Synthesis and characterization of new pectin derivative with antitumor property

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Introduction: The pectin (Pec) is a linear polysaccharide consisting essentially of α-(1-4)-D-galacturonic acid residues. The Pec is widely used as gelling agent due to thickening properties and also in drug delivery systems due to their excellent biocompatibility and to be sensitive to pH-changes. Furthermore, it is believed that Pec helps to reduce cholesterol level in blood, aids the reduction of glucose uptake, facilitates the excretion of toxins and divalent metals in urine [1] and possesses anti-tumor activity [2]. Attention has been paid to unsaturated derivatives of polysaccharides since they can be crosslinked forming biodegradable and, often, biocompatible hydrogels, in order to various applications as biomaterials [3]. The purposes of this study were to prepare a new unsaturated Pectin derivative through modification with maleic anhydride (MA) and to evaluate the cytotoxic activity of Pec-MA towards cancer cells of colon (Caco-2) targeting to apply as antitumor biomaterial. Thus, the as-obtained derivative (Pec-MA) contains ester linkages and terminal carboxylic groups. **Materials and Methods:** *synthesis of Pec-MA*: dried Pec (1.0 g) was dissolved in DMF (10 mL), while 3.0 g of MA was dissolved in DMF (30 mL). Both solutions were maintained under stirring at room temperature up to solubilization. Then, the MA solution was added to the Pec solution. The reaction was subjected to heating and maintained at 70 °C under stirring for 24 h. Finally, the resulting solution was precipitated in acetone (200 mL), separated by filtration, re-dissolved in distilled water and placed in cellulose tubes to dialysis. The dialysis was performed against deionized water at pH 6.0-6.5 for four days by changing the water twice a day, and then the material was frozen and lyophilized at -55 °C for 72 h. The final product was labeled as Pec-MA. The Pec-MA was characterized through NMR (^{1}H and ^{13}C), FTIR, TGA/DTG and WAXS. Zeta potentials (ZP) were measured on Pec and Pec-MA particles obtained after crosslinking process. Cytotoxicity tests of Pec and Pec-MA against Caco-2 cells were performed as described by Skehan *et al*. [4]. The cells were seeded in 96-well tissue plates at a density of 8×10^5 cell ml^{-1} in 100 µl medium for 24 h in the CO_2 incubator. After incubation for 48 h, the cell monolayers were washed with 100 µl phosphate buffered saline (PBS) sterile, fixed with trichloroacetic acid and stained for 30 min with 0.4% (wt/vol) sulforhodamine B dissolved in 1% acetic acid. The dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris [tris (hydroxymethyl)aminomethane] base for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. **Results:** The presence of vinyl carbons in the Pec-MA structure was evidenced by ^{1}H NMR spectroscopy due the appearance of two new asymmetric peaks at 6.30 and 6.60 ppm. The peak at 6.30 ppm was assigned to vinyl hydrogen atoms adjacent to the carboxylic acid groups of pectin chains, while the peak at 6.60 ppm was attributed to the vinyl hydrogen adjacent to the ester groups. The presence of signal at 136 ppm on ^{13}C NMR spectrum evidenced the presence of vinyl carbons on Pec-MA. The incorporation of maleic acid (MA) in the Pec structure was also confirmed by FTIR spectroscopy. FTIR spectra of Pec and Pec-MA show discrete changes for instance the shift of band at 1750 to 1736 cm^{-1} that was attributed to existence of conjugated esters on Pec-MA structure. The incorporation of MA in the Pec structure increased the proportion of -COOH groups in the Pec-MA as compared to raw Pec. GPC analyses showed that molecular weight of Pec-MA (2.3×10^5 Da) is lower than Pec (2.9×10^5 Da). The surface potential of Pec-MA was -22.8 mV while the surface potential of Pec is -13.0 mV. This difference was most related to the higher amount of carboxylate groups in the structure of Pec-MA as compared to Pec. WAXS profile showed that Pec possesses more organized structure than Pec-MA. TGA/DTG curves showed that thermal stability of Pec is little bit higher than Pec-MA ($\Delta T_{dec} \approx 18$ °C). The Pec-MA showed considerable cytotoxic effect towards Caco-2 cells after 48 h incubation, with an average cytotoxic concentration (CC_{50}) in the range of 25 µg/ml. On the other hand, the CC_{50} for Pec is 140 µg/ml. This result demonstrated that the Pec-MA is much more effective for inhibiting the growth of tumor cells of colon cancer as compared to Pec. Thus, it is suggested that the insertion of MA on Pec results in favoring cytotoxic effect on the sick cells, potentiating the antitumor activity. The higher amount of carboxylate groups on Pec-MA contribute to this behavior mainly lowering the surface potential. This result is in agreement with published studies in which the good property of polymers copolymerized with MA in exhibit antitumor activity is reported [5].

Conclusions: controlled reaction conditions allow preparing a new biomaterial based on pectin-maleate derivative. The cytotoxicity assays towards Caco-2 cells revealed that the Pec-MA was effective to inhibit the growth of tumor cells of colon cancer as compared to Pec.

References: [1] Ogonczyk, D. *et al.,* Biomicrofluids. 2011;5: 013405. [2] Ovodov, Y. Russ. J. Bioorg. Chem. 2009;35:269. [3] Oh, J.K. *et al.,* Prog. Polym. Sci. 2009;12:1261. [4] Skehan, P. *et al.,* J. Nat. Cancer Inst. 1990;82:1107-12. [5] Karakus, G. *et al.,* J. Appl. Polym Sci. 2011;122:2821.