Journal of Molecular Graphics and Modelling 60 (2015) 98–107

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Interaction of menthol with mixed-lipid bilayer of stratum corneum: A coarse-grained simulation study

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ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 10 June 2015
Accepted 11 June 2015
Available online 18 June 2015

Keywords:
Menthol
Penetration enhancer
Mixed-lipid bilayer
Molecular dynamics simulation
Coarse-grained

ABSTRACT

Menthol is a widely used penetration enhancer in clinical medicine due to its high efficiency and relative safety. Although there are many studies focused on the penetration-enhancing activity of menthol, the details of molecular mechanism are rarely involved in the discussion. In this study, we present a series of coarse-grained molecular dynamics simulations to investigate the interaction of menthol with a mixed-lipid bilayer model consisting of ceramides, cholesterol and free fatty acids in a 2:2:1 molar ratio. Taking both the concentration of menthol and temperature into consideration, it was found that a rise in temperature and concentration within a specific range (1–20%) could improve the penetration-enhancing property of menthol and the floppiness of the bilayer. However, at high concentrations (30% and more), menthol completely mixed with the lipids and the membrane can no longer maintain a bilayer structure. Our results elucidates some of the molecular basis for menthol's penetration enhancing effects and may provide some assistance for the development and applications of menthol as a penetration enhancer. Furthermore, we establish a method to investigate the penetration enhancement mechanism of traditional Chinese medicine using the mixed-lipid bilayer model of stratum corneum by molecular dynamics simulations.

1. Introduction

Transdermal drug delivery system is key field in pharmaceutical research. It has several advantages over oral administration, such as bypassed first-pass liver metabolism and gastrointestinal irritation, improved bioavailability, better patient compliance and reduced side effects [1–4]. However, human skin can selectively and effectively inhibits chemical penetration [5]. The most important control element is generally the stratum corneum (SC), whose barrier property hampers the application of transdermal drug delivery [6,7]. Thus, it is important to promote the drug penetration property through SC in the development of transdermal drug delivery procedures.

The most common approach to drug penetration enhancement is through employing permeation enhancers (PEs). Compared with frequently-used chemical PEs (sulfoxides, alcohols, azone etc.), volatile oil of traditional Chinese medicine, especially terpenoids, may offer advantages for being less toxic to the body [8]. Menthol, a monoterpenoid component extracted from traditional Chinese medicine (TCM) *Mentha arvensis* var. *piperasceus*, has traditionally been used for the treatment of febrile diseases, muscle aches, sprains and other similar conditions using alone. In recent years, it has also been widely used as a PE in clinical medications. As previously reported, menthol can efficiently promote the penetration of both hydrophilic and lipophilic drugs, such as quercetin, ondansetron hydrochloride, salicylate, zidovudine, etc. [9–12]. Given the "yaofuheyi" characteristic (means that an agent in a preparation not only have the effect of drug but also can be used as an excipient) and good penetration enhancing efficiency of menthol, it turned out to be a representative volatile oil of TCM used as a PE.

To elucidate the mechanisms of penetration enhancement of menthol, a lot of previous research has been done. Many researchers have inferred from in-vitro transdermal experiments that the penetration enhancement mechanism of menthol is possibly to disrupt and transform the SC lipids from its original state into a less ordered packing form [9,13]. There are also some observations of menthol's effect on skin by various type of microscopy

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http://dx.doi.org/10.1016/j.jmgm.2015.06.005
1093-3263/

such as light microscopy, scanning electron microscopy and transmission electron microscopy. [14,15], indicating the rarefaction and shedding of SC caused by menthol. However, the penetration enhancement mechanism of menthol has rarely been discussed from molecular level due to the restrictions of the experimental methods applied and the complexity of the transdermal process. New techniques and methods need to be adopted to gain further insight on this issue.

Molecular dynamics (MD) simulation is an effective and intuitional technique based on Newtonian mechanics. It is becoming increasingly popular in studies on the interactions of molecules within the lipid bilayer systems, providing us with information that cannot be obtained by laboratory experiments [16]. Furthermore, we can explore larger temporal and spatial scales during a simulation by altering molecular resolution, an approach generally known as mesoscopic or coarse-grained (CG) [16,17]. Martini force field, the most widely used CG force field developed by Marrink and his coworkers in 2007, has been widely used in many studies of biomolecules, such as lipids, polymers, proteins, carbohydrates, etc. [18–22]. Several studies have been done to discuss the property and feasibility of different membrane models using Martini CG force field [23–27]. The results show that the loss in chemical specificity of the models can be kept to a minimum under the CG approach and a larger system and longer time-scale can be obtained as well. Therefore, MD simulation using Martini CG force field is an ideal method for investigating the interactions of molecules within bio-membranes and to elucidate the molecular mechanisms of adsorption and penetration.

In this work, a number of MD simulation studies were carried out on a mesoscopic level to investigate the interaction of menthol with a mixed skin-lipid model of SC. Both the concentrations of menthol and temperature effects were taken into consideration, aiming to explain the molecular mechanisms of menthol's penetration enhancing effect and thus provide some assistance for the development and application of menthol as a penetration enhancer.

2. Simulation method

2.1. Force field

All the simulation studies in this work were carried out on a mesoscopic level by CG method to obtain longer time and length scales. The interaction parameters used in the simulation were based on the Martini force field, which was provided by the website of Martini [28]. The Martini model is based on a four-to-one mapping (on average four heavy atoms are represented by a single interaction center), but for ring structures, a two/three-to-one mapping method is used [18]. There are 18 types of normal sites and corresponding S-sites (for ring structures) in Martini force field, which reserves the main information of molecules and attain longer temporal and larger spatial scales in simulation as well.

2.2. Bilayer model of SC

SC is mainly composed of corneocytes and a specialized mixture of lipids, which is often found in a "brick and mortar" form, with the corneocytes (bricks) surrounded by the continuous lipid layers (mortar) [29,30]. The lipids, mainly comprised of ceramides (CER), free fatty acids (FFA) and cholesterol (CHOL), are organized in lamellar layers which are essentially impermeable and thus form the main barrier against penetration [31]. Given the complexity of the composition of the skin lipids and the consensus over their molecular architecture, many researchers have developed skin-lipid 'substitutes' to explore the interaction of molecules within SC using MD simulation, which has a barrier function similar to skin tissue [32]. Both single-component models [33–36] and mixed skin-lipid models with different compositions and contents [37–40] have been investigated. Das et al. demonstrated that the simulated model bilayers comprising of CER2 (ceramide NS, the predominant ceramide in skin lipid with a C24:0 fatty acid tail, short for CER in the following passage), FFA (C24:0) and CHOL at ratio close to 2:2:1 has the most desirable properties of good barrier function and stability against mechanical stress [38,39]. Therefore, it can be used to investigate the interactions and elucidate the molecular mechanisms of the penetration enhancement effect of menthol.

Table 1
Bond distributions of menthol by AA and CG methods.

Bond	AA	CG	RSD*%
1–2	0.2483	0.2428	2.2
2–3	0.2892	0.2787	3.6
2–4	0.2490	0.2464	1.0
3–4	0.2903	0.2964	2.1

* Relative standard deviation.

2.3. CG molecule models

This assay mainly involves five molecules, menthol, CER, CHOL, FFA and water. The parameter files of the CG models of CER, CHOL, FFA and water are available in the Martini website (Fig. 1a–d showing the mapping methods respectively), however, menthol has never been parameterized to a CG model.

We developed the CG model of menthol (CG-men) according to the CG recipe published in the Martini website [41], and then validated the new CG-men. The atomistic structure was mapped via Visualizer and Mesocite modules in Materials Studio 5.5 (Accelrys Inc., supported by CHEMCLOUDCOMPUTING) software package [42]. Fig. 1(e) showing the mapping method and some parameters of CG-men. Menthol is a ring-like amphiphilic molecule with a hydrophilic hydroxyl group and some hydrophobic methyl groups. We took a polar bead (SP1) to represent the hydrophilic group and three apolar beads (SC1) to take the place of the hydrophobic parts.

Because the key to developing a new CG model is the 'Coarse-grain target atomistic data', we calculated the bond distributions and radial distribution functions (RDFs) of menthol-octanol in a mixed-system consisting of a certain amount of menthol, water and octanol. Both of them were calculated by all atom (AA) method and CG method to validate our CG-men. RDFs provide a measure of the probability that, given the reference particle α at the origin of an arbitrary reference frame, there will be a particle β with its center located in a spherical shell of infinitesimal thickness at a distance, r, from the reference particle α [42]. Thus, the cumulative number RDF would indicate the total average number of particle β around α within a distance of r [43]. The results show that all the bond distributions (Table 1), the RDFs and cumulative number RDF of menthol–octanol of CG model (Fig. 2) are similar to that in AA representations, which demonstrats our CG-men to be a valid model.

2.4. Initial structures

The bilayer model of SC in this study is composed of CER, CHOL and FFA in a 2:2:1 molar ratio, whose properties have been validated by Das and his coworkers in 2009 [38,39]. The bilayer systems with different menthol concentrations in water are built using the Packmol package [45] and figures depicting lipid molecules are generated with Visual Molecular Dynamics (VMD) [46].

Fig. 3(a) shows a side view of a fragment of the blank bilayer, which packed CER (252 lipids), CHOL (252 lipids), FFA (126 lipids) and water (4882 beads) in a box of $18 \times 18 \times 10$(nm). Next, the