Tumoricidal effect of O-carboxymethyl chitin, N,O-carboxymethyl chitosan and 2-phtalimido chitin evaluation with human tumour cell line

Rym Salah[1], Djaber Tazdaït[2], and Nabil Mameri[3]

***Abstract*—**Chitin is a nitrogen-containing polysaccharide, related chemically to cellulose. It is a principal constituent of the exoskeleton 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 chitosan, O-carboxymethyl chitin, N,O-carboxymethyl chitosan and 2-phtalimido chitin. Also, cytotoxic activities of chitin derivatives were evaluated using the human cell line, MRC-5. Furthermore, anticancer activities of chitin derivatives were evaluated using the human leukaemia cell line, THP-1. The specific cytotoxicity of chitin derivatives to the tumour cell line was demonstrated, and the high antitumor effect of chitin derivatives was established. Furthermore, a specific interaction was suggested.

***Keywords*—**Anticancer activity, O-carboxymethyl chitin, N,O-carboxymethyl chitosan, 2-phtalimido chitin, THP-1 and MRC-5 cell lines.

I. Introduction

Two centuries after its discovery, it is widely accepted that chitin is an important biomaterial in many aspects. It is a linear polysaccharide formed by β-(1,4)-linked N-acetylglucosamine units [1]. Chitin is one of the most abundant renewable biopolymer on earth that can be obtained as a cheap renewable biopolymer from marine sources [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]-[6]. Cytotoxic drugs continue to play a major role in cancer therapy [7]. 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 [8]. Chemical modification of chitin is difficult in general, because chitin is a highly crystalline material with a strongly hydrogen-bonded network structure [9]-[10]. The purpose of this work is the determination of the antitumour activities of chitin derivatives using the human leukaemia cell line, THP-1. The specific cytotoxicity of chitin derivatives to the tumour cell line was studied. In our knowledge, there is no study on the anticancer activities of O carboxymethyl chitin, N,O carboxymethyl chitosan, and 2 phtalimido chitin evaluation on human leukaemia cell line, THP-1.

Rym Salah[1] is with the Mouloud Mammeri University of Tizi-Ouzou, Algeria (corresponding author's phone: +213699370987; e-mail: rymsalah4@gmail.com).

Djaber Tazdaït[2] is with the Mouloud Mammeri University of Tizi-Ouzou, Algeria.

Nabil Mameri[3] is with the University of Technology of Compiègne, France.

II. Materials And Methods

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

A. Test Materials

Shrimp shells were acquired from a seafood restaurant. It was established 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).

The conversion of chitin to chitosan involved deacetylation using NaOH. The parameters used (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 (50% by weight) was mixed at fixed temperature 100 °C under constant stirring [4].

O-carboxymethyl chitin was prepared by reacting chitin with NaOH and SDS (Sodium Dodecyl Sulphate) at 20°C during 12 hours, then adding isopropanol and monochloroacetic acid at 25°C.

Carboxymethylation of chitosan was carried out by stirring chitosan in NaOH for 65 min then monochloroacetic acid was added dropwise to the reaction mixture and the reaction was continued for 3 h at 60 °C with stirring [11].

2-phtalimido chitin was chemically as described by [12].

B. Cell Lines

MRC-5 cell line is a normal human foetal lung fibroblastic cell line. It was obtained from Pasteur Institute of Algeria. These cells were maintained in MEM (Minimum Essential Medium) supplemented with 10 % of FBS (Foetal Bovine Serum), 2 mM of glutamine, 100 UI/mL of penicillin and 100

µg/mL of streptomycin. Cultures were maintained in a humidified atmosphere with 5.5 % CO_2 at T = 37 °C.

THP-1 cell line is a human monocytic leukaemia cancer cell line. It was obtained from Algerian Pasteur Institute. These cells were maintained in RPMI 1640 medium (Roserv Park Memorial Institute) supplemented with 10 % of FBS, 2 mM of glutamine, 1.5 mg/mL of glucose, 100 µg/mL of streptomycin and 100 UI/mL of penicillin. Cultures were maintained in a humidified atmosphere with 5.5 % CO_2 at 37 °C.

C. MTT Assay

Enzyme-based method using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) rely on a reductive coloring reagent and dehydrogenase in a viable cell to determine cell viability with a colorimetric method. This method is easy-to-use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. Among the enzyme-based assays, the MTT assay is the best known method for determining mitochondrial dehydrogenase activities in the living cells. In the method, MTT is reduced to a purple formazan by NADH. However, MTT formazan is insoluble in water, and it forms purple needleshaped crystals in the cells. Therefore prior to measuring the absorbance, an organic solvent is required to solubilize the crystals.

A monolayer of THP-1 cells were cultured during one day in plate flasks (capacity 75 mL) containing 25 mL 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. After 44 h of treatment, 10 µL of MTT (10 mg/mL in distilled water) was added in each well. The 96-well plate was incubated for 4 h at 26 °C in dark followed by the addition of 100 µL of DMSO (dimethylsulfoxide) in each well. Then, the 96-well plate was incubated for 30 min at 26 °C in dark and optical density was read at 490 nm on an ELISA reader.

III. Results And Discussion

The cytotoxic effects of chitin derivatives on a human normal foetal lung fibroblastic cell line, MRC-5 have been evaluated. The results (Fig. 1) indicate that chitin derivatives exhibited no cytotoxic effects at concentrations inferior or equal to 2000 µg/ml.

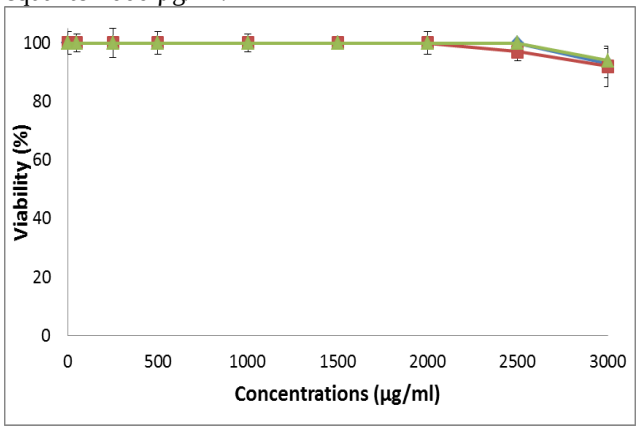

Fig. 1 Cytotoxic activity of O carboxymethyl chitin ( ), N,O carboxymethyl chitin ( ) and 2 phtalimido chitin ( ) against MRC-5 cell line

The antitumour effects of O-carboxymethyl chitin, N,O-carboxymethyl chitosan and 2-phtalimido chitin on the human leukaemia cell line, THP-1 have been evaluated. The influence of chitin derivatives was determined, using concentrations inferior to 2000 µg/mL, on THP-1 cell line. The results, presented in Fig. 2, indicate that chitin derivatives have the potential to suppress 100% of the growth of THP-1 tumour cells at concentrations equal or superior to 1500 µg/mL. The lowest IC_{50} (Inhibition Concentration 50) value was 445 µg/mL for carboxymethyl chitin (Table 1).

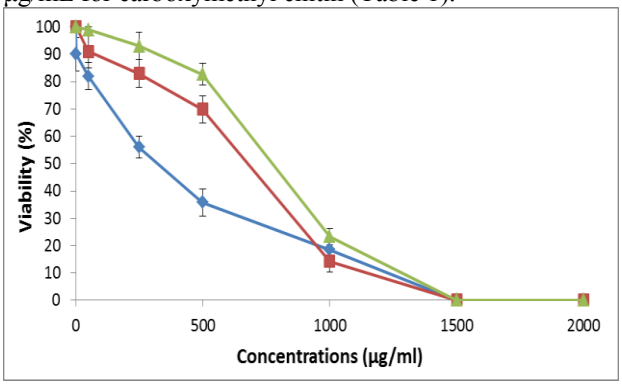

Fig. 2 Cytotoxic activity of O carboxymethyl chitin ( ), N,O carboxymethyl chitin ( ) and 2 phtalimido chitin ( ) against THP-1 cell line

TABLE 1
INHIBITORY CONCENTRATIONS 50 FOR CHITIN AND DERIVATIVES AGAINST THP-1 CELL LINE

Comounds	IC_{50} (µg/mL)
O carboxymethyl chitin	445
N,O carboxymethyl chitosan	689
2 phtalimido chitin	777

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 [13]-[14]. 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 [15].
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 [16].
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 [17].
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 [18].

5. Chitin derivatives can interact with a chitin binding protein, expressed on the cell membrane of THP-1 cell line, like YKL-40 protein [4].

YKL-40, also known as chitinase 3-like 1 (CHI3L1) or human cartilage glycoprotein 39 (HC gp-39), is a 40 kDa mammalian glycoprotein and member of glycosyl hydrolase family 18. THP-1 cells express YKL-40 [19]-[20]. However, MRC-5 cells do not express YKL-40 glycoprotein [21]-[22]. The highest plasma YKL-40 levels have been found in patients with metastatic disease [20]-[23].

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