

Cytotoxic Activity of Chitin Derivatives Evaluation with Human Tumour Cell Lines

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Abstract: Chitin is a long-chain polymer of a N-acetylglucosamine, a derivative of glucose, and is found in many places throughout the natural world. It is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans. Due to their biocompatibility and less toxic nature, it has been developed as new physiologically bioactive materials since they possess various biological activities. Chemical extraction of chitin from shells, of the white shrimp *Parapenaeus longirostris* (Lucas, 1846), produces a chitin with a high viscosity, a low molecular weight and a high degree of deacetylation. In the present study, chemical modifications of chitin produced low molecular weight carboxymethyl chitin, chitooligosaccharides and 2-phtalimido chitosan. Also, anticancer activities of chitin derivatives were evaluated using the human rhabdosarcoma cell line, RD, the human leukaemia cell line, THP-1, and the human larynx cancer cell line, HEp-2. The cytotoxic effects of chitin derivatives were also evaluated using a normal human foetal lung fibroblastic cell line, MRC-5. The specific cytotoxicity of chitin derivatives to tumour cell lines was demonstrated, and the high antitumor effect of chitin derivatives was established. Furthermore, a specific interaction was suggested.

Keywords: anticancer activity, cell lines, chitooligosaccharides, HEp-2, low molecular weight carboxymethyl chitin, MRC-5, 2-phtalimido chitosan, RD, THP-1.

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 antitumour activities of chitin derivatives using the human rhabdosarcoma cell line, RD, the human leukaemia cell line, THP-1, and the human larynx cancer cell line, HEp-2. An electrostatic interaction-activity relationship was evaluated. The specific cytotoxicity of chitin derivatives to tumour cell lines was studied using the normal human foetal lung fibroblastic cell line, MRC-5.

2. Materials and methods

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

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* (Lucas, 1846).

Chitin was extracted from shell waste of the white shrimp *Parapenaeus longirostris* (Lucas, 1846) by sequential treatments with HCl (demineralisation) and NaOH (deproteinisation) [10]. Chitosan was prepared by deacetylation of chitin [11]. Low molecular weight carboxymethyl chitin was prepared by method of Tokura, et al., (1983) [12]. 2-phtalimido chitosan was chemically prepared from chitosan [13]. Chitooligosaccharides was produced by depolymerisation of chitosan [14].

2.2. Cell lines

HEp-2 cell line, which is a human larynx cancer cell line, was obtained from Algerian Pasteur Institute. These cells were maintained in MEM (Minimum Essential Medium) supplemented with 10% of FBS (foetal bovine serum), 2mM of glutamine and 100 UI/mL of penicillin. Cultures were maintained in a humidified atmosphere with 5.5% CO₂ at 37°C.

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/mL of penicillin and 100µg/mL of streptomycin. Cultures were maintained in a humidified atmosphere with 5.5% CO₂ at 37 °C.

RD cell line, which is a human rhabdosarcoma cell line, was obtained from Algerian Pasteur Institute. These cells were maintained MEM supplemented with 10% of FBS, 2mM of glutamine and 100 UI/mL of penicillin. Cultures were maintained in a humidified atmosphere with 5.5% CO₂ at 37°C.

THP-1 cell line, a human monocytic leukaemia cell line, was obtained from Pasteur Institute of Algeria. These cells were maintained in RPMI 1640 (Roserv Park Memorial Institute) supplemented with 10% of FBS, 2 mM of glutamine, 1.5 mg/mL of glucose and 100 UI/mL of penicillin and 100µg/mL streptomycin.

2.3. Cytotoxicity assay

MRC-5 cells were cultured for one day in a 75mL flask plates containing sufficient cells in adequate medium. The next day, that growth medium was replaced with exposure medium un-amended and amended with varied concentrations of test agents (0, 1, 50, 250, 500, 1000, 1500, 2000, 2500 and 3000 µg/mL). After 24 h of exposure, cytotoxicity was assessed with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test.

2.4. Antitumour assay

Tumour cells were cultured for one day in a 75mL flask plates containing sufficient cells in adequate medium. The next day, that growth medium was replaced with exposure medium un-amended and amended with varied concentrations of test agents. After 24 h of exposure, cytotoxicity was assessed with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test [15].

3. Results and discussion

The cytotoxic effects of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides on a human normal foetal lung fibroblastic cell line, MRC-5 have been evaluated. The results (Fig. 1) indicate that low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides exhibited no cytotoxic effects at concentrations inferior or equal to 2000 µg/mL.

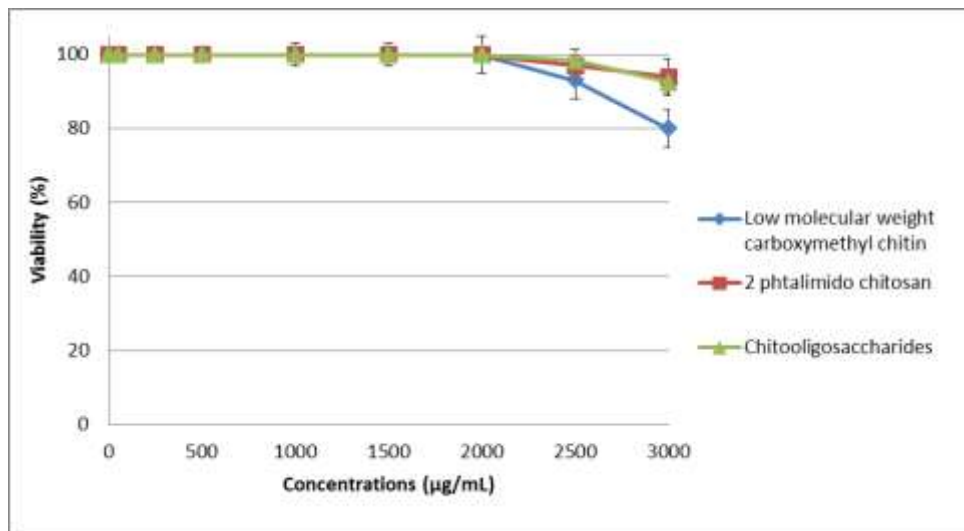


Fig. 1: Cytotoxic activity of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides against MRC-5 cell line.

The influence of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides on the growth of THP-1 cancer cell line was determined using noncytotoxic concentrations ($\leq 2000 \mu\text{g/mL}$) of each compound on normal human lung fibroblasts, MRC-5. The results, presented in Fig. 2, indicate that chitooligosaccharides have the potential to suppress 100% of the growth of THP-1 tumour cells at concentrations equal or superior to $250 \mu\text{g/mL}$. On the other hand, low molecular weight carboxymethyl chitin has the potential to suppress 100% of the growth of THP-1 tumour cells at $500 \mu\text{g/mL}$. However, 2 phtalimido chitosan has the potential to suppress 100% of the growth of THP-1 tumour cells at $1500 \mu\text{g/mL}$. These results suggest that low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides are able to develop an inhibitory effect on the proliferation of the THP-1 cell line. Indeed, chitooligosaccharides were highly tumour-suppressive.

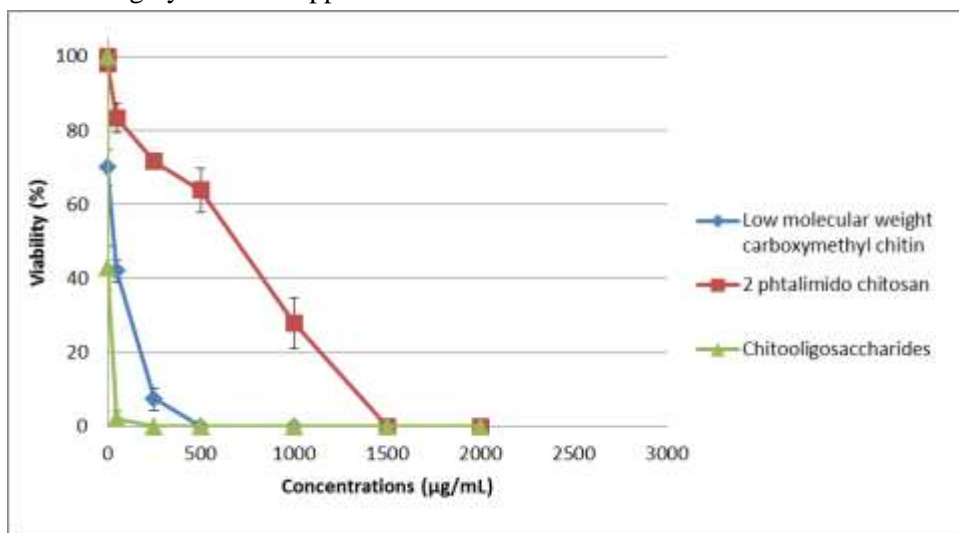


Fig. 2: Antitumour activity of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides against THP-1 cell line.

Chitooligosaccharides were highly tumour-suppressive. The IC_{50} value of chitooligosaccharides was $15 \mu\text{g/mL}$ for chitooliosaccharides (Table 1). These results suggest that tumour suppression increases significantly with the decrease of the molecular weight.

TABLE 1: Inhibitory concentrations 50 for chitin derivatives against RD cell line.

Compounds	IC ₅₀ (µg/mL)
Chitooligosaccharides	15
Low molecular weight carboxymethyl chitin	124
2 phtalimido chitosan	685

The cytotoxic effects of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides on the human rhabdosarcoma cell line, RD have been evaluated. The influence of chitin derivatives was determined, using concentrations inferior to 2000µg/mL, on RD cell line. The results, presented in Fig. 3, indicate that 2phtalimido chitosan has the potential to suppress 36% of the growth of RD tumour cells at concentrations equal or superior to 2000µg/mL. However, chitooligosaccharides have the potential to suppress 100% of the growth of RD tumour cells at 1500µg/mL. Also, low molecular weight carboxymethyl chitin has the potential to suppress 100% of the growth of RD tumour cells at 500µg/mL. These results suggest that chitin derivatives are able to develop an inhibitory effect on the proliferation of the RD cell line.

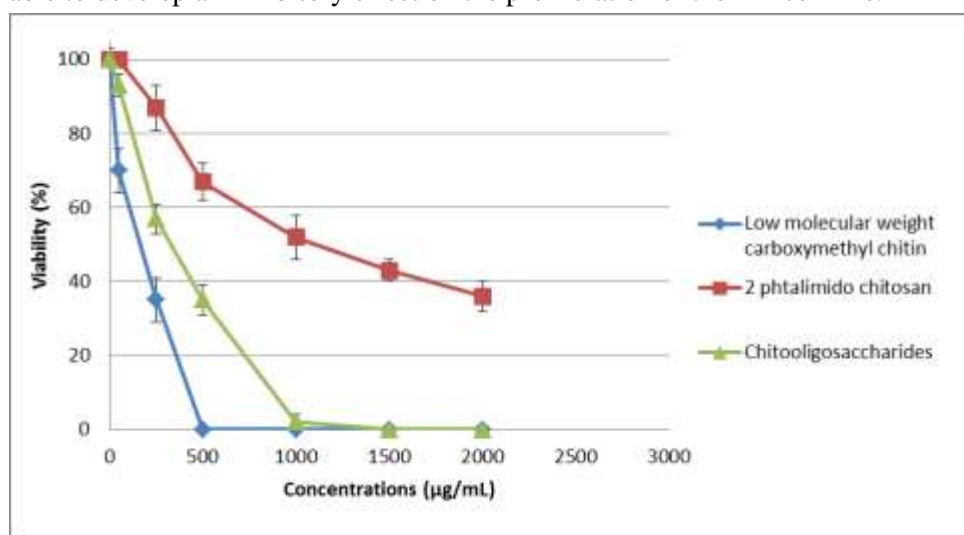


Fig. 3: Cytotoxic activity of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides against RD cell line.

The lowest IC₅₀ value was 216µg/mL for low molecular weight carboxymethyl chitin (Table 2).

TABLE 2: Inhibitory concentrations 50 for chitin derivatives against RD cell line.

Compounds	IC ₅₀ (µg/mL)
Low molecular weight carboxymethyl chitin	216
Chitooligosaccharides	843
2 phtalimido chitosan	1330

The cytotoxic effects of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides on the human larynx cancer cell line, HEp-2 have been evaluated. The influence of chitin derivatives was determined, using concentrations inferior to 2000µg/mL, on HEp-2 cell line. The results, presented in Fig. 4, indicate that 2 phtalimido chitosan has the potential to suppress 55% of the growth of HEp-2 tumour cells at concentrations equal or superior to 2000µg/mL. However, chitooligosaccharides have the potential to suppress 100% of the growth of HEp-2 tumour cells at 1000µg/mL. Conversely, low molecular weight carboxymethyl chitin has the potential to suppress 100% of the growth of HEp-2 tumour cells at 250µg/mL. These results suggest that chitin derivatives are able to develop an inhibitory effect on the proliferation of the HEp-2 cell line.

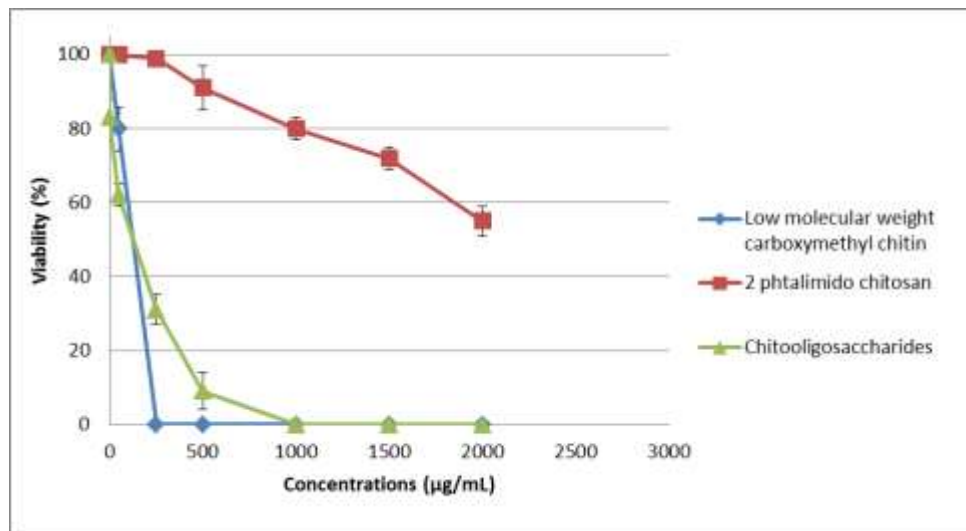


Fig. 4: Cytotoxic activity of low molecular weight carboxymethyl chitin, 2-phtalimido chitosan and chitooligosaccharides against HEP-2 cell line.

The lowest IC₅₀ value was 125µg/mL for low molecular weight carboxymethyl chitin (Table 3).

TABLE 3: Inhibitory concentrations 50 for chitin derivatives against HEP-2 cell line.

Compounds	IC ₅₀ (µg/mL)
Low molecular weight carboxymethyl chitin	125
Chitooligosaccharides	157
2 phtalimido chitosan	-

The antitumor effect of chitin derivatives may be explained by an electrostatic interaction between the charges of the anticancer products utilized and charged functional residues existing on internal components and the tumour cell surface [16, 17]. Such structure-activity relationships suggest the existence of biological systems which could recognize the configurationally structures of compounds.

The exact mechanism of action of chitin derivatives is still unknown, but different mechanisms can be proposed:

1. Chitin derivatives can increase the permeability of cell membrane, and ultimately disrupt cell membranes with the release of cellular content [18].

2. Chitin derivatives can precipitate and stack on the cell surface, thereby forming an impervious layer around the cell. Such a layer can be expected to prevent the transport of essential solutes and may also destabilize the cell wall beyond repair thereby causing severe leakage of cell constituents and ultimately cell death [19].

3. The cationic nature of chitin derivatives causes it to bind with sialic acid in phospholipids of the cell membrane, consequently restraining the movement of the cell constituents [20].

4. Chitin derivatives can bind on the cell membrane to form a film around the cells, so the transport of nutrient into the cells is disturbed [21].

5. Chitin derivatives can interact with a chitin binding protein, expressed on the cell membrane of HEP-2 and RD cell lines, like YKL-40 protein [22].

4. Conclusion

Chitin derivatives are attractive target for selective anticancer drug development. In fact, chitin derivatives prepared from the white shrimp *Parapenaeus longirostris* (Lucas, 1846) showed a great and specific anticancer effect on the human rhabdosarcoma cell line, RD, the human leukaemia cell line, THP-1, and the human larynx cancer cell line, HEP-2. Thus, chitin derivatives have promising roles in natural cancer prevention and treatment.

The higher order structure of active chitin derivatives, and the details of their mechanisms of action in the host are now under investigation, especially to clarify the entity intervening between polysaccharides and tumour.

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