A Self-Microemulsifying Drug Delivery System to Overcome Intestinal Resveratrol Toxicity and Presystemic Metabolism

KATARINA BOLKO SELJAK,[1] KATJA BERGINC,[2] JURIJ TRONTELJ,[3] ALENKA ZVONAR,[1] ALBIN KRISTL,[3] MIRJANA GAŠPERLIN[1]

[1]Faculty of Pharmacy, Chair of Pharmaceutical Technology, University of Ljubljana, Slovenia
[2]Lek Pharmaceuticals d.d, Ljubljana, Slovenia
[3]Faculty of Pharmacy, Chair of Biopharmaceutics and Pharmacokinetics, University of Ljubljana, Slovenia

Received 12 May 2014; revised 9 July 2014; accepted 16 July 2014

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24114

ABSTRACT: A mixed lipid-mixed surfactant self-microemulsifying drug delivery system (SMEDDS) was developed to exploit the health benefits of resveratrol, a Biopharmaceutical Classification System Class 2 natural polyphenol, subject to extensive intestinal presystemic metabolism. SMEDDS with a mixed lipid phase (castor oil/Capmul MCM 1:1) and a mixed surfactant phase (Kolliphor EL/Kolliphor RH 40 1:1) was developed and evaluated for its self-emulsifying properties and *in vitro* dispersion. The impact of SMEDDS on the permeability properties of resveratrol and its metabolite fluxes through the rat intestine and Caco-2 cells was monitored. The inhibitory effect of selected SMEDDS components on the efflux transporters multidrug resistance-associated protein and P-gp as well as cytotoxicity was assessed on Caco-2 cells. The formulation allowed for high resveratrol loading (122.5 mg/g SMEDDS), excellent self-emulsifying properties, and very rapid release. When formulated in SMEDDS, resveratrol metabolite efflux significantly declined. The formulation (SMEDDS without incorporated resveratrol) and its individual components did not compromise *in vitro* cell vitality and integrity. Mixed lipid-mixed surfactant SMEDDS is a prospective formulation to improve resveratrol biopharmaceutical, pharmacokinetic, and toxicological properties, leading the way to resveratrol use not only as a supplement but also as a pharmacological drug. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: Caco-2 cells; dissolution; drug delivery systems; lipids; phase diagram; self-emulsifying; surfactants; gastrointestinal; solubility

INTRODUCTION

Daily consumption of plant-derived polyphenolic phytochemicals in the Mediterranean diet has been associated with a diverse range of health benefits.[1,2] Resveratrol is the main flavonoid found in wine, grapes, and peanuts,[1] conferring antioxidant, anticancer, anti-inflammatory, and phytoestrogenic activities,[2] which decrease the incidence of degenerative, cardiovascular, and cancerous pathologies and consequently the mortality rate, commonly known as the "French paradox."[1,3] Owing to these health-promoting effects, the annual out-of-pocket costs spent on resveratrol food supplements are expected to increase in the future.[4] A recent review article from Cottard et al.[5] provides support for the further use of resveratrol not only as a food supplement but also as a drug in human medicine.

To exploit the pharmacological effects of resveratrol, its absorption must ensure sufficient plasma concentration and distribution into the target tissues. Unfortunately, extensive *in vitro* and *in vivo* animal and human studies have shown that resveratrol plasma levels after peroral administration of pure resveratrol or its dietary sources (i.e., wine) are significantly below therapeutic levels (plasma concentrations were below 5 ng/mL; approximately 1.7%–1.9% of plasma species are in the form of transresveratrol with F_{abs} estimated at 16%–17% of dose per os) because of the extensive first-pass intestinal and hepatic metabolism of parent resveratrol into two resveratrol glucuronides, resveratrol sulfate, and dihydro analogs of glucuronides and sulfate.[6–10] Because resveratrol metabolites have been identified as the main species in plasma, the supposedly preventive and curative properties exerted by continuous flavonoid consumption therefore most probably originate from direct or indirect pharmacological activities of the corresponding metabolites.

To exploit resveratrol bioactivities linked to its health promotion, strategies to attain higher plasma levels of resveratrol or its metabolites after peroral application should be explored. Because of the unproblematic resveratrol permeability, enhancement of its water solubility and/or dissolution rate was shown as a reasonable approach to a satisfying bioavailability.[11] This can be achieved by including specific pharmaceutical excipients in resveratrol formulations or by using novel drug delivery systems.[12,13] In this way, higher amounts of resveratrol would be available for absorption. This could also saturate the resveratrol intestinal first-pass metabolism, resulting in higher amounts of resveratrol absorbed and consequently metabolized in the liver, thus increasing the probability of metabolite pharmacological activity and/or their interconversion into resveratrol at the site of action.[6] Eventually, continuous oral application of resveratrol products would ensure resveratrol/metabolite tissue accumulation. Namely, elimination of resveratrol metabolites via bile leads to their conversion by intestinal bacteria back into resveratrol, which can be reabsorbed during enterohepatic circulation.[7,14]

In our previous work, we showed the potential of mixed lipid phase self-microemulsifying drug delivery systems (SMEDDSs) to improve resveratrol solubility and release rate.[15] Moreover, the developed mixed lipid phase SMEDDS were also consisted of lower surfactant content and no added cosolvents, making

Correspondence to: Mirjana Gašperlin (Telephone: +386-1-4769634; Fax: +386-1-4258031; E-mail: mirjana.gasperlin@ffa.uni-lj.si)

Journal of Pharmaceutical Sciences

them also appropriate for filling into hard gelatin capsules. It is generally accepted that a synergistic effect on ease of microemulsion formation *in vivo* can be obtained by using a combination of surfactants over a single surfactant in SMEDDS. We therefore aimed to merge the advantages of mixed lipid phase SMEDDS with those of mixed surfactant phase SMEDDS to further improve resveratrol absorption by enhancing its biopharmaceutical properties (solubility, dissolution rate, and first-pass metabolism). A novel SMEDDS was developed and extensively investigated for its resveratrol delivery potential. SMEDDSs are classified as isotropic mixtures of lipids and surfactants that form o/w microemulsions under dispersion with intestinal fluids following oral application.[16] The drug bioavailability is thereby mostly improved via enhanced drug solubilization and rate of drug release.[17] When appropriately formulated, the incorporated drug (i.e., resveratrol) will remain dissolved within the nanosized lipid droplets during its passage through the gastrointestinal tract (GIT). Higher amounts of dissolved resveratrol could thus be absorbed. Simultaneously, SMEDDS ingredients could additionally affect intestinal efflux transporters in the apical membrane of absorptive cells, thereby affecting the intestinal absorption process as well as presystemic metabolism of resveratrol and/or its metabolites. Furthermore, it has been reported that lipidic resveratrol drug delivery systems could attenuate reported cytotoxic effects of resveratrol solution.[13,18,19]

In this study, resveratrol was incorporated into SMEDDS composed of mixed lipid phase and mixed surfactant phase with the aim of lowering the cytotoxic effects of high-dosage resveratrol formulation. We propose that resveratrol remains incorporated within the lipid droplets of microemulsions formed in the GIT, thereby restricting its contact with the enterocyte's membranes and consequently diminishing its cytotoxicity. The *in vitro* performance of the SMEDDS formulation developed was assessed by monitoring its impact on permeability properties of resveratrol and its metabolites through the rat intestine and Caco-2 cells. Cytotoxicity and inhibitory effect of selected SMEDDS components on efflux transporters were also examined.

MATERIALS AND METHODS

Materials

The model active ingredient, resveratrol, was purchased from Merck (Darmstadt, Germany). Both surfactants—polyoxyl 35 castor oil (Kolliphor EL) and polyoxyl 40 hydrogenated castor oil (Kolliphor RH40)—were supplied from BASF (Ludwigshafen, Germany). Castor oil was purchased from Lex (Koper, Slovenia), and glyceryl caprylate/caprate (Capmul MCM EP) was obtained as a sample from Abitec (Columbus, OH). Components for the Caco-2 incubation medium as well as fluorescein (FLU) sodium salt and rhodamine 123 (Rho123) hydrate were obtained from Sigma–Aldrich (Buchs, Switzerland). Salts used to prepare the Ringer buffer and all other chemicals used in this study were of analytical grade. The cytotoxicity was assessed with kits from Promega (Madison, WI) to monitor the release of lactate dehydrogenase (LDH; CytoTox 96® Non-Radioactive Cytotoxicity Assay), intracellular proteins (Coomassie [Bradford™] Assay Kit) and ATP (CellTiter-Glo® Assay).

Construction of Pseudoternary Phase Diagram

For development of the pseudoternary diagram of the lipid phase, surfactant, and water, the water titration method was utilized. The lipid phase (equal mass parts of Capmul MCM and Castor oil) was mixed with the surfactant phase (equal mass parts of Kolliphor EL and Kolliphor RH40) at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The resulting mixtures were diluted with purified water under moderate agitation in a water bath kept at 37.0 ± 0.1°C. Visual assessment of samples was carried out following each dropwise addition of 5% water. Transparent liquid samples were regarded as microemulsions, and cloudy dispersions were considered coarse emulsions. The occurrence of a viscous gel was also noted. Afterwards, a drop of liquid sample was tested with cobalt paper to discern between w/o and o/w dispersion systems.[20]

SMEDDS Preparation and Determination of Its Saturated Resveratrol Solubility

Self-microemulsifying drug delivery system was prepared by weighing the excipients (20% each of Capmul MCM and castor oil; 30% each of Kolliphor EL and Kolliphor RH40) and blending them at room temperature to achieve a homogenous and transparent mixture. Afterwards, SMEDDS was loaded with resveratrol by placing a total of 5 mL of SMEDDS with added excess of resveratrol in a beaker. The resulting mixture was magnetically stirred at 21.0 ± 0.2°C for 48 h, while being protected from light. It was later transferred to a vial and ultracentrifuged (Thermo Fischer Scientific, Waltham, MA) at 178880 g for 20 min. Saturated resveratrol solubility in SMEDDS was determined after a sample of supernatant was adequately diluted with 70% (v/v) ethanol, filtered through a 0.45-μm membrane filter and analyzed by HPLC (see *Reverse-Phase HPLC Analysis of Resveratrol*). The assay was conducted in duplicate. For further research, SMEDDS was loaded with approximately 80% of saturated resveratrol solubility in SMEDDS (∼10 mg resveratrol/100 mg of SMEDDS formulation).

Self-Emulsification Properties

Assessment of SMEDDS self-emulsification properties was carried out by measuring the time required for 1 g of formulation to form a homogenous dispersion in 250 mL of aqueous media, magnetically stirred at 100 rpm and 37.0 ± 0.2°C. The aqueous media utilized were purified water, hydrochloric acid solution with pH 1.2, or phosphate buffer solution with pH 6.8. In addition, a photon correlation spectrometer (Zetasizer Nano; Malvern Instruments, Inc., Southborough, MA) was used to measure droplet size diameter of the resulting dispersions.

Dynamic Viscosity of SMEDDS and Its Constituents

A Physica MCR 301 rheometer (Anton Paar, Graz, Austria) was used to assess the dynamic viscosity of samples. A 1.15-mL sample was used with a cone and plate measuring system (cone radius 24.981 mm, cone angle 2.001°), kept at a temperature of 37.0 ± 0.1°C.

In Vitro Dispersion of SMEDDS

Dispersion of resveratrol incorporated in SMEDDS was determined using a USP XXII apparatus 2 (paddle) system, commonly used for the evaluation of the drug dissolution. However, in the case of SMEDDS, this system cannot elucidate between free drug and the drug potentially remaining in the (dispersed)

 DOI 10.1002/jps.24114