realized inside the cells and carrageenan is usually a mix of mostly high molecular polysaccharides, we may conclude that the carrageenan entering cells is probably an unlikely event.

The lack of the direct mutagenic effects of the carrageenans themselves (Nantes et al., 2014) and their antimutagenic activity may be considered as an argument for their use as a base for creation of the functional food products or diets with the potential antioxidant and antimutagenic activity.

8. Conclusion

In the present work we have analyzed apparently all the published results of the studies that were devoted to the investigation of antiproliferative and antitumor activities of carrageenans, the sulfated polysaccharides of marine origin produced by red algae. At present there are not too many such sources of data, even less than forty. But, nevertheless, on the base of the main goal that is creation of the new drug or pharmaceuticals for the therapy of malignant tumors, some deductions can be made and some problems that are to be solved may be formulated. If we are going to set such a goal than obviously the high molecular carrageenans as well as the oligocarrageenans with the number of the galactose residues higher than 10 − 20 units may be excluded from the further investigation.

The highest cytotoxic effects registered in the several cancer lines as it was mentioned above were demonstrated in the tests with κ-carrabiose, which half-inhibiting concentrations were similar to those of the officially approved antitumor drugs with alkylating properties. The next important point is the fact that any carrageenan regardless of the alga type it was isolated from is not a sole molecule with the constant molecular weight. Carrageenans are composed of the polysaccharide molecules with different sizes and hence they may be described as the ones possessing some average molecular weight. Besides, chemical structure of such biopolymers varies in dependence on the alga type, growth phase, season of harvesting, and even the method of extraction. All these factors pose the very complex task of experimental compound standardization, which is the mandatory condition for registration and approval of the pharmaceuticals as well as making possible an obtaining the similar results through the different studies performed by different researchers. Reduction of the galactose residue number in the selected carrageenan samples may make this task less complicated whereas all other factors should still be taken into account.

In the gastrointestinal tract carrageenan demonstrates high resistance to the microbial and enzyme degradation, is not absorbed into systemic circulation, and pass through the whole digestive tract without alterations and cannot be detected in blood because of the large size of the native molecules (Uno et al., 2001; Weiner, 2014; Weiner et al., 2015). Determination of the carrageenan molecule size that would contribute their absorption into a system circulation requires investigation of the polysaccharide pharmacokinetics. Indications concerning localization of a tumor that is supposed to be a focus of the possible therapy would depend on the results of these studies. Also, the spectrum of the testing cell lines should be substantially expanded considering the varying sensitivity of the tumor cells to the influence of different pharmaceutical agents. Now the spectrum of the tumor cells available for testing of the oligocarrageenan effects does not exceed ten cell lines and it is rather possible that comparative antiproliferative activity of the κ-, λ-, and ι-oligocarrageenans or their mixtures will be substantially different than then data obtained up to date.

Quite promising areas in the study of the biological activity of carrageenans are considered their possible use as the agents for antitumor immunity activation, the adjuvants of the therapeutic vaccines, and the antimutagenic agents.

Declaration of Competing Interest

The authors declare that they have no competing financial interests.

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