Phytomedicine 23 (2016) 1764–1770

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Original Article

Selecting optimum protein nano-carriers for natural polyphenols using chemoinformatics tools

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ARTICLE INFO

Article history:
Received 27 July 2016
Revised 30 September 2016
Accepted 26 October 2016

Keywords:
Curcumin
Resveratrol
Gelatin nanoparticles
Albumin
Docking
Molecular dynamics

ABSTRACT

Background: The normal fate of any natural product with a therapeutic potential is to be formulated into an effective medicine. However, the conventional methods of selecting the suitable formulations or carriers based on the formulator experiences, trials and errors as well as materials availability do not usually yield the optimal results.
Hypothesis: We hypothesize the possibility of the virtual optimum selection of a protein carrier for two polyphenolic compounds widely investigated for their chemopreventive effects; resveratrol and curcumin using a combination of some chemoinformatics tools.
Methods: Two protein-based nanoparticles namely; albumin and gelatin nanoparticles were compared as carriers for the two selected phytochemicals; resveratrol and curcumin. Resveratrol-albumin, resveratrol-gelatin and curcumin-albumin results were gathered from the literature. While, a new combination (formulation), comprising curcumin as the cargo and gelatin nanoparticles as the carrier, was prepared and evaluated as a potential medicine for breast cancer. Combined chemoinformatics tools, namely; molecular dynamics and molecular docking were used to determine the optimum carrier for each of the two chemopreventive agents.
Results: A new curcumin-gelatin nanoparticulate formulation was prepared and proven cytotoxic after an application period of 48 h on MCF-7 breast cancer cell-lines scoring an IC_{50} value of 64.8 µg/ml. The utilized chemoinformatics tools comprising the molecular dynamics simulations of the protein nanoparticulate drug-carriers followed by the molecular docking of phytochemical drugs on these carriers could capture the optimum protein carrier for each of the tested phytochemical and hence propose a successful formulation.
Conclusion: This study presents one in a series that proves the novel addressed concept of the utilization of computational tools rather than wet-lab experimentation in providing better selection of drug-carrier pairs aiming for better formulations and the subsequent successful therapeutic effects.

Introduction

Since the pre-historic era, humans have utilized naturally-driven bioactive agents to treat a plethora of diseases. Recently, natural products have been explored for the prevention and treatment of serious diseases such as cancer (Mehanny et al., 2016a,b). These products included phytochemicals such as: β-carotene, curcumin, epigallocatechin gallate, genistein, resveratrol, gingerol, and capsaicin. These agents specifically have been proven to have strong chemotherapeutic actions. However, the exploitation of these agents was challenged by their instabillity, poor (or sometimes extremely poor) aqueous solubility and the subsequent poor bioavailability limiting their use (Bharali et al., 2011). In the current study, resveratrol and curcumin were selected as examples of chemopreventive natural products. Resveratrol is considered a promising chemopreventive phytochemical which was first isolated from the roots of *Veratrum glandiflorum* O. Loes, then isolated from

Abbreviations: DMSO, Dimethyl Sulphoxide; GNPs, Gelatin Nanoparticles; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMEM, Dulbecco's modified Eagle Medium; TEM, transmission electron microscopy.

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http://dx.doi.org/10.1016/j.phymed.2016.10.020
0944-7113/

grapes, peanuts, mulberries and another 70 plant species. It has been shown to inhibit the growth of various cancer cells in culture as well as in implanted tumors *in vivo* (Kundu and Surh, 2008; Singh et al., 2015). Curcumin is a major bioactive component isolated from the rhizomes of *Curcuma longa* L. (Zingiberaceae) and has been the scope of many publications for its wide range of pharmacological activities but limited clinical applications due to its poor aqueous solubility, multidrug pump P-pg efflux, extensive *in vivo* metabolism and rapid elimination (Patil et al., 2015). The anti-cancer activity of curcumin has been exhibited in breast, ovarian, colon and lung cancer (Saxena and Hussain, 2013; Patil et al., 2015; Yang et al., 2015; Wang et al., 2016).

Nanotechnology is a fast developing science gaining more grounds in medicine and therapy everyday. Nanoparticles have emerged as versatile nano-carriers for the specific and targeted delivery of drugs to organs and tissues which is particularly relevant in cancer therapy where most of the biological processes occur at the nanometer level (Li et al., 2012). Hence, the nanoparticle nanotechnology has been greatly appreciated as a potential tool for cancer diagnosis and treatment (Bharali et al., 2011).

In this context, the search for the optimum smart materials, that can best accommodate the active chemotherapeutic agents at the nano level, is an ongoing process. Accordingly, protein carriers have been sought of due to their many advantages including their biodegradablility, biocompatiblity, non-antigenicity, low cost and availability. Moreover, the surface of protein nanoparticles can be modified with site-specific ligands, cationized with amine derivatives or coated with polyethyl glycols to achieve targeted and sustained release drug delivery (Hathout and Omran, 2016). Compared to other colloidal carriers, protein nano-carriers are better stable in biological fluids to provide the desired controlled and sustained release of entrapped drug molecules (Sahoo et al., 2015). Amongst the commonly used proteins, albumin and gelatin reside at the top due to their abundant resources.

To this end, a usual question rises; what is the optimum proteinaceous carrier material for a specific drug? The conventional answer could be obtained by testing several protein-drug pairs in the wet laboratories till reaching the most suitable carrier. Nevertheless, another question has been raised; can computers and softwares, specifically, chemoinformatics tools, answer this question and thereby replace the exhausting and resources consuming wet-lab trials? We have recently answered this question and confirmed the possible usage of several chemo-informatics tools in optimizing and predicting the loading of drugs in several carriers (Metwally and Hathout, 2015a,b).

Therefore, in the current study we hypothesize that chemoinformatics tools such as molecular dynamics simulations utilized to build virtual protein nano-carriers followed by the molecular docking of the investigated phytochemicals on these virtually built carriers can select the optimum protein-based nanoparticles material that can best accommodate naturally discovered chemotherapeutic agents (polyphenolic compounds having close chemical structures). This included the phytoalexin; resveratrol, and the diferuloylmethane; curcumin, (Fig. 1A and B) based on their physical and chemical potential interactions with the proposed nano-carriers. Several software packages and docking scores were evaluated in order to validate the hypothesis. In order to test this hypothesis, the mass of the loaded polyphenol per 100 mg carrier of resveratrol on each of albumin and gelatin nanoparticles was collected from the literature. Similarly, the loaded mass of curcumin on albumin nanoparticles was obtained. To the best of our knowledge, curcumin-gelatin nanoparticles, was not previously produced, and hence, we attempted to prepare this new formulation to compare to the curcumin-albumin counterpart.

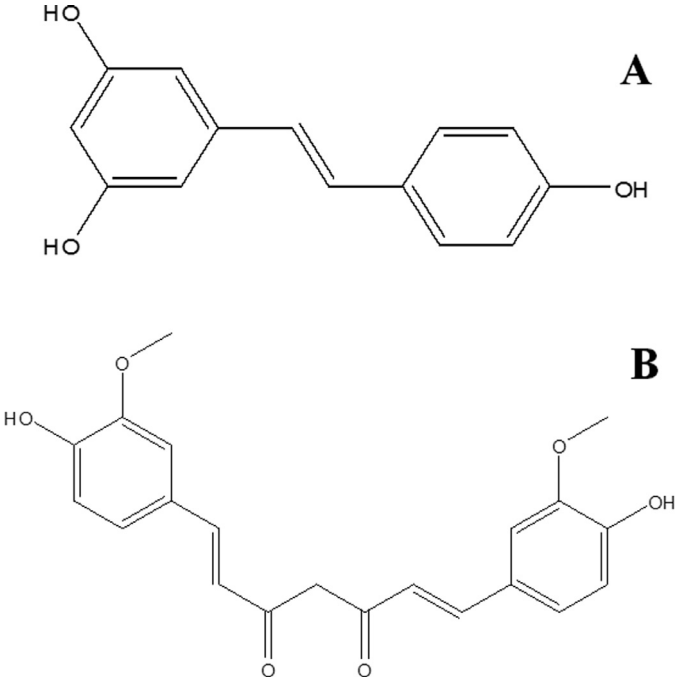

Fig. 1. The chemical structure of (A) Resveratrol and (B) Curcumin.

Methodology

Materials

Curcumin, gelatin, gluteraldehyde solution (25%, w/v) and glycine were purchased from Sigma-aldrich, Taufkirchen, Germany. Dimethyl Sulphoxide (DMSO), Trypan blue and MTT were purchased from Sigma (St.Louis, MO, USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza, Belgium.

Methods

Data mining using scientific literature databases

The mass of drug loaded per 100 mg protein of 2 different drugs; resveratrol (Li et al., 2012; Karthikeyan et al., 2013) and curcumin (Jithan et al., 2011) entrapped in protein-based nanoparticles were gathered from the scientific literature databases using PubMed, Scopus and Web of Science.

Preparation of curcumin-loaded gelatin nanospheres

Curcumin-loaded gelatin nanospheres were prepared using the single desolvation method (the same method that was previously used to prepare the curcumin-loaded albumin nanoparticles (Jithan et al., 2011)). Briefly, 10 mg curcumin were dissolved in 3 ml absolute ethanol. Meanwhile, gelatin (200 mg) was dissolved in 2 ml de-ionized water at 40 °C. The alcoholic solution was then added to the aqueous solution drop-wise till a colloidal dispersion was obtained. Afterwards, 0.03 ml gluteraldehyde (25%, w/v) were added to the dispersion to cross-link the formed nanoparticles and prevent their swelling. Stirring for 1 h was performed. Consequently, the colloidal dispersion volume was completed to 25 ml using deionized water. Finally, the prepared nanospheres were separated by centrifugation at 13,000 rpm for 25 min (Abozeid et al., 2016).

Characterization of the prepared curcumin-loaded gelatin nanospheres

Particle size measurements. The particle size and polydispersity index of the prepared polyphenol-loaded nanospheres was measured using dynamic light scattering (DLS) (Malvern zetasizer, Nano ZS, Malvern, Worcestershire, UK) after re-dispersion of the centrifuged