Development and Evaluation of a Novel Microemulsion Formulation of Elacridar to Improve its Bioavailability

RAMOLA SANE, RAJENDAR K. MITTAPALLI, WILLIAM F. ELMQUIST

Department of Pharmaceutics, Brain Barriers Research Center, University of Minnesota, Minneapolis, Minnesota 55455, USA

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ABSTRACT: The study objective was to develop a formulation of elacridar to overcome its dissolution-rate-limited bioavailability. Elacridar is a P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) inhibitor that has been used to improve the brain distribution of drugs that are substrates of P-gp and BCRP. The chronic use of elacridar is restricted because of the poor solubility leading to poor oral bioavailability. A microemulsion formulation using Cremophor EL, Carbitol, and Captex 355 (6:3:1) was developed. The elacridar microemulsion was effective in the inhibition of P-gp and Bcrp in Madin–Darby canine kidney II-transfected cells. Friend Leukemia Virus Strain B (FVB) mice were used to determine the bioavailability of elacridar after a 10 mg/kg dose of elacridar in the microemulsion, intraperitoneally (i.p.) and orally (p.o.); and the absolute bioavailability was determined to be 1.3 and 0.47, respectively. Coadministration of elacridar microemulsion i.p. with p.o. erlotinib in FVB mice improved the erlotinib brain penetration threefold. The current study shows that a microemulsion formulation of elacridar is effective in improving the bioavailability of elacridar and is an effective inhibitor of P-gp and Bcrp, *in vitro* and *in vivo*. It offers an alternative to the suspension and allows a decrease in the dose required to achieve a significant inhibitory effect at the blood–brain barrier. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1343–1354, 2013

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INTRODUCTION

One of the major hurdles that has to be overcome for effective treatment of brain disorders is the blood–brain barrier (BBB). Efflux transporters, such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), work at the level of the BBB to actively efflux drugs from the brain, thus limiting distribution to their target site. Pharmacological inhibition of these efflux transporters may overcome this inadequate delivery of drug therapy to the brain. Elacridar (GF 120918) was initially developed as a multidrug resistance reversal agent to restore sensitivity of tumor cells to chemotherapeutics such as doxorubicin.[1] It is a potent inhibitor of P-gp and BCRP.[2,3] This inhibitory effect has been also used to improve the oral (p.o.) bioavailability of drugs that are substrates for P-gp and BCRP in humans, such as topotecan and paclitaxel, by limiting the efflux of substrate drugs into the intestinal lumen.[4–6] Elacridar has also been used to study the influence of P-gp and Bcrp in preclinical models on the brain distribution of substrate drugs such as morphine, amprenavir and several tyrosine kinase inhibitors.[7–11] These studies have shown that inhibition of these efflux proteins at the BBB using elacridar is an effective way to enhance the brain distribution of drugs that are substrates for P-gp and BCRP. This strategy could result in improved efficacy of several drugs that have a site of action in the brain, but have central nervous system (CNS) distribution limited by the BBB. Therefore, the use of elacridar as a drug delivery adjuvant has the potential to enhance efficacy of these drugs by improving their distribution to target sites within the CNS.

Abbreviations used: BBB, blood–brain barrier; CNS, central nervous system; P-gp, p-glycoprotein; BCRP, breast cancer resistance protein; FVB, Friend Leukemia Virus Strain B; LC–MS/MS, liquid chromatography–tandem mass spectrometry; AUC, area under the curve; i.v., intravenous(ly); i.p., intraperitoneal(ly); p.o., per os.

Correspondence to: William F. Elmquist (Telephone: +612 625 0097; Fax: +612 626 2125; E-mail: elmqu011@umn.edu)

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Coadministration of elacridar as an adjuvant treatment presents several challenges, in both preclinical and clinical applications. The bioavailability of elacridar in the mouse after a p.o. or intraperitoneal (i.p.) dose is only about 22% and 1%, respectively.[12] Unfavorable physicochemical properties, such as poor solubility and high lipophilicity, result in dissolution-rate-limited absorption from the gut lumen.[13] Therefore, remarkably large doses of elacridar, as much as 100–500 mg/kg, have been administered p.o. in preclinical species to achieve plasma concentrations that can be effective for inhibition of P-gp and Bcrp.[7,14] Moreover, given the magnitude of these doses, chronic multidose regimens are problematic. The poor solubility of elacridar also limits its development as an injectable. These are major obstacles in the practical use of elacridar in both preclinical animal models and in clinical applications. Because the p.o. absorption of elacridar is dissolution-rate limited,[13] improving the dissolution rate would be an important strategy to overcome this limitation. Several approaches could be used to improve the dissolution rate of elacridar, including the preparation of a microemulsion, a solid dispersion, a water-soluble prodrug, as well as the use of surfactants, cyclodextrins, or lipids.

Given the success of some microemulsions in increasing bioavailability, and reducing variability in absorption,[15] we chose the microemulsion approach to improve the absorption of elacridar. Microemulsions are thermodynamically stable, transparent or translucent, optically isotropic colloidal dispersions, with low viscosity, and fine droplet size (<100 nm). Microemulsions could be generated using self-microemulsifying drug delivery systems (SMEDDS), which are composed of a mixture of surfactant, cosurfactant, and lipid, which upon agitation with water form microemulsions.[16] Microemulsions improve the absorption of drugs by improving dissolution.[17,18] SMEDDS and microemulsions have been widely used to improve the bioavailability of poorly soluble drugs. Neoral (cyclosporine A), Fortovase (saquinavir), and Norvir (ritonavir) are examples of commercially available SMEDDS, which have been employed to improve bioavailability of these poorly soluble drugs.

Given its high lipophilicity and poor bioavailability, elacridar is a good candidate for a microemulsion formulation. The objective of this study was to develop and characterize a microemulsion formulation of elacridar, assess its systemic bioavailability, and determine its pharmacokinetics in plasma and brain. An improvement in the bioavailability of elacridar may result in a practical multiple-dosing regimen, one that will allow chronic inhibition of the transporters at the BBB.

MATERIALS AND METHODS

Chemicals and Reagents

Elacridar (GF 120918) [N-(4-(2-(1,2,3,4-tetrahydro-6, 7-dimethoxy-2-isoquinolinyl)ethyl)phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide] of molecular weight 563.64 g/mol was purchased from Toronto Research Chemicals (Ontario, Canada). Cremophor EL, Cremophor RH40, Solutol HS, Captex 355, and Captex 300 were obtained from Abitech (Janesville, Wisconsin). Carbitol [2-(2-ethoxyethoxy) ethanol] was purchased from Sigma–Aldrich (St. Louis, Missouri). Erlotinib was purchased from LC Laboratories (Woburn, Massachusetts). [^{14}C]-dasatinib was a kind gift sample from Bristol-Myers Squibb (Princeton, NJ). All other chemicals used were reagent grade or high-performance liquid chromatography (HPLC) grade from Sigma–Aldrich.

Solubility

The solubility of elacridar in various components was determined as follows: a small quantity of elacridar (~1 mg) was added to a 100 μL of vehicle in a closed microcentrifuge tube. The tubes were shaken on an orbital shaker at 37°C at 200 rpm for 24 h to allow for equilibration. At the end of 24 h, the tubes were removed from the shaker and centrifuged at 10600g for 5 min to separate the solubilized fraction from the undissolved material. The supernatant was pipetted, diluted, and concentration of elacridar was determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Construction of Phase Diagrams

A series of mixtures was prepared with various ratios of Solutol HS and Cremophor EL. The phase behavior of the system was studied at surfactant to cosurfactant ratios of 8:1, 4:1, and 2:1. Aliquots of each surfactant–cosurfactant mixtures were mixed with oil and then purified water. Mixtures were mixed by vortexing at room temperature. The samples were assessed visually and determined to be either coarse emulsions, gels, or clear and transparent microemulsions. The microemulsion region was plotted on a ternary phase diagram. The microemulsion region represents clear and optically isotropic systems. The nonmicroemulsion region represents the dispersed and turbid systems. These were identified by visual inspection.

Emulsion Droplet Size Analysis

The droplet size of the microemulsion was measured by the dynamic light scattering technique using a

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