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Cancer chemopreventive and anti-inflammatory activities of chemically modified guar gum

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Abstract

Guar gum (G) is a simple characterized branched polysaccharide, which is frequently used in food industries. We prepared the gum *C*-glycosylated derivative (GG), and its sulphated derivative (SGG), aiming to characterize their cancer chemopreventive, and anti-inflammatory properties. Estimation of cancer chemopreventive activity, specifically anti-initiation, including the modulation of carcinogen metabolism and the antioxidant capacity, revealed that GG was a potent anti-initiator, where it inhibited not only the carcinogen activator enzyme, cytochrome P450 1A (CYP1A), but also induced the carcinogen detoxification enzymes glutathione-*S*-transferases (GSTs), while SGG inhibited both CYP1A and GSTs. SGG was an effective radical scavenger than GG against hydroxyl, peroxyl, and superoxide anion radicals. GG and SGG were found to modulate the macrophage functions into an anti-inflammatory pattern. Thus, both enhanced the macrophage proliferation and phagocytosis of fluorescein isothiocyanate (FITC)–zymosan; however, they also inhibited strongly the nitric oxide generation and tumor necrosis factor-α secretion in lipopolysaccharide (LPS)-stimulated RAW macrophage 264.7. Unexpectedly, both GG and SGG dramatically inhibited the binding affinity of FITC–LPS to RAW 264.7, as indicated by flow cytometry analysis. GG and SGG exhibited a significant anti-proliferative activity against human hepatocellular carcinoma cells (Hep G2), and only SGG was specifically cytotoxic for human breast carcinoma cells (MCF-7), but neither was significantly cytotoxic for human lymphoblastic leukemia cells (1301). SGG led to a major disturbance in cell cycle phases of Hep G2 cells as indicated by concomitant arrest in S- and G2/M-phases, a disturbance that was associated with an induced cell death as a result of necrosis, but not apoptosis in both GG- and SGG-treated cells. Taken together, the modified gums could be used as an alternative of G in health food industries to provide cancer prevention in risk populations.

Keywords: Guar gum; Cytochrome P450; Cancer chemoprevention; Anti-initiating; Anti-inflammatory; Anti-promoting; Macrophage function; Apoptosis; Necrosis

1. Introduction

In cancer multi-stage cascade, normal cells undergo initiation, promotion, and progression processes. Understanding of the cellular and molecular basis of the carcinogenesis cascade provides a targeted approach for cancer chemoprevention, which aims to halt or reverse the development and progression of precancerous cells through use of non-cytotoxic doses of nutrients and/or pharmacological agents [1]. Identification of new effective cancer chemopreventive agents has become an important worldwide strategy in cancer prevention.

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Guar gum (G) is simple, non-ionic and best-characterized branched polysaccharide extracted from *Cyamopsis tetragonoloba* seeds. It is also called guaran, clusterbean, Calcutta lucern, Gum cyamopsis, Cyamopsis gum, Guarina, Glucotard, Guyarem, and others. The composition of G was found to be 75% soluble fiber, 7.6% insoluble fiber, 2.16% crude protein, 0.78% total lipids, 0.54% ash, and 9.55% moisture [2]. Gum is a high molecular weight galactomannan consisting of a (1 → 4)-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose (i.e. 1 → 6-linked-α-D-galactopyranose) [3]. Generally, galactomannans properties depend on their chemical structure, such as chain length, availability of *cis*-OH groups, steric hindrance, degree of polymerization, and additional substitutions [4], which resulted in considerable variations in their functional characteristics. Many sulphated polysaccharides were reported to have in vitro antiviral and anticoagulant activity, which was attributed to the presence of the negatively charged sulphate groups [5,6]. In previous studies, G was found to have the ability to bind toxic substances to carry them out of the body, and to significantly decrease the levels of blood sugar, cholesterol, triglycerides, and lipids in normal and diabetic rodents [2,7]. Depending on its viscosity properties after its hydration, G has a wide variety of non-food and food uses as a stiffener in ice cream, yogurt, bakery, and soups; as a stabilizer for cheeses, puddings, and cream; and as a meat binder [8,9]. G is also available in the health stores as a part of a formula for healthy bowel activity, weight loss, and diabetes control.

The object of this study was to modify a cheap, widely used, and biologically inactive polymer into a biologically active one. Therefore, the present work aimed to prepare the guar gum *C*-glycosylated derivative (GG) and to submit GG to an additional sulphation to afford SGG. The ultimate goal was to investigate the possible cancer chemopreventive activity of those derivatives, aiming to use them as alternatives of G in health food industries, to provide potential cancer chemopreventive and/or anti-inflammatory properties for high-risk populations.

2. Material and methods

2.1. Preparation and analysis of guar gum derivatives

G was purchased from Sigma (USA), as well as all reagents and solvents. GG was prepared according to [10]. A solution of G (200 mg/20 ml H_2O), $NaHCO_3$ (200 mg), and pentane-2,4-dione (300 μl) was stirred under reflux at 100 °C for 20 h. The solution was diluted and neutralized with Dowex 50 resin (H^+ form). The resin was filtered off and dialyzed against distilled water, and then 100 mg $NaBH_4$ was added to the retentate. The reaction mixture was stirred for 24 h at room temperature; the excess of $NaBH_4$ was neutralized by glacial acetic acid. The solution was dialyzed against running distilled water for 48 h and repeated precipitation with absolute ethanol, and then lyophilized. The sulphation of GG derivative to prepare SGG was performed by stirring of 0.3 g of GG with 2 ml of dry formamide for 24 h at room temperature. A complex of SO_3·formamide was prepared by dropping 5 ml of $HClSO_3$ in 20 ml of formamide in an ice-water bath before incubation with the GG-mixture for 24 h at room temperature. The reaction was stopped by cooling and neutralized by 30% NaOH. The mixture was dialyzed against running distilled water, then lyophilized.

2.1.1. Chemical analysis of GG and SGG

The total carbohydrate of prepared GG and SGG was determined by phenol–sulphuric acid assay [11], and the total protein was assayed according to [12]. The sugar composition was determined after complete hydrolysis with H_2SO_4 (2 mol/l) at 100 °C for 8 h. The mixture was neutralized with $BaCO_3$, centrifuged, filtered, neutralized with Dowex 50 resin (H^+ form), and concentrated. The paper chromatography of hydrolysates was carried out by the descending method on Whatman No. 1 paper using butanol–acetone–water (4:5:1). The sugars were detected by aniline phthalate [13]. The sulphate content was determined after hydrolysis of SGG with HCl [14] and liberated sulphate ions were determined by the $BaCl_2$ turbidimetric method [15].

2.2. Cell culture

Several cell lines were used in this study including: human lymphoblastic leukemia (1301), a generous gift from The Training Center of DakoCytomation, Elly, UK; human hepatocarcinoma (Hep G2); human breast carcinoma (MCF-7), both were a generous gift from Prof. Mohamad A. Ali, Virology Laboratory, NRC, Cairo, Egypt; and RAW murine macrophage (RAW 264.7) was purchased from ATCC, USA. Cells were routinely cultured in Dulbeco's Modified Eagle's Medium (DMEM), except RAW 264.7 cells, which were grown in RPMI-1640. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 unit/ml penicillin G sodium, 100 unit/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained