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Anticancer activity of chemically prepared shrimp low molecular weight chitin evaluation with the human monocyte leukaemia cell line, THP-1

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ABSTRACT

In the present study, anticancer activities of chitin, chitosan and low molecular weight chitin were evaluated using a human tumour cell line, THP-1. A molecular weight–activity relationship and an electrostatic interaction–activity relationship were determined. The cytotoxic effects of chitin and derivatives were also evaluated using a normal human foetal lung fibroblastic cell line, MRC-5 and the specific cytotoxicity of chitin and derivatives to tumour cell lines was demonstrated. The high antitumour effect of low molecular weight of chitin was established.

1. Introduction

Chitin is a linear polysaccharide joined by β-(1,4)-linked N-acetylglucosamine (GlcNAc) units [1]. It is the second most abundant natural polymer after cellulose [2]. Their unique properties, biodegradability, biocompatibility and non-toxicity, make them useful for a wide range of applications. Although chitin has very strong functional properties in many areas, the water-insoluble property of α-chitin is disadvantageous for its wide application [3]. In the research field of chitin, functional property has been developed for pharmaceutical and new drug candidate [4,5]. Cytotoxic drugs continue to play a major role in cancer therapy [6]. However, cytotoxic drugs produce side effects, especially the destruction of lymphoid and bone marrow cells. Therefore, strategic improvements in cancer therapy are needed to ameliorate efficiency while decreasing side effects. Most biological activities of chitin are attributed to their free amino groups [7]. Chemical modification of chitin is difficult in general, because chitin is a highly crystalline material with a strongly hydrogen-bonded network structure [8,9]. The purpose of this work is the determination of the anticancer activities of chitin, chitosan and low molecular weight chitin using a human tumour cell line, THP-1. A molecular weight–activity relationship and an electrostatic interaction–activity relationship were evaluated. The cytotoxic effects of chitin and derivatives were also treated using a normal human foetal lung fibroblastic cell line, MRC-5 and the specific cytotoxicity of chitin and derivatives to tumour cell lines was studied. The function of YKL-40 glycoprotein was also investigated.

2. Materials and methods

All chemicals used in this study were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

2.1. Test materials

Shrimp shells were obtained from a seafood restaurant. It was confirmed that all shells were from a single species of shrimp *Parapenaeus longirostris*.

Abbreviations: CHI3L1, chitinase 3-like 1; ^{13}C NMR, 13carbon nuclear magnetic resonance; DNA, desoxyribonucleotid; FBS, foetal bovine serum; FPLC, fast performance liquid chromatography; FT-I, Fourier transform infrared; HC gp-39, human cartilage glycoprotein 39; HL-60 cells, human leukaemia-60 cells; HPAEC, high performance anion exchange chromatography; HT 1080, human tumour HT 1080; IC_{50}, inhibitory concentrations 50; LPS, lipopolysaccharids; MEM, minimum essential medium; PMA, phorbol 12-myristate 13-acetate; RPMI 1640, Roserv Park Memorial Institute; R.S.D., relative standard deviation.

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2.1.1. Chitin extraction

For extraction of chitin, the shrimp shells were washed under running warm tap water to remove soluble organics, adherent proteins and other impurities. The shells were then collected and boiled in water for 1 h to remove the tissue, followed by drying in an oven (Prolabo, model Volca MC18, French) at 160 °C for 2 h to make them more brittle and to breakdown the crystalline structure of chitin [10]. At the end, the dried shells were ground into a fine powder using a standard grinder (Model KU-2, PredomMesko, SkarzyskoKam., Poland).

After that, the powder was demineralized to remove the calcium carbonate. For that, only dilute hydrochloric acid (1.5 M) was used to prevent hydrolysis of chitin [11] within 30 min of incubation. The ratio of dried shells to acid solution used during the extraction of chitin was 1/15 (w/v). The experiments were carried out at room temperature under constant stirring of 150 rpm. The decalcified shells were collected on a 250 μm sieve, washed to neutrality with tap water, rinsed with deionised water, and then oven-dried at 80 °C overnight. The rate of demineralization was evaluated by determining ash contents in the solid.

The demineralized powder was then deproteinized by sodium hydroxide. The sodium hydroxide concentration was 2 M, the reaction time was 120 min and the temperature was 45 °C. At the end of this process the material was filtrated, washed and dried, as previously described in the demineralization process [12]. In order to evaluate the extent of deproteinization, the protein concentration in the supernatant was determined according to Biuret's method [13].

2.1.2. Chitosan preparation

Chitosan was prepared by deacetylation of chitin. The conversion of chitin to chitosan involved deacetylation using the process suggested by Kurita et al. [14]. The parameters employed (i.e. reaction duration, temperature and concentration of alkaline reagent) were as follows: a suspension of 1 g of chitin in 50 mL of aqueous sodium hydroxide, as deacetylation reagent (50% by weight) was mixed at fixed temperature 100 °C under constant stirring. After 3 h, the solid was filtered, washed with water and 80% (v/v) alcohol until the filtrate was neutral. Then it was oven-dried at 80 °C overnight [15].

2.1.3. Low molecular weight chitin preparation

Low molecular weight chitin and low molecular weight chitosan were obtained by hydrolysis of chitin. For that, 1 g chitin was hydrolysed by 50 ml 7 N HCl at 70 °C during 3 h.

2.2. Analytical methods

Dried chitin and derivatives samples (1 mg) were dispersed, separately, in 100 mg of anhydrous KBr and pressed. The IR spectra were recorded at room temperature in the wavenumber range of 400–4000 cm^{-1} and referenced against air with a Nicolet 380 FTIR instrument (Thermoelectron Corporation). A total of 32 scans were averaged for each sample at 4 cm^{-1} resolution.

Average molecular weights of low molecular chitin and low molecular chitosan were measured by FPLC, which incorporated a Amersham Bioscience Instrument and a Superdex™ 200 10/300 GL Tricorn™ high performance column.

The viscosity averaged molecular weights of chitin and chitosan were determined using the Mark–Houwink equation:

$$\eta = K(M_v)^a \quad (1)$$

where η is the intrinsic viscosity of chitin and chitosan, K and a are constants that depend on the polydispersity of chitin and chitosan and the solvent system used, and M_v is the viscosity averaged molecular weights.

The values of constants were previously determined to be $K = 0.24\ cm^3/g$ and $a = 0.69$ at 25 °C for chitin, and $K = 0.078\ cm^3/g$ and $a = 0.76$ at 25 °C for chitosan [16].

HPAEC analyses were carried out on a Dionex ICS-3000 system consisting of a SP gradient pump, an ED electrochemical detector with a gold working electrode, an Ag/AgCl reference electrode and Chromeleon version 6.5 (Dionex Corp., USA). All eluents were degassed by flushing with helium for 30 min.

Separations were performed 25 °C on a CarboPac PA-1 column (4 mm × 250 mm) connected to a CarboPac PA-1 guard column (4 mm × 50 mm Dionex).

^{13}C NMR analyses were achieved at 80 °C with a Bruker Advance 300 spectrometer of 300 MHz equipped with $^{13}C/^{1}H$ dual probe. The ^{13}C NMR experiment was recorded with a spectral width of 3000 Hz, an acquisition time of 1.36 s, a pulse width of 7 μs, a relaxation time of 1 s and a number of 256 scans. The HOD signal was presaturated by a presaturation sequence.

2.3. Cytotoxicity assay

2.3.1. Cytotoxicity assay of MRC-5 cell line

MRC-5 cell line, a normal human foetal lung fibroblastic cell line, was obtained from Pasteur Institute of Algeria. These cells were maintained in MEM supplemented with 10% of FBS, 2 mM of glutamine, 100 UI cm^{-3} of penicillin and 100 μg cm^{-3} of streptomycin. Cultures were maintained in a humidified atmosphere with 5.5% CO_2 at $T = 37$ °C [17].

A monolayer of MRC-5 cells were cultured during one day in plate flasks (capacity 75 cm^3) containing 25 cm^3 of adequate medium. The next day, the growth medium was replaced with exposure adequate medium un-amended and amended with varied concentrations of test agent (1, 50, 250, 500, 1000, 1500, 2000, 2500 and 3000 μg/ml). After 24 h of exposure, cytotoxicity was assessed by means of the Trypan blue exclusion test [18]. All the experiments and measurements were done in triplicate and arithmetic averages were taken throughout the data analysis and calculations.

2.3.2. Cytotoxicity assay of THP-1 cell line

THP-1 cell line, a human monocytic leukaemia cell line, was obtained from Pasteur Institute of Algeria. These cells were maintained in RPMI supplemented with 10% of FBS (foetal bovine serum), 2 mM of glutamine, 1.5 mg/ml of glucose and 100 UI cm^{-3} of penicillin and 100 μg/ml streptomycin [19].

A monolayer of THP-1 cells were cultured during one day in plate flasks (capacity 75 cm^3) containing 25 cm^3 of adequate medium. The next day, the growth medium was replaced with exposure adequate medium un-amended and amended with varied concentrations of test agent (1, 50, 250, 500, 1000, 1500 and 2000 μg/ml). After 24 h of exposure, cytotoxicity was assessed by means of the Trypan blue exclusion test [20]. All the experiments and measurements were done in triplicate and arithmetic averages were taken throughout the data analysis and calculations.

2.4. Trypan blue exclusion assay

About 50 μl of cell suspension was sampled and mixed with an equal volume of 0.5% Trypan blue. The total number of live cells was then counted using a haemocytometer under light microscopy [21].

The cytotoxic activity was calculated by the following relation established by [22]:

$$\text{Cytotoxic activity}\,(\%) = 100 \times \frac{\%\,\text{sample} - \%\,\text{control}}{100 - \%\,\text{control}} \quad (2)$$