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Original article

Nanoparticle and liposome formulations of doxycycline: Transport properties through Caco-2 cell line and effects on matrix metalloproteinase secretion

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ABSTRACT

Nanoparticle and liposome formulations containing doxycycline or doxycycline and sodium taurocholate (NaTC) were developed in this study. The anticancer effects of doxycycline and penetration properties from those formulations through Caco-2 cell monolayers were investigated. Matrix metalloproteinases (MMPs) have been reported to play a role in the negative prognosis of many malignant tumors including glioblastoma multiforme (GBM). This study is presented to demonstrate that these developed nanoparticle and liposome formulations of doxycycline are capable of inhibiting MMP-2 release from cultured Caco-2 cells. In this study, Caco-2 cells were used as model cell cultures. A MTT test was performed to determine the effect of doxycycline on the viability of Caco-2 cells. Doxycycline nanoparticles were prepared using emulsion polymerization and doxycycline liposomes were prepared using the dry film hydration method. Transport studies of doxycycline through Caco-2 cells were investigated. MMP-2 was found to be inhibited more with doxycycline if NaTC is present in the formulation. NaTC was also found to be useful to increase penetration due to the inhibition of efflux by interacting with p-glycoproteins, in addition to the penetration enhancing effect as a result of opening tight junctions. These developed formulations were proposed to use for the treatment of tumors and GBM.

1. Introduction

The most frequently observed malignant glioma tumor seen in adults is the glioblastoma multiforme (GBM). Neither chemotherapy nor radiotherapy has been successful. The major physiological processes characterized for malignant gliomas are: growth/proliferation, angiogenesis, and invasion [1]. GBM is considered a grade IV malignant tumor. GBM (WHO Grade IV) is also the most frequent and malignant astrocytic brain tumor [2–4]. A high proliferation rate, exponential growth and diffusely infiltrate adjacent brain tissues are the main characteristics of these tumors [5,6]. Due to microscopic invasion in the surrounding normal brain tissue, GBM is still an incurable disease that easily relapses [7]. Surgery, radiation, and chemotherapy are recognized as conventional treatment options for GBM [2,7–9]. However, because of these highly invasive tumor cells profile, along with their resistance to chemotherapeutic agents and radiation, many treatment methods still remain ineffective [2,10,11]. Through the degradation of the components of the extracellular matrix (ECM), including laminin and fibronectin, glioblastomas have a high infiltrative ability, show rapid cell proliferation, and aggressively invade the surrounding normal brain tissue. Several different proteases were found to contribute to the invasion of malignant gliomas, such as matrix metalloproteinases (MMPs), and serine and cysteine proteases. Among these, MMPs have received greater attention. MMPs are characterized for the degradation of a broad range of ECM components, as well as the elevation of MMPs, such as MMP-2 and -9 were determined to have a high concentration in human glioblastoma tissues [12,13].

In the turnover of ECM and cell migration, MMPs are very important as a family of proteases [14–16], and most MMPs require proteolytic cleavage for activation since they are secreted as proenzymes. Fetal tissue development, postnatal tissue repair and certain pathological conditions such as periodontitis, autoimmune disorders of the skin and dermal photoaging, rheumatoid arthritis, osteoarthritis, chronic ulcerations, uterine involution, wound healing, bone resorption, and tumor progression and metastasis are the various pathophysiological conditions in which the gelatinases of MMPs have been identified to have a role [14,17,18]. It is around the basal lamina capillaries, facilitate angiogenesis, and neurogenesis that gelatinases degrade molecules [15]. It is frequently co-expressed in human cancers that two

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gelatinases, MMP-2 and MMP-9, degrade basement membrane type IV collagen and appear to be essential for cellular invasion [17,19–24]. Gelatinase A (MMP-2), gelatinase B (MMP-9) and matrilysin are the most common MMPs found in colorectal or gastrointestinal cancer cells [19]. Cytotoxic chemotherapies, in combination with MMP inhibitors designed to block tumor cell migration, such as marimastat and prinomastat, have undergone phase II clinical trials [19,22]. However, there was no prolongation of patient survival or radiographic responses reported. The reason for such disappointing results remain unclear; thought there is a possibility that the results are due to insufficient inhibition of MMPs or alternative mechanisms of tumor cell migration, as it may be necessary to perform separate determinations of drug efficacy to stop or slow down growth/proliferation, angiogenesis, and invasion [23]. Therefore, effective inhibition of MMP-2 appears to be necessary for better therapy.

Doxycycline was obtained by modification of the oxytetracycline molecule, which is a tetracycline antibiotic [24,25]. Doxycycline has a broad-spectrum of activity against aerobic and anaerobic gram-positive and gram negative bacteria, rickettsia, chlamydia [26]. Doxycycline has also been studied in patients as an anticancer agent, because of its antitumor properties [17,26,27]. It has been shown that doxycycline predominantly inhibits MMP-2 and MMP-9, which is reported to be one of the more potent and well-tolerated matrix metalloproteinase inhibitors (MMPIs) in the tetracycline family [22]. Doxycycline was found to inhibit of mitochondrial protein synthesis [1,28]. It has also been reported that doxycycline has anti-angiogenesis effects [24] and anti-invasion effects, postulated via the inhibition of MMPs [29], and no major toxicity has been reported with long-term use. Similarly, it has been found to be effective in a variety of tumor cell lines including breast, colon, mesothelioma, osteosarcoma, renal, prostate, and melanoma cell lines [30–34] as a non-specific MMP inhibitor. Chelation of a zinc ion by doxycycline at the active site of the enzyme has been also reported [35], but the exact mechanism of MMP inhibition is not entirely clear. It is reported as a very attractive agent for further experiments and it has been highlighted that tumor migration and invasion [36] can be stopped or reduced. It has been reported that MMP inhibition by doxycycline can be possible within the normal therapeutic dose range (less than 10 μg/mL) [37,38]. No report has been found related to the direct doxycycline effect on MMP-2 activity or invasion capacities of the tumors' GBM cell growth. As a cytotoxic agent or an MMP inhibitor, the present data showed that doxycycline can be a good candidate for further experiments to achieve better treatment of GBM [36], therefore smart and effective doxycycline formulations still need to be developed.

Liposomes enclose aqueous compartments and are microscopic vesicles composed of one or more lipid bilayers, and can entrap hydrophilic molecules inside or within the lipid bilayers [39–42]. Natural and synthetic phospholipids and cholesterol are usually the main components of these vesicle formulations [43] and can act as biocompatible, biodegradable, non-immunogenic, and non-toxic drug carriers [44,45]. They are capable of reducing the side effects of active molecules and enhancing the accumulation of the drug at the administration site [46]. Controlled drug release and reduction of toxicity are the main advantages [47,48]. By using liposomes, a number of novel approaches have been proposed, particularly for targeted drug delivery [49].

Ranging in size from 10–1000 nm, nanoparticles were made of natural or artificial polymers [50,51] and the drug of interest was dissolved or entrapped or adsorbed or attached and/or encapsulated into or onto the nanomatrix [51,52]. Nanoparticles were used to deliver active drug molecules to the site of action to enhance the effect. Preparation of these systems was reported to be simple and rather easy to scale-up [53]. Allowing an efficient drug accumulation at the targeted sites in the body, the advantages of nanoparticles include reduced drug toxicity, improved biodistribution, and therapeutic efficacy that allows sustained drug release at the targeted site over a period of days or even weeks after administration [50,53]. Liposomes and nanoparticles have been largely used for brain-targeted drug delivery systems, as well [53]. The human adenocarcinoma cell line Caco-2 [54] has been developed as a model for the intestinal epithelium [55,56] where these Caco-2s were originally isolated from the human colon adenocarcinoma by Fogh et al. (1977) [57]. Caco-2 cells are widely accepted and used in in vitro models to predict intestinal absorption by epithelial cells [58,59] and exhibit enterocyte-like characteristics, but interestingly it has been indicated that Caco-2 cells can be also used to predict blood-brain barrier (BBB) permeation potential [60,61]. Recent results in the literature show that doxycycline inhibits MMP-2 from cultured Caco-2 cells [62]. Therefore, the Caco-2 cell line was selected to examine and to test the permeation ability of doxycycline. This will also show the BBB permeation potential of doxycycline, and MMP-2 inhibition capability.

In this study, liposome and nanoparticle formulations of doxycycline were prepared. The effect of absorption enhancer [sodium taurocholate (NaTC)] was investigated on the permeability of doxycycline through Caco-2 cells. NaTC has been used as an absorption enhancer because it has been also found to be act as efflux and p-glycoprotein inhibitor [59] for BBB that may help doxycycline penetrate barriers faster.

2. Material and methods

2.1. Materials

Doxycycline, sodium taurocholate (NaTC), cholesterol and MTT (3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide) were purchased from Sigma (USA). Dipalmitoylphosphatidylcholine (DPPC) was provided by Across Organics, Belgium. Dulbecco's modified Eagle's medium (DMEM) was purchased from Biochrom, Germany. The ELH-MMP2-001 Elisa kit was purchased from RayBio, Germany. Polyvinyl alcohol was provided by Wecker-Germany, and Eudragit-RS 100 was provided by Röhm Gmb & Co.KG, Germany.

3. Methods

3.1. In vitro studies

3.1.1. Preparation of doxycycline liposomes

Multilamellar doxycycline liposomes were prepared using the dry film hydration method [63]. Doxycycline, cholesterol and DPPC (1:1) were dissolved with chloroform and evaporated under a vacuum at ~ 44 °C. The film was hydrated by a phosphate buffer (pH 7.4) and liposomes were centrifuged at 15,000 rpm at 25 °C for 60 minutes. Supernatants and liposomes were then separated.

3.1.2. Preparation of doxycycline nanoparticles

The method was adopted from the literature [64]. Eudragit-RS-100 was used as a polymer. Polymer (2 g) was dissolved in methanol at room temperature then injected slowly (0.5 mL/min) into aqueous phases containing doxycycline and 0.4% polyvinyl alcohol (PVA). The mixture was stirred at 8000 rpm by Ultra-turrax® for 5 minutes. After evaporation, nanoparticle suspensions were ultracentrifuged at 15,000 rpm for 35 minutes at 25 °C. Supernatants and nanoparticles were separated. Doxycycline-NaTC nanoparticles were prepared using the same method. NaTC was added to the doxycycline-containing phase during the preparation, and the rest of the method was exactly the same.