International Journal of Pharmaceutics 521 (2017) 150–155

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Increased intestinal permeation and modulation of presystemic metabolism of resveratrol formulated into self-emulsifying drug delivery systems

G. Mamadou[a,b,*], C. Charrueau[c], J. Dairou[d], N. Limas Nzouzi[b], B. Eto[b], G. Ponchel[a]

[a] Institut Galien Paris-Sud, CNRS UMR 8612, Université Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, France
[b] TransCell-Lab Laboratory, Faculty of Medicine Xavier Bichat, University of Paris Diderot – Paris7, Paris, France
[c] Unité de Technologies Chimiques et Biologiques pour la Santé UTCBS UMR 8258 CNRS/U1022 INSERM, Université Paris Descartes, Sorbonne Paris Cité, Paris, France
[d] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, Université Paris Descartes, Sorbonne Paris Cité, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 13 February 2017
Accepted 14 February 2017
Available online 17 February 2017

Keywords:
Resveratrol
Polyphenol
Nanoemulsion
Intestinal absorption
Ussing chamber

ABSTRACT

Despite various beneficial biological properties, resveratrol lacks therapeutic applications because of poor bioavailability due to variable absorption and extensive metabolism. The present study aims at evaluating the capability of self-emulsifying drug delivery systems (SEDDS) to enhance resveratrol permeation across rat intestine and to modulate its presystemic metabolism. For that purpose, semi-solid (SS) and liquid (L) SEDDS were prepared and dispersed in an aqueous buffer to produce nanoemulsions (NE). The jejunal absorptive transepithelial fluxes (J_{ms}) of resveratrol elicited by these formulations (SS-NE and L-NE) and presystemic metabolization were determined on Ussing chambers. The absorptive fluxes through the intestinal epithelium from the nanoemulsions ($J_{ms} = 20.5 \pm 3.1\ \mu g\ h^{-1}\ cm^{-2}$ SS-NE; $28.9 \pm 2.9\ \mu g\ h^{-1}\ cm^{-2}$ L-NE) were significantly increased compared to an ethanolic control solution ($J_{ms} = 3.4 \pm 0.3\ \mu g\ h^{-1}\ cm^{-2}$, $p < 0.05$). No significant variations of conductance were observed after two hours of contact between the formulations and the mucosa. Simultaneously, the presystemic metabolization pattern was modified in the case of the nanoemulsions compared to the control solution. In conclusion, our data suggests that oil-in-water nanoemulsions prepared from SEDDS dispersions of medium-chain lipids could be promising formulations for enhancing oral delivery of resveratrol.

1. Introduction

Resveratrol or 3, 5, 4′-trihydroxy-*trans*-stilbene is a natural product. In the past decade, the small polyphenol resveratrol has received widespread attention as either a potential therapy or as a preventive agent for numerous diseases. Studies using purified enzymes, cultured cells, and laboratory animals have suggested that resveratrol has anti-aging, anti-carcinogenic, anti-inflammatory, and anti-oxidant properties (Das and Das, 2007) that might be relevant to chronic diseases and/or longevity in humans.

Resveratrol is also protective against oxidative stress, inflammation (Das and Das, 2007) and the development of cardiovascular diseases (Chen et al., 2002), diabetes (Venturini et al., 2010), neurodegenerative diseases (Vingtdeux et al., 2008), and cancer (Kris-Etherton et al., 2002). Resveratrol plays a prominent role in the prevention of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, cerebral ischemia as well as Huntington's disease because resveratrol enters the blood stream after the formation of glucuronide conjugates and can readily pass through the blood-brain barrier (Baur and Sinclair, 2006). Resveratrol (10–100 μM) is reported to exert neuroprotective effects in several studies (Richard et al., 2011).

It constitutes one of the primary components in red wine and is claimed to be an essential factor for explaining the French Paradox, a term frequently used to summarize the apparently paradoxical epidemiological observation that French people have a relatively low incidence of cardiovascular diseases despite having a diet relatively rich in saturated fats (Liu et al., 2007; Sun et al., 2008). Resveratrol is present in skin and seeds of more than 70 different

* Corresponding author at: TransCell-Lab Laboratory, Faculty of Medicine Xavier Bichat, University of Paris Diderot – Paris7, Paris, France.
E-mail address: godefroymamadou@wanadoo.fr (G. Mamadou).

http://dx.doi.org/10.1016/j.ijpharm.2017.02.036
0378-5173/

plant species, including grapes, berries, grains, tea, and peanuts (Chen et al., 2002; Soleas et al., 1997). It is synthesized in the pericarp of grape berries, epidermis of grape berry leaf, and in the stalks and kernels of the berries.

Resveratrol exists in two geometric isomers with *trans* and *cis* configuration (Fig. 1). *trans*-resveratrol is considered to be a non-toxic stereoisomer and the bioactive one, responsible for the reported beneficial health effects (Orallo, 2006).

Beside unfavourable physico-chemical characteristics including poor water solubility and limited chemical stability (Vian et al., 2005; Wenzel and Somoza, 2005), several studies conducted on the pharmacokinetics of resveratrol indicate that its variable absorption and rapid and extensive presystemic and systemic metabolism results in low oral bioavailability (Wenzel and Somoza, 2005). Planas et al. (2012), Mattarei et al., (2014) have shown extensive implication of ABC transporters in absorption and presystemic metabolization of resveratrol. In this context, numerous formulations have been described in the literature to overcome resveratrol bioavailability limitations, including for example nanoparticles for controlled drug delivery (Rajan and Raj, 2013), resveratrol-cyclodextrin-liposomes (Soo et al., 2016), or colloidal mesoporous silica nanoparticles (Summerlin et al., 2016). The most promising delivery perspectives are obtained with colloidal carriers (Amri et al., 2012; Summerlin et al., 2015 Summerlin et al., 2015). Among the large variety of proposed formulations, self-emulsifying drug delivery systems (SEDDS) consisting of ternary mixtures of oil, surfactant and cosurfactant able to form oil-in-water nanoemulsions upon aqueous dilution have been recently demonstrated to significantly increase resveratrol uptake by cultured cells (Amri et al., 2014). Resveratrol-SEDDs also showed higher hypoglycemic and hypolipemic effects in diabetic-induced rats than unprocessed resveratrol (Balata et al., 2016). The present study was performed to evaluate the capacity of nanoemulsions obtained from SEDDS to enhance intestinal permeation of resveratrol and to modulate its presystemic metabolism by using the Ussing chamber technique.

An original SEDDS formula previously established *via* ternary phase diagrams construction and based on a Miglyol® 812/Montanox® 80/ethanol combination (Amri et al., 2014) was evaluated *versus* a formulation based on a commercially ready-to-use SEDDS-generating excipient, *i.e.* Gelucire® 44/14, combined with the solubilizer Labrasol®, both excipients being known to improve oral bioavailability of poorly water soluble lipophilic active ingredients.

2. Materials and methods

2.1. Materials

trans-Resveratrol (mw = 228 g/mol) and ethanol 96% v/v were obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Medium-chain triglycerides (Miglyol® 812) were purchased from Sasol (Witten, Germany). Lauroylmacrogolglyceride with melting point of 44 °C and HLB of 14 (Gelucire® 44-14), and polyoxylglycerides (Labrasol®), were generous gifts from Gattefossé, (Saint-Priest, France). Polysorbate 80 (Montanox® 80 VG PHA) was purchased from Seppic (Paris, France).

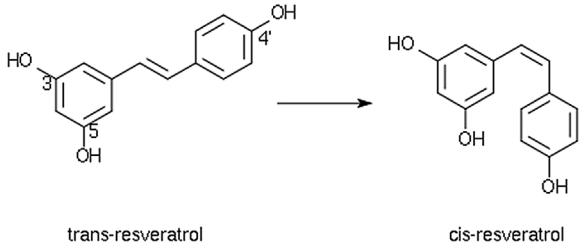

Fig. 1. Chemical structures of *trans* and *cis*-resveratrol.

2.2. Preparation of SEDDS

2.2.1. Liquid SEDDS

A ternary combination composed of Miglyol® 812 (20%), Montanox® 80 (70%) and 200 mM resveratrol in ethanol 96% v/v (10%) was prepared as previously described (Amri et al., 2014).

2.2.2. Semi-solid SEDDS

Gelucire® 44-14 (63%) and Labrasol® (27%) were melted at 50 °C in a water bath and resveratrol (10%) was added to the molten base under stirring for 5 min. The mixture was then cooled at room temperature.

2.3. Preparation of nanoemulsions from SEDDS

The liquid (L) and semi-solid SEDDS (SS) were dispersed in the aqueous buffer used in Ussing chamber experiments and described below in Section 2.5.2 under gentle stirring at room temperature in order to achieve a concentration of 0.2 mg/mL (0.9 mM) in the resulting nanoemulsions (L-NE and SS-NE). An ethanolic solution of resveratrol at the same concentration (0.9 mM) was used as a control (C).

2.4. Droplet size and zeta potential determination

The droplet size distribution and zeta potential values of the nanoemulsions resulting from the self-emulsification of the SEDDS in the aqueous buffer described below in Section 2.5.2 were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Orsay, France). All measures were repeated three times, and the values of z-average diameters (nm), polydispersity index (PI), and zeta potential (mV) were recorded and expressed as mean ± SD.

2.5. Permeation study in ussing chambers

2.5.1. Animals

Mature male Sprague-Dawley rats, weighing 180–250 g, were obtained from Janvier SAS (Route des Chênes, Le Genest-St-Isle, St Berthevin, France), housed in individual cages and fed with standard laboratory chow (UAR, Villemoisson s/Orge, France).

The study was conducted in accordance with the accepted principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, and all efforts were made to minimize animal suffering and the number of animals used. Ethics approval was obtained from University of Paris-Sud.

The studies were conducted in fasted animals. Food was withdrawn 18 h before the experiments, but the animals had access to drinking water. The animals were then euthanized by CO_2 inhalation, and their small intestine was removed and rinsed free of intestinal content by flushing with ice-cold Buffer's solution. The animals' stomachs were empty. The tissues (jejunum) were opened along the mesenteric border and mounted as flat sheets between the two halves of acrylic Ussing chambers, as previously described (Dossou-Yovo et al., 2014).

The Ussing chambers were protected from light to avoid isomerization of *trans*-resveratrol to *cis*-resveratrol (Nour et al., 2012; Figueiras et al., 2011).