synthesis of water-soluble quaternized chitosans and their antitumor activity

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***Abstract*-water-soluble low-molecular-weight chitosan(LCTS) was prepared by hydrogen peroxide oxidation method and their quaternized derivatives(QLCTS) was also synthesized using previous methods. Their antitumor activity was studied, and the results showed that the LMW and its quaternized derivatives inhibited the growth of pancreatic carcinoma cell strain PANC-1 in vitro, and the inhibitory rate reached 59.2%.**

Keywords-water-souble; quaternized chitosan; antitumor activity

I. INTRODUCTION

Pancreatic cancer is a disease in which malignant cancer cells form in tissues of the pancreas. About 90% of all pancreatic cancers are ductal adenocarcinomas with an overall 5 years survival rate of less than 5%[1-2]. It is difficult to detect and diagnose because there are no obvious signs in the early times. Pancreatic exits behind other organs, so it is difficult to handle with. So it is the most lethal human malignancies, and it the forth leading reason of cancer related death in adults[3]. Though there are several methods, such like radiation therapy and chemotherapy, these treatments obviously have severe shortcomings [4-5]. It is more and more interesting to find some nature drugs such as polysaccharide[6-10].

Chitosan, one of the most abundant natural polysaccharides, with a special designation of poly[β-(1→4)-2-acetamido-2-amino-2-deoxy-D-glucopyranose], is obtained on an industrial scale by the alkaline deacetylation of chitin [11]. It has been attracting people's attention for its unique physiochemical characteristics and bioactivities [12-14]. With the development of the study of chitosan on biomedicine, the antitumor activity of chitosan and its dervaties been studied accordingly.

II. MATERIALS AND METHODS

*A. Ma*terials

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). Its degree of deacetylation was 97%, and the viscosity-average molecular weight was 3.0×10^5 aldehydes, sodium iodide (NaI), sodium borohydride ($NaBH_4$), and iodomethane (CH_3I) were purchased from the Sigma–Aldrich Chemical Co. The other reagents were all analytical grades and were used without further purification. The IR spectra were measured on a Jasco-4100 FT-TR spectrometer with KBr disks. The average viscometric molecular weight of chitosan was estimated from the intrinsic viscosity determined in the solvent 0.1M CH_3COOH/0.2M NaCl using the Marke-Houwink parameter α=0.96, $K\eta$=1.424 at 25℃ when the intrinsic viscosity is expressed in mL g^{-1}.

B. Preparation of low-molecular –weight water-soluble chitosan

LCTS were obtained by using hydrogen peroxide oxidation of chitosan as follows: 5g chitosan were dispersed in 100 mL 6% hydrogen peroxide at 60℃. Then it was precipitated in acetone and filtered, washed with acetone three times, and dried at 60 ℃ in vacuum for 12 h.[15]

C. Preparation of quaternized chitosan

QLCSs were synthesized as follows[16]: 3 g LCTS was dissolved in 100 mL of water, and various aldehydes (3-fold excess to mole mass of chitosan) were added respectively, with stirring at room temperature. After 2 h, 10% $NaBH_4$ (1.5-fold excess relative to mole mass of aldehydes) was added, and the solutions reacted for 2 h. The mixture was precipitated in acetone and filtered. The N-alkyl chitosans were obtained after drying at 60 ℃ in vacuum for 12 h. N-alkyl chitosan (1 g) was dispersed in 50 mL of N-methyl-2-pyrrolidone for 12 h at room temperature. Then, 0.5 mL NaOH (1 M), 1 g NaI and 4 mL CH_3I were added. Each reaction was carried out with stirring at 50 ℃ for 20 h. The product was obtained by precipitation with excess acetone, and the QLCTSs were obtained by drying at 60 ℃ in vacuum for 12 h (Scheme 1).

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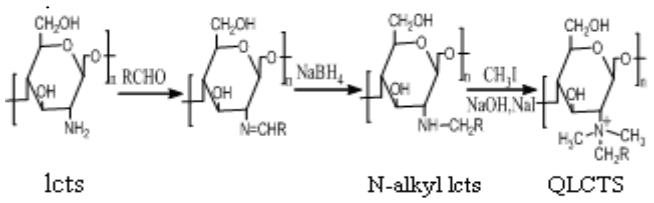

Scheme1: synthesis routes of QLCTS.

EDMLCS: R=-CH_3; PDMLCS: R=-CH_2CH_3; SALLCT: R=2-OH-C_6H_4C-

D. Infrared spectroscopy

LCTS and QLCTS were prepared in the form of potassium bromide (KBr) disks were studied. Each sample (10 mg) was dried to constant weight at 60℃, and then blended with 100 mg of KBr. The mixture was compacted under the infrared lamp to keep dry. The spectra of the sample in the forms of KBr disk were obtained using an FT-IR spectrometer with a frequency range of 4000-400 cm^{-1} .

E. cell lines and culture

The pancreatic carcinoma cell strain PANC-1 maintained with RPMI 1640 medium containing 10% fetal bovine serum and 100 ng/mL, each, of penicillin and streptomycin at 37℃ in a humidified atmosphere with 5% CO_2.

F. Growth inhibiton assay

The inhibition of the samples on the growths of PANC-1were evalutiated in vitro by MTT assay. Briefly, the PANC-1 cells (5×10^4) were incubated in 96-well plates containing 0.100 ml of the culture medium at 37℃ in a humidified atomssphere with 5% CO_2. Cells were permitted to adhere for 24h, then washed with 0.100 mL of phosphate-buffered saline (PBS). One hundred microlitres of different concentrations of LCTS and QLCTS (0.125, 0.250, 0.500, 1.000 mg/mL), prepared in culture medium, were added to each well. After 48 h of exposure, the samples-containing medium was removed, washed with 0.100 mL of PBS and replaced by fresh medium. The cells in each well were then incubated in culture medium with 0.020 mL of a 5 mg/mL solution of MTT for 4 h. After the media were removed, 0.150 mL of DMSO was added to each well. Absorbance at 570 nm (maximum) was determined by a Power Wave X Microplate ELISA Reader (Bio-TeK Instruments, Winoski, VT). The inhibition rate (IR) was calculated according to the formula below:

Growth inhibition rate(%)=

(1-absorbance of experimental group/absorbance of bland control group)×100%

G. Statistical analysis

The data were presented as means±standard deviations of three determinations. Statistical analyses were performed using student's t-test and one way analysis of variance. Multiple comparisons of means were done by the LSD (least significance difference) test. All computations were done by employing statistical software (SAS, version 8.0) .

III. RESULTS AND DISCUSSION

A. Preparation of low-molecular –weight water-soluble chitosan

Table1. Characterization of low molecular weight chitosan

	Mw(×10^3)	DDA(%)	Yield(%)
LCTS	13.3	89.0	47

Compared with the chitosan, its molecule weight has greatly decreased to 13.3×10^3, and has a good water soluble ability. The yield is relatively low.

B. Infrared spectroscopy

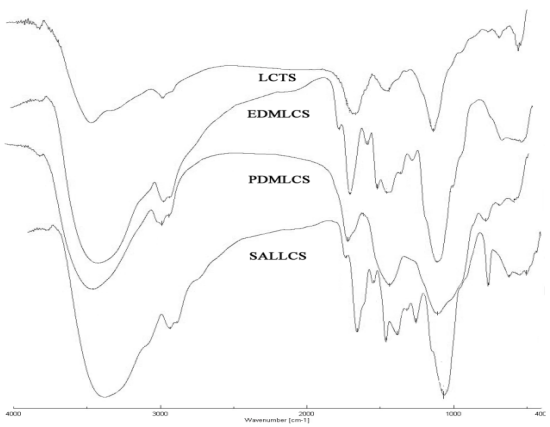

Figure 1: FT-IR spectra of LCTS and QLCTSs.

In the FT-IR spectra of LCTS and the QLCTSs(Figure 1), LCTS was typically characterized by absorption regions as follows[17]: the major peaks of chitosan at about 896, 1087, 1590 and 3420 cm^{-1} belonging to pyranose ring, glucoside, amine and hydroxyl groups, respectively, were identifiable. After quaternization, new peaks appeared at about 1660 cm^{-1}, which were assigned to the quaternary ammonium salt. There were peaks at about 1415-1430 cm^{-1}, which were ascribed to the characteristic absorb of N-CH_3 [18]. The spectrum data indicated that the QLCTSs were obtained.

C. *Growth inhibition on the panc-1 cell line.*

Table2: Growth inhibition of samples at different concentrations against the panc-1 cancer cell line in vitro

	inhibition rate(%)			
	0.125mg/mL	0.250mg/mL	0.500mg/mL	1.000mg/mL
LCTS	13.8	15.4	17.2	22.5
EDMLCS	14.3	16.2	14.7	15.1
PDMLCS	23.4	28.5	24.9	19.8
SALLCS	22.5	20.8	41.4	59.2

Table2 shows the growth inhibition of samples at different concentrations against the panc-1 cancer cell line in vitro . as it shows, the SALLCS has the best inhibition at 1.000mg/ml and reached the 59.2%.

CONCLUSION

In this study, there kinds of quaternized low molecule weight chitosans were synthesized and the antitumor ability was also evaluated against panc-1cell line. From the results, it showed that different QLCTS had different inhibition rate. It suggested the structure had very important relation with the cancer cell. It also proved that chitosan and its quaternized derivatives had some cubic effects against some kind of cancer diseases.As it is well known, the bioactivity of chitosan, is mainly attributes to the active hydroxyl and amino groups. It is a perspective route to develop new drugs for cancer therapy such as chitosan quaternized derivatives.

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