Synthesis, Characterization and Pharmacological Assessment of Cellulose Nanowhiskers Based Drug Delivery System

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Abstract

 Doxorubicin represents the most powerful antineoplastic agent available in most of cancer treatment protocols. It acts by different mechanisms, mainly by intercalation with cellular DNA, production of reactive oxygen species ROS and inhibition of topoisomerase TOP IIα

 The use of Doxorubicin is associated with world wild problem which is cancer cell resistance. Therefore Cellulose Nanowhiskers (CNWs) as a novel nancarriers were extracted and incorporated chemically with Doxorubicin as an attempt to increase its intracellular concentration and reduced its resistance. CNWs were extracted from commercial cotton, structurally modified and linked with a biological linker in a way to be capable for incorporation with Doxorubicin (DOX). The nanosized particles had been characterized by Atomic force microscopy(AFM), Scanning electron microscopy (SEM) and X-ray diffraction (XRD).The synthesis of the target compounds (I-III) had been successfully achieved and the characterization and identification were took place using Fourier transform infrared spectroscopy (FT-IR) and 1H-Nuclear magnetic resonance (1H-NMR) spectroscopy.

 The In vivo study of the antitumor effect of the final compound was evaluated using mammary adenocarcinoma cell line (AM3)-induced mice, which approved that compound (III) produced significant reduction in tumor size with suspended antitumor effect compared to the control group (DOX).

الخلاصة :

يعتبر الدوكسوروبيسين من الادوية الشائعة الاستعمال كمضاد فعال للسرطان والمتواجد بكثرة في معظم بروتوكولات العلاج العالمية للسرطان. يعمل هذا الدواء كمضاد سرطان بعدة آليات, أهمها التداخل مع الحامض النووي للخلية السرطانية ,انتاج مشتقات الاوكسجين النشطة وكذلك من خلال تبيط انتاج انزيم التوبوايزومريز(2) . يعاني هذا الدواء من مشكلة كبيرة وهي (مقاومة الخلايا السرطانية) له والتي تعتبر من اكثر المشاكل المسسببة بأنحدار استعماله عالميآ, لذلك في هذا البحث تم تحضير السيليلوز نانووايسكر كناقل نانوي آمن للدوكسوروبيسين بعدما تم استخلاصه ومعالجته كيميائيآ. ويعتبر هذا العمل بمثابة محاولة لزيادة مستويات الدوكسوروبيسين داخل الخلايا السرطانية و بالتالي تقليل نسبة المقاومة له. تم استخلاص السيليلوز نانووايسكر من القطن الطبي التجاري بواسطة التحلل الحامضي وتمت معاملته كيميائيآ و ثم تمت إضافة مركب اميني يعمل عمل ذراع بايولوجية رابطة لأجل جعل المركب الناقل مهيئآ تمامآ للربط مع الدواء.

تمت دراسة تأثير المركب المصنع كمضاد سرطان محمل على الناقل النانوي داخل جسم الكائن الحي من خلال دراسة فعاليته على مجموعة من الفئران المختبرية الحاملة لخلايا سرطانيه ( سرطان الغدد الثديية) حيث وجدَ ان فعالية المركب المصنع تضاهي فعالية الدوكسوروبيسين وحده من خلال ملاحظة النقصان الكبير في حجم الأورام الخاص بالفئران التي تمت معالجتها بالمركب المصنع وبصورة مستمرة مقارنةً مع الفئران المعالجة بالدوكسوروبيسين وحده. .

Key words Cellulose nanowhiskers(CNWs) , Nanoparticles, GABA, Doxorubicin

1- Introduction

 In the past, treatment strategies diagnosed many pathological cases as incurable fatal diseases(1) but now days numerous medication approaches have been developed to treat such complicated cases, some of the newly introduced medications found to have serious side effects (2) and others were absolutely useless within the biological fluids i.e. cannot withstand the acidic media or enzymatic hydrolysis of the gastrointestinal tract(GIT) if they give orally, making them absolutely worthless in vivo (3).Thus many studies focused on introducing better medication with improved delivery and targeting abilities (4).

 Within the previous few decades; a great attention focused on the nanotechnology science as a broad area for research and development (5). Applying nanotechnology to medicine, nanoparticles are designed to alter or mimic biological processes (8). Nanoparticles are solid, colloidal, with a size between 10nm -1000 nm, in the medical field less than 200nm is desirable (9). There is a huge increase in the medical applications of nanotechnology mainly due to the unique properties of the nanoparticles such as high surface area, hydrophilicity and long circulation time as compared with small molecules. It’s very known that the efficiency of most of drug delivery systems is directly proportional to the particle size [except intravenous and solution preparations]. The small particle size of nanoparticles and high surface area increase their solubility, bioavailability, enhance the ability to cross blood brain barrier (BBB) and enhance the entrance to the pulmonary systems (13). The characterization of the nanoparticles determine the size, shape and surface changes of the tested nanoparticles using different microscopic techniques including Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (55). Cellulose represents one of the most abundant renewable material on the planet, could be used in future as an oil-replacing based feed stocks. In the mid-19th century the first plastic materials were derived from cellulose as a cellulose nitrate. The need for natural raw materials increased from the Second World War when the supplies of raw materials were limited (64). Cellulose produced by the condensation polymerization of glucose monomers in plants, bacteria and some animals (tunicates).

2. Experiment

All chemicals used in this work were used without further purification as they received from the suppliers; Sigma-Aldrich, Adamas and Merck. General methods used for the analysis of the synthesized products are: Atomic force microscopic analysis was done in University of Baghdad-Collage of Science- Department of Chemistry. Infrared spectra were recorded as KBR disc by using FT-IR spectrophotometer in University of Baghdad- College of science – Department of Chemistry. The 1H-NMR spectra was performed at University of Moscow-Russia. Instrument Model: Bruker 400 MHz. This microscopic technique performed in University of Baghdad- College of science – Department of Chemistry. X-ray diffraction performed in University of Baghdad- College of science – Department of Chemistry. The preliminary anticancer study was performed at the Iraqi center for cancer and medical genetic researches (ICCMGR)

2.1. Preparation of Cellulose nanowhiskers (CNWs)

 In this method, about 60 g of commercial cotton was mixed with 1L of boiling hydrochloric acid (2.5 N HCl) solution and stirred for 45 min. at 450C to yield a thick suspension of hydrolyzed cotton wool .This process represents the hydrolysis step, which was stopped by the addition of excess amount of distilled water. To remove the acidic media, the suspension was subjected to centrifugation at 6000rpm for 10 min. until the turbidity of the supernatant appeared. The acidic supernatant was removed and the process was replicated. The resultant suspension containing the CMWs was dialyzed against tap water MWCO=12000-14000 until the medium became neutral.

 Then the neutral suspension was placed in ultrasonic bath for 10 min. and centrifuged again to collect the CNWs in form of cloudy supernatant, this process was continued to collect CNWs as much as possible until the supernatant became clear. The collected supernatant was lyophilized and collected as a white pure powder (123).

2.5.2. General Procedure for Synthesis of Compound (II):

 This reaction performed in a 250 mL round bottom flask, in which 10 mL of dichloromethane containing (2.00 %, w/v) of CNWs, (10 equiv. per glucose unit) of Gamma aminobutyric acid (GABA) and (1.22 g, 1.00 mmol) of P-dimethyl aminopyridine (DMAP) were stirred in an ice bath to reach 0°C , during stirring (1.87 g, 8.00 mmol) of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and (0.50 mmol) of sulfo-N-hydroxysuccinimide (NHS) were added drop wise to the previous solution. After 5 min. the ice bath was removed and the reaction continued at room temperature with continuous stirring for 3 hrs. While the reaction was performed, some of N-acyl urea were precipitated, which could be easily removed by Buchner. The resultant solution was washed two times with 10 mL of 0.5N HCl and again with 2 x 10 mL of 0.1N of sodium bicarbonate (NaHCO3). Some of N-acyl urea derivatives may precipitate again, which were removed by the filtration of the two layers. The organic layer was dried with magnesium sulfate (MgSO4) and the compound (II) was collected as a white powder (124).

2.2.4. General Procedure for Synthesis of compound (III):

. A 10 mL solution of DOX (5.5g, 10.18mmol) dissolved in benzene was added gradually to a solution of compound (II) (2.15g, 5.09mmol) in in 5mL benzene. The mixture was refluxed for 10 hr. until no water appeared and the solvent was removed by rotary evaporator. Recrystallization of the precipitate took place with ethyl acetate to obtain pure product (125).

2.3. Preliminary anticancer study:

 In vivo anticancer effect of the synthesized nanocarrier grafted with DOX was evaluated on laboratory mice with induced mammary adenocarcinoma (AM3). The evaluation of anticancer effect based on measuring the degree of the reduction in the size of the tumor in comparison to DOX as standard.

2.3.2. Methods:

Laboratory female mice weighing (60 ± 10 g) aged 6-8 weeks were supplied and housed in Iraqi center for cancer and medical genetic researches under standardized conditions for 10 days for acclimatization. Animals were fed commercial chaw and had free access to water. On day 0 of the experiment, MR3 cells (6×106, in 0.2 ml of 1:1 phosphate buffer and five full antibiotics) were injected subcutaneously into the mice. The mice were then randomly divided into groups of five mice each, to receive different treatment agents. The test solutions were prepared in DMSO, and were then diluted with 0.9% sodium chloride solution (final DMSO concentration=0.1). The solutions were injected i.p. at a volume of 0.01 mL/g of body weight on the 10th and 17th day after tumor

transplantation. The control mice received a 10% DMSO solution at a volume of 0.01 mL/g of body weight according to the same schedule.