International Journal of Pharmaceutics 361 (2008) 251–252

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Note

Ethyl docosahexaenoate decreased Neoral® absorption due to particle size enlargement

Vilasinee Hirunpanich, Erika Sugiyama, Hitoshi Sato*

Department of Pharmacokinetics/Pharmacodynamics, Faculty of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

ARTICLE INFO

Article history:
Received 17 March 2008
Received in revised form 6 May 2008
Accepted 29 May 2008
Available online 5 June 2008

Keywords:
Ethyl docosahexaenoate
Cyclosporine
Microemulsion
Neoral®
Particle size

ABSTRACT

We recently reported that docosahexaenoic acid (DHA) enhanced the bioavailability of cyclosporine A (CsA) in a conventional oil formulation by inhibiting CYP3A-mediated gut metabolism. The aim of this study was to evaluate the effect of ethyl docosahexaenoate (DHA-EE), the commercially available form of DHA, on the absorption of CsA from its microemulsion formulation, Neoral®, in rats. AUC_{∞}, $AUC_{0-10\,h}$ and C_{max} of CsA decreased significantly when DHA-EE was co-administered, indicating that CsA absorption was diminished by DHA-EE. The results using a laser nanoparticle size analyzer exhibited approximately 60-fold shifting of the microemulsoin particle size from 0.042 μm to 2.46 μm, when 1 mg/ml DHA-EE was added to the microemulsion solution *in vitro*. Considering that the absorption of microemulsified drugs may decrease with increase in the particle size, the observed pharmacokinetics change of CsA may be caused by microemulsion enlargement, due to physicochemical interactions with DHA-EE. Possible interactions between DHA-EE and emulsified drugs might be of clinical importance.

We have reported that docosahexaenoic acid (DHA) enhanced the bioavailability of cyclosporine (CsA) in a conventional oil formulation by inhibiting cytochrome P450 (CYP) 3A-mediated gut metabolism (Hirunpanich et al., 2005), which prompted us to investigate the effect of ethyl docosahexaenoate (DHA-EE), which is the commercially available form of DHA, on the pharmacokinetics of CsA dosed as the new microemulsion formulation, Neoral®.

The US Food and Drug Administration (FDA) recently approved the highly concentrated omega-3-acid ethyl esters (Lovaza™; Reliant Pharmaceuticals, Inc., USA), which contains 375 mg DHA-EE per 1-g capsule, for the treatment of hypertriglyceridemia with a dose of 4 g/day (FDA, 2004). Neoral® and DHA-EE could be used together in clinical settings, since CsA has a side effect of hyperlipidemia. We therefore evaluated the effects of DHA-EE on the pharmacokinetics of CsA when given as a microemulsion formulation to rats. In addition, the change of particle size of microemulsions was measured in the presence and absence of DHA-EE *in vitro*, to determine their physicochemical interactions. For these purposes, the two experiments were designed as follows.

1. Effect of DHA-EE on CsA absorption from microemulsion formulation

Male Wistar rats weighing 230–260 g were fasted overnight with free access to water. The animal experiments were performed in accordance with the Guideline for Animal Experiments of Showa University.

Neoral® (Novartis, Japan) was diluted in water to obtain the CsA concentration of 3.5 mg/ml. DHA-EE (Sigma, USA), was prepared freshly by suspending in 1% carboxymethyl cellulose sodium salt (CMC-Na) (Wako Pure Chemical Industries, Japan) to get a concentration of 100 mg/ml. The rats were cannulated at the femoral artery for blood sampling under light ether anesthesia, kept in a Bolman cage, and used after recovery from anesthesia.

DHA-EE (100 mg/kg) or CMC-Na (control) was orally pre-administered 15 min before Neoral® (3.5 mg/kg) administration. Then, blood samples (150 μl) were collected into plastic tubes containing EDTA at 0.5 h, 1 h, 2 h, 4 h, 8 h and 10 h, thoroughly mixed, and stored at 4 °C until analysis by a monoclonal antibody fluorescence polarization immunoassay (TDxFLx, Dainabot Co. Ltd., Japan).

Pharmacokinetic parameters of CsA, *i.e.* the area under the time–concentration curve from zero to infinity (AUC_{∞}), the area under the time–concentration curve from 0 h to 10 h ($AUC_{0-10\,h}$), half-life ($T_{1/2}$), the maximum blood concentration (C_{max}), volume of distribution (V_{dss}/F), total clearance (CL_{tot}/F), and time to reach C_{max}

* Corresponding author. Tel.: +81 3 3784 8611; fax: +81 3 5498 1148.
E-mail address: sato-tky@umin.net (H. Sato).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.05.036

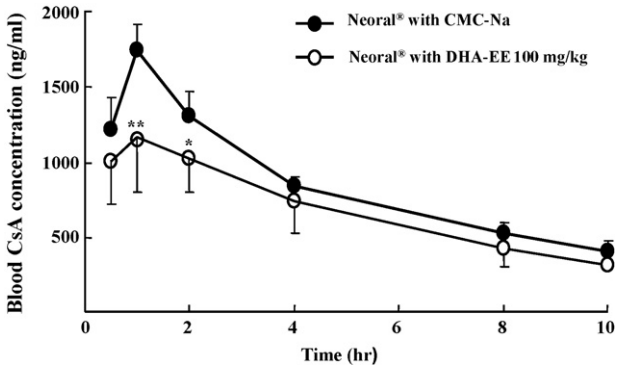

Fig. 1. Pharmacokinetic profiles of CsA after oral administration of Neoral® to rats. Ethyl docosahexaenoate (DHA-EE, 100 mg/kg) or CMC-Na (control) was pre-administered orally 15 min before Neoral® administration (3.5 mg/kg). Each point is the mean from 5 rats, and the vertical bar represents the standard deviations. The asterisks (*, **) indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively, when compared to the control group according to a Student's *t*-test.

Table 1
Pharmacokinetic parameters of CsA (3.5 mg/kg) given as Neoral® with and without DHA-EE in rats (mean ± S.D.; $n = 5$)

Parameters	Control (without DHA-EE)	Pre-administered with DHA-EE (100 mg/kg)
AUC_{∞} (ng h/l)	10.7 ± 1.04	9.03 ± 1.20*
$AUC_{0-10\,h}$ (ng h/l)	7.15 ± 0.88	5.99 ± 0.65*
$T_{1/2}$ (h)	4.82 ± 1.50	4.84 ± 0.79
CL_{tot}/F (ml/(min kg))	5.53 ± 0.60	6.57 ± 1.04
V_{dss}/F (l/kg)	2.27 ± 0.58	2.72 ± 0.37
T_{max} (h)	1.20 ± 0.45	1.00 ± 0.00
C_{max} (ng/l)	1.76 ± 0.39	1.17 ± 0.17**

(*, **) Significant differences at $p < 0.05$ and $p < 0.01$, respectively, when compared to the control group according to a Student's *t*-test.

(T_{max}), were estimated by a non-compartmental analysis using WinNonlin™ (version 4.0.1, Pharsight Corporation, USA).

2. Effect of DHA-EE on particle size of CsA microemulsions

Neoral® was diluted in water to get a CsA concentration of 1 mg/ml. DHA-EE (0.5 mg/ml and 1.0 mg/ml) or CMC-Na solution (control) was introduced into the microemulsion solution and vortexed for 5 s. Then, the mean particle size of each microemulsion solution was measured in duplicate by using a laser nanoparticle size analyzer (SALD-7100, Shimadzu, Japan).

We previously reported that DHA increased the blood concentration of CsA given as a conventional oil formulation, and the consistent results were obtained when DHA-EE was employed instead of DHA (results not shown), indicating that DHA and DHA-EE show essentially the same effect on CsA bioavailability. Opposite to these results, co-administrations of DHA-EE and Neoral® significantly decreased the blood CsA concentrations (Fig. 1) and AUC_{∞}, $AUC_{0-10\,h}$, and C_{max} decreased significantly by 15%, 16% and 34%, respectively (Table 1), while CL_{tot}/F increased by approximately 20%. In contrast, the pharmacokinetic parameters of T_{max}, $T_{1/2}$, V_{dss}/F did not change. Thus, DHA-EE decreased obviously the absorption of CsA from the microemulsion formulation, but did not change the distribution or hepatic clearance of CsA.

Table 2
Particle size of Neoral® with or without DHA-EE, determined by a laser nanoparticle analyzer (mean ± S.D.; $n = 4$)

	Particle size (μm)
Neoral® only (control)	0.042 ± 0.014
Neoral® + 1% CMC-Na	0.044 ± 0.015
Neoral® + 0.5 mg/ml DHA-EE	0.198 ± 0.175
Neoral® + 1.0 mg/ml DHA-EE	2.46 ± 0.13**

** Significant difference at $p < 0.01$, when compared to the control group according to a Student's *t*-test.

Particle size is one of the important physicochemical properties of microemulsion for the evaluation of its stability and *in vivo* fate of microemulsion (Charman et al., 1992). Several studies reported that gastrointestinal uptake of CsA microparticles was significantly affected by particle size and the uptake efficiency of small particles is supposed to increase CsA absorption (Desai et al., 1996; Zidan et al., 2007). In this study, DHA-EE changed the physical characteristics of microemulsion system, as assessed by both a great increase in the average particle size of CsA microemulsion from 0.042 μm to 2.46 μm (Table 2) and change of the visual turbidity of the microemulsion solution from transparent to opaque.

It has been reported that fatty acids with long length carbon chains (C10 and C12) exhibited low binding capacity with drug molecules in the microemulsion system and interfered with the microemulsion, resulting in a decreased critical micelle concentration (James-Smith et al., 2008). Based on this finding, it is possible that DHA-EE (a C-22 fatty acid) may incorporate into the oil phase of the microemulsion, leading to disruption or malfunctioning of the microemulsion system. The same explanation may be applied to the fact that AUC and C_{max} of CsA were decreased by 13% and 33%, respectively, when either high or low fat meal was given to human subjects prior to Neoral® dosage (Novartis, 2005), the mechanism of which has not been elucidated so far.

In summary, we first provided an *in vivo* evidence to show that DHA-EE decreases the intestinal absorption of CsA from microemulsion formulation, probably by significantly increasing the microemulsion particle size. The interactions between DHA-EE and emulsified drugs might be of clinical importance. Molecular mechanisms of this interaction should be further clarified.

References

Charman, S.A., Charman, W.N., Rogge, M.C., Wilson, T.D., Dutko, F.J., Pouton, C.W., 1992. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm. Res. 9, 87–93.

Desai, M.P., Labhasetwar, V., Amidon, R.J., Levy, R.J., 1996. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm. Res. 13, 1838–1845.

FDA announcement on qualified health claims for omega-3 fatty acids, November 10, 2004; accessible *via* http://www.fda.gov.

Hirunpanich, V., Katagi, J., Sethabouppha, B., Sato, H., 2005. Demonstration of docosahexaenoic acid as a bioavailability enhancer for CYP3A substrates: in vitro and in vivo evidence using cyclosporine in rats. Drug Metab. Dispos. 34, 305–310.

James-Smith, M.A., Shekhawat, D., Cheung, S., Moudgil, B.M., Shah, D.O., 2008. Effect of chain length on binding of fatty acids to Pluronics in microemulsions. Colloids Surf. B: Biointerf. 62, 5–10.

Novartis, The insert package for Neoral® (T2005-23), August 2005; accessible *via* http://www.pharma.us.novartis.com/product/pi/pdf/neoral.pdf.

Zidan, A.S., Sammour, O.A., Hammad, M.A., Megrab, N.A., Habib, M.J., Khan, M.A., 2007. Quality by design: understanding the product variability of a self-nanoemulsified drug delivery system of cyclosporine A. J. Pharm. Sci. 96, 2409–2423.