RELATIVE BIOAVAILABILITY OF ARTESUNATE AND DIHYDROARTEMISININ: INVESTIGATIONS IN THE ISOLATED PERFUSED RAT LIVER AND IN HEALTHY CAUCASIAN VOLUNTEERS

KEVIN T. BATTY, KENNETH F. ILETT, SHANE M. POWELL, JAYE MARTIN, AND TIMOTHY M. E. DAVIS

Department of Pharmacology, University of Western Australia, Nedlands, Western Australia; Department of Medicine, University of Western Australia, Fremantle Hospital, Fremantle, Western Australia; Clinical Pharmacology and Toxicology Laboratory, The Western Australian Centre for Pathology and Medical Research; School of Pharmacy, Curtin University of Technology, Bentley, Western Australia

Abstract. The aim of this study was to evaluate the bioavailability of artesunate (ARTS) and dihydroartemisinin (DHA). When the single-pass isolated perfused rat liver model was used, the hepatic bioavailability of ARTS (39 μM) was 0.20 and the bioavailability of DHA in ARTS and DHA (35 μM) perfusions was 0.12 and 0.11, respectively. Thus, the combined bioavailability of ARTS and DHA in the ARTS perfusions (0.32) was three-fold higher than the bioavailability of DHA. In the human study, eight healthy volunteers were randomized to receive oral ARTS (135 mg; 352 μmol) or oral DHA (120 mg; 422 μmol). The area under the curve (AUC0–6h) of DHA following ARTS (2.9 μmol.hr/L) was higher than the AUC0–6h of DHA following DHA administration (1.2 μmol.hr/L; P < 0.005). The dose-corrected relative oral bioavailability of DHA for DHA compared with ARTS was 43%. Thus, the use of conventional oral dosage regimens of ARTS appears preferable to oral administration of DHA.

INTRODUCTION

The antimalarial efficacy of artemisinin and its semi-synthetic derivatives is well established. However, aspects of the pharmacology of this group of antimalarials, such as the pharmacokinetic properties, have yet to be fully elucidated. Detailed pharmacokinetic data for artesunate (ARTS) and its active metabolite dihydroartemisinin (DHA) have been reported in adults and children with malaria, and in healthy volunteers. These studies indicate that ARTS has an elimination half life (t1/2) of 2–5 min and DHA has a t1/2 in the order of 40–60 min. Given the short t1/2 of ARTS, it is likely that the antimalarial efficacy of this drug is due primarily to the action of DHA.

Dihydroartemisinin is one of the least expensive and most potent artemisinin antimalarials, with the recommended dose being 120 mg initially (approximately 2.0–2.5 mg/kg), then 60 mg a day for 4–6 days. A dose of 120 mg DHA (approximately 2.5 mg/kg) is equivalent, in molar terms, to 160 μmol/kg of ARTS, it is likely that the antimalarial efficacy of this drug is due primarily to the action of DHA.

There are limited bioavailability data for formulations of ARTS, and none for DHA. We have demonstrated in patients with uncomplicated falciparum malaria that the relative bioavailability of DHA when ARTS is given orally is approximately 80%. In an evaluation of two generic formulations of ARTS in healthy volunteers, it was reported that the relative bioavailability of a Chinese product was 63% that of a Belgian product. More recently, Newton and others, using a non-selective, sensitive bioassay technique, found that the apparent oral bioavailability of combined ARTS-DHA after oral ARTS administration in uncomplicated malaria decreased from 61% in the acute phase to 31% during convalescence, a median of seven days later.

We have investigated the bioavailability of ARTS and DHA in both the isolated perfused rat liver (IPRL) model and in a human volunteer study. In the IPRL study, our aim was to compare the availability of DHA with the combined availability of ARTS and DHA when ARTS was perfused. The complementary volunteer study was designed to evaluate the relative bioavailability of ARTS and DHA when given by oral administration to healthy Caucasian volunteers. The volunteer study included a detailed assessment of the adverse effects of ARTS and DHA, including electrocardiographic monitoring. Other than a case report, there have been no studies of the pharmacokinetics of ARTS or DHA in Caucasians. Based on the limited published pharmacokinetic data, our hypothesis was that ARTS and DHA would have similar relative bioavailabilities.

MATERIALS AND METHODS

Isolated perfused rat liver preparation. This study was approved by the University of Western Australia Animal Experimentation and Ethics Committee. Five-week old male Wistar rats (n = 7; Animal Resources Centre, Canning Vale, Western Australia) were anesthetized with sodium pentobarbitone (70–90 mg/kg, Nembutal, Boehringer Ingelheim Pty. Ltd., North Ryde, New South Wales, Australia), and IPRL preparations were established according to standard techniques, as described previously. Briefly, the perfusate was composed of 10% human O+ red blood cells (WA Blood Transfusion Service of the Australian Red Cross, Perth, Western Australia), 1% bovine serum albumin (Fraction V, Code A-3350; Sigma, St. Louis, MO), and 30 μM sodium taurocholate (Sigma) in modified Krebs-Henseleit buffer. Livers were perfused at a rate of 10 mL/min for an equilibration period of 15–20 min, and 100% O2 was fed into the oxygenator throughout the perfusion. Liver viability was assessed by visual appearance, portal vein pressure, bile flow rate, and oxygen consumption. Bile was collected into pre-weighted microcentrifuge tubes that were weighed at the end of each experiment. Bile flow rate was calculated from the weight of bile produced during the experiment, assuming a specific gravity of 1 g/mL. Oxygen consumption was determined from blood gas determinations of inflow and out-
flow perfusate samples (500 μL) that were drawn into blood gas syringes (Bard-Parker 1-mL arterial blood gas sampling kits; Becton-Dickinson, Rutherford, NJ) at 45-min intervals during the experiments.17 Blood gas analyses were performed using a NOVA StatProfile™ Plus 5 blood gas analyzer (NOVA Biomedical, Waltham, MA). At the completion of each perfusion, bolus doses of 3H-H2O and 14C-sucrose (both isotopes from Amersham Life Sciences, Buckinghamshire, United Kingdom) were introduced into the portal vein cannula of the IPRL circuit to determine the cellular (functional) volume of the liver (Vf).17,19

DHA (4 mg/mL in ethanol; Mediplantex Company, Hanoi, Vietnam) and ARTS (5 mg/mL in ethanol; Guilin No. 2 Pharmaceutical Factory, Guangxi, People’s Republic of China) were added to each perfusate reservoir (400 mL) to give total concentrations of approximately 35 and 39 μM, respectively. The order of perfusion was randomized according to a pre-determined schedule, with drug-free perfusate passed through the liver for 15 min between each single-pass experiment. Samples (2 mL) were drawn from the perfusate reservoir and collected from the hepatic outflow cannula at 0, 15, 20, and 30 min. The perfusate was centrifuged (10,000 × g for 1 min) and the supernatant was stored at −25°C until analyzed by high performance liquid chromatography (HPLC).20 Bile was collected for 45-min periods composed of the 30-min perfusion and subsequent 15-min washout. The chemical stability of ARTS was adequate for these experiments, since approximately 5% loss of potency after 45 min (10% loss at 95 min) at 36.5°C in 0.9% sodium chloride solution has been demonstrated (Batty KT, Pharmacokinetic studies of artemunate and dihydroartemisinin, PhD Thesis, University of Western Australia, Nedlands, Western Australia, 1999).

Volunteer study. Eight healthy non-smoking Caucasian volunteers were recruited. A complete clinical assessment including analysis of serum electrolytes, proteins, and creatinine, full blood count, liver and thyroid function tests and electrocardiogram, as well as medication history, was conducted prior to the study. No subject had a history of cardiovascular or neurologic disease and none had taken an artemisinin derivative previously. Females were required to have a negative pregnancy test result before recruitment. All subjects had a mean content of 45 mg.3 The stability of ARTS was 13.3% and 12.6% at 1,380 nM and 5,120 nM, respectively, and 14.0% and 8.9% for DHA at 1,030 nM and 4,320 nM, respectively.

Pharmacokinetic and statistical analysis. Perfusate and plasma samples were assayed using a previously validated HPLC assay with limits of quantitation for ARTS and DHA of approximately 80 nM and 70 nM, respectively.20 The between-run coefficients of variation for HPLC analysis of ARTS were 13.3% and 12.6% at 1,380 nM and 5,120 nM, respectively, and 14.0% and 8.9% for DHA at 1,030 nM and 4,320 nM, respectively.

Pharmacokinetic parameters from the IPRL data were determined using non-compartmental analysis.22 Intrinsic clearance (CLint; Equation 1; Appendix 1) was calculated from the equation for the venous equilibrium model of hepatic elimination.24 The fraction of the dose (fD; Equations 2 and 3; Appendix 1) of precursor (ARTS), which forms the metabolite (DHA), was estimated using both the venous equilibrium and parallel tube models of hepatic elimination.25

For the human study, a power calculation based on previous data3 indicated that eight volunteers would be required to demonstrate a clinically significant (30%) difference in relative oral bioavailability of DHA (power = 80%, α = 0.05). The mean (95% confidence interval) content of the DHA tablets (n = 6) was 20.0 (19.4, 20.6) mg and ARTS tablets had a mean content of 45 mg.3 The stability of ARTS (780 nM and 4,560 nM) and DHA (1,060 nM and 6,160
nM) in plasma has been assessed in our laboratory for up to 12 months at −25°C and found to be within ± 7.6% of replicate samples stored at −80°C. Pharmacokinetic parameters (area under the curve from zero to six hours (AUC0–6 hr), Cmax, and tmax) were determined from the plasma concentration-time data using non-compartmental analysis.24 Relative bioavailability of DHA was calculated using Equation 4 (Appendix 1). Where applicable, pharmacokinetic parameters derived for DHA assumed complete bioconversion from ARTS. Differences between means were analyzed by the Student’s t-test. Data are expressed as mean ± SD unless otherwise indicated.

**RESULTS**

**Isolated perfused rat liver (IPRL) study.** Demographic data for the rats and the indices of liver viability are given in Table 1. The cellular volume of the livers (Vl) was determined to be 4.7 mL, this being 57 ± 8% of the wet weight of the livers. Vl was used as the denominator in all subsequent pharmacokinetic calculations (Table 2). Portal vein pressure and bile flow were stable throughout the IPRL experiments (Table 1) and consistent with established limits.17,18,26 Oxygen consumption (normal range > 1.9 μmol O2/min/g of liver) increased by approximately 15% after the equilibration period, but there was no significant difference in oxygen consumption between the ARTS and DHA perfusions (Table 1).

Although the bioavailability of DHA was similar when ARTS and DHA were perfused through the liver, an additional 20% of the dose of ARTS was available post-perfusion as the precursor (Table 2). Clearance of DHA was approximately 11% higher than clearance of ARTS. However, due to a wide spread of data, intrinsic clearance was not different between the two drugs. The estimates of fsl (fraction of the dose of precursor (ARTS) which forms the metabolite (DHA)) from the venous equilibrium and parallel tube models were 1.84 ± 1.28 and 0.54 ± 0.11, respectively.

**Volunteer study.** Demographic, hematologic, and biochemical data are summarized in Table 3. All results were within laboratory reference ranges and there were no significant changes in indices over the period of study. The only adverse effect reported in the symptom diaries with a temporal relationship to drug administration was mild headache.

This was documented on six occasions (37%; n = 16). ARTS was associated with four of these reports. One volunteer recorded a headache on both study days but suggested it was due to the research laboratory environment. Four of the volunteers reported a headache on the first study day and in two cases the headache was associated with discomfort from the intravenous cannula.

None of the subjects had a QTc in standard lead II that exceeded 0.55 sec on any electrocardiogram taken during the study. As shown in Figure 1, the changes in mean QTc-II and mean maximum QTc following both ARTS and DHA were not significantly different. Although there was a 35% increase in the mean corrected QTc dispersion following ARTS (0.026 ± 0.008 to 0.035 ± 0.013 sec; P = 0.17) and a 12% decrease following DHA (0.024 ± 0.006 to 0.021 ± 0.006 sec; P = 0.24), the power of these observations was less than 20% and sample sizes of 30 and 64 would have been required to demonstrate statistical significance for the ARTS and DHA results, respectively.

Pharmacokinetic data are summarized in Table 4 and shown in Figure 2. The results for ARTS were highly variable and indicated a low oral bioavailability for the parent drug. However, based on the AUC data in our previous reports (5.3 μmol·hr/L and 4.1 μmol·hr/L for 5 μmol/kg doses of ARTS in falciparum and vivax malaria, respectively), the apparent oral bioavailability of DHA from the ARTS dose in the present study was in the order of 35–48%. The dose-corrected relative bioavailability of DHA for oral DHA compared to oral ARTS was 43% (Table 4), suggesting an apparent oral bioavailability of DHA of 15–21%.
FIGURE 1. Electrocardiogram data from healthy volunteers before, during, and after artesunate (ARTS) and dihydroartemisinin (DHA) dosing. There were no significant differences in any parameters (see Results). Pre-med = medical examination one week prior to the study; ARTS0 = immediately prior to oral ARTS dose; ARTS2 = 2 hr after oral ARTS dose (soon after expected peak concentration); DHA0 = immediately prior to oral DHA dose; DHA2 = 2 hr after oral DHA dose; Post-med = medical examination 1 week after study.

FIGURE 2. Plasma concentration-time profile for dihydroartemisinin (DHA) following 135 mg (352 μmol) of oral artesunate (ARTS) (○) and 120 mg (422 μmol) of oral DHA (●) administered to eight healthy Caucasian volunteers. Values are the mean and SD.

DISCUSSION

The Caucasian volunteer data from the present study demonstrate that orally administered ARTS provides a two-fold greater relative bioavailability of DHA than oral DHA per se. Based on previous studies in Vietnamese patients,6 and in the absence of pharmacokinetic data for ARTS or DHA in Caucasian patients with malaria, our study suggests that a 150 mg oral dose of ARTS produces an AUC for DHA equivalent to that after a 240 mg oral dose of DHA. In addition, since the Cmax was significantly lower and the tmax longer for DHA than for ARTS, oral ARTS is likely to provide higher peak plasma concentrations up to 1 hr earlier than DHA after oral administration. These findings suggest that ARTS has pharmacokinetic advantages over DHA.

Malaria infection may itself have a significant effect on the pharmacokinetics of the artemisinin derivatives. As a hemisuccinate ester of DHA, ARTS is susceptible to hydro-

TABLE 4
Pharmacokinetic parameters for artesunate (ARTS) and dihydroartemisinin (DHA) following oral ARTS (150 mg*; 390 μmol) or oral DHA (120 mg, 422 μmol) to eight healthy volunteers†

<table>
<thead>
<tr>
<th></th>
<th>Oral ARTS</th>
<th>DHA</th>
<th>Oral DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>62</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>2.3 ± 0.3</td>
<td>–</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Dose (μmol/kg)</td>
<td>5.9 ± 0.7</td>
<td>–</td>
<td>7.1 ± 0.8</td>
</tr>
<tr>
<td>AUC0–6 hr (μmol.hr/L)</td>
<td>0.40 ± 0.30</td>
<td>2.9 ± 1.2§</td>
<td>1.4 ± 0.8§</td>
</tr>
<tr>
<td>Cmax (nM)</td>
<td>290 (242, 607)</td>
<td>1,920¶ (1,455, 2,580)</td>
<td>558¶ (538, 995)</td>
</tr>
<tr>
<td>tmax (min)</td>
<td>39 (20, 79)</td>
<td>69¶ (50, 142)</td>
<td>152¶ (126, 209)</td>
</tr>
<tr>
<td>Relative bioavailability (DHA)</td>
<td>0.43 ± 0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Actual dose = 135 mg (352 μmol); see Materials and Methods.
† Data are given as mean ± SD or median (interquartile range) as appropriate. AUC = area under curve.
‡ Insufficient data from two volunteers for determination of pharmacokinetic parameters.
§ P < 0.01, oral ARTS versus oral DHA for dose-corrected AUC (2.9 ± 1.2 μmol.hr/L versus 1.4 ± 0.8 μmol.hr/L, respectively, by Student’s paired t-test.
¶ P < 0.01, oral ARTS versus oral DHA for corrected Cmax (1,920 nM and 465 nM, respectively; Wilcoxon signed rank test) and tmax (Wilcoxon signed rank test).
lysis by gut wall, plasma, and tissue esterases in vivo to form the active metabolite DHA. Previous studies in patients with malaria indicate that oral ARTS must be well absorbed, since the mean relative bioavailability of DHA following oral administration of ARTS is at least 60%. After correcting for dose, mean AUC values for DHA in patients with malaria were 4.1–6.1 μmol/hr/L per 100 mg of oral ARTS. In contrast, in volunteers and convalescing patients, including subjects in the present study, mean AUC values for DHA were two-fold lower (1.3–3.1 μmol/hr/L per 100 mg of oral ARTS).8 An investigation of the reasons for this observation was beyond the scope of the present study.

The significantly lower bioavailability of DHA following DHA administration observed in the present study suggests that the metabolite is itself subject to efficient pre-systemic clearance. Recent reports from animal17 and human studies (Maggs JL and others, unpublished data; Ilett KF and others, unpublished data) demonstrate that dihydroartemisinin-glucuronide (DHA-G) is the principal metabolite of DHA in the liver and this may contribute to the explanation of the low bioavailability of DHA.27 Ester pro-drugs may increase the bioavailability of xenobiotics 28,29 and, in the case of DHA, glucuronidation may be prevented by administering the pro-drug ARTS. Plasma and tissue esterases appear to hydrolyze ARTS efficiently, as demonstrated by the short plasma t1/2 of ARTS and the finding that DHA often is the only drug detected in the plasma following oral ARTS administration. Nevertheless, we found that AUC and Cmax for DHA following ARTS were significantly higher than after oral DHA. Furthermore, tmax was significantly shorter, suggesting that absorption of ARTS is more efficient than absorption of DHA. These findings are consistent with a recent study in Vietnamese volunteers in whom oral bioavailability of DHA and ARTS were 45% and 80%, respectively, indicating a relative bioavailability of approximately 55%. In the same study, the relative oral bioavailability of DHA was significantly higher (88%) in patients with falciparum malaria, suggesting that first pass metabolism of DHA may be reduced in malaria infection. Although we cannot exclude poor absorption as a reason for the low bioavailability of DHA, in the present study, we propose that the principal reason for the 2–3-fold higher relative bioavailability of DHA following ARTS administration is protection of the metabolite from pre-systemic glucuronide conjugation.

Our in vitro data relating to liver metabolism of ARTS and DHA showed that total clearance was significantly lower for ARTS compared with DHA, albeit by only 10%, but intrinsic clearance of the two drugs was not significantly different. An important finding from the IPRL experiments was that relative total bioavailability of ARTS was approximately three-fold higher than the bioavailability of DHA, a difference of similar magnitude to that found in the volunteer study. Furthermore, consistent with established principles, the bioavailability of DHA delivered to the liver as the metabolite was similar to the apparent bioavailability of DHA when delivered as the prodrug ARTS.

An explanation for the higher hepatic bioavailability of ARTS compared with DHA may in part be a function of the mechanism of xenobiotic glucuronidation and is likely to be concentration-dependent. Glucuronidation occurs in the hepatocytes, and for conjugates with molecular weights greater than 300–400 (molecular weight of DHA-G = 460), the glucuronides are eliminated predominantly in the bile. ARTS is highly water-soluble and unlikely to be taken up avidly by hepatocytes, but is considered to be hydrolyzed rapidly to DHA, most likely by tissue esterases. Since DHA has relatively low solubility in water, it would be able to cross cell membranes and be conjugated. Thus, we conclude that at the concentration used in the present study, efficient hydrolysis of ARTS to DHA occurred, with subsequent glucuronide conjugation of DHA.

Formation of DHA from ARTS is usually considered to be complete (i.e., fm = 1.0), although we found fm ≥ 1.0 with the venous equilibrium model, and fm = 0.5 with the parallel tube model. While these results cannot support or refute the suggestion that fm = 1.0 for the conversion of ARTS to DHA in vivo, our data are consistent with the previous finding that the venous equilibrium model over-estimates fm and the parallel tube model under-estimates fm. In a recent study from our group, observed plasma DHA profiles in patients receiving ARTS by intravenous infusion were very close to those predicted under the assumption of complete conversion of ARTS to DHA.

Although the primary focus of this study was evaluation of ARTS and DHA bioavailability, we were able to gather side-effect data in a controlled setting. Both drugs were well tolerated and the high incidence of mild headaches appears to have been due to physical discomfort in the majority of cases. Mean values for the QTc interval in standard lead II, maximum QTc and QTc dispersion increased over the 2-hr period after ARTS administration, but in no case was the change statistically significant. Smaller trends were observed following DHA in all parameters except QTc dispersion. Interestingly, the post-dose decreases in QTc reported previously in 113 patients with acute malaria who were treated with ARTS or artemether are in contrast to data from a recent clinical safety review, in which it was stated that prolonged QT intervals occurred in approximately 1.1% of 2,638 patients treated with artemisinin derivates who had electrocardiographic assessments. While our data are consistent with those of the latter retrospective study, the effects are relatively small compared to those resulting from therapy with other antimalarial drugs such as quinine. In addition, there is no evidence that the QT effects of any antimalarial drug are associated with an increased incidence of cardiac arrhythmias in patients with severe falciparum malaria. Larger prospective studies are needed to evaluate associations between artemisinin administration and ECG changes.

Our results indicate that DHA has a high hepatic extraction ratio and a significantly lower relative bioavailability than ARTS. We have demonstrated that, to achieve similar AUCs in Caucasian patients, the molar dose of oral DHA should be twice that of oral ARTS. However, due to the pharmacokinetic advantages of ARTS, specifically the achievement of higher and earlier peak plasma concentrations of DHA, we recommend administration of oral ARTS in favor of oral DHA formulations that are currently available. We conclude that it is appropriate to use established dosage regimens of 2–4 mg/kg/day ARTS for the treatment of uncomplicated malaria.

Acknowledgments: We acknowledge the assistance of Neville...
BIOAVAILABILITY OF ARTESUNATE AND DIHYDROARTEMISININ

Butcher (Department of Pharmacology, University of Western Australia), Tony Hall and Merilyn Faulkner (Haematology Department) and Michelle Lever (Biochemistry Department, The Western Australian Centre for Pathology and Medical Research, Nedlands) for assistance in this study. Dr Hany Ghabrial (Department of Medicine, University of Melbourne) provided technical advice and surgical training (to Kevin T. Batty).

Financial support: This work was supported by a Project Grant from the National Health and Medical Research Council (NHMRC) of Australia (Timothy M. E. Davis and Kenneth F. Ilett). Kevin T. Batty was a recipient of an NHMRC Dora Lush (Biomedical) Scholarship.

Authors' addresses: Kevin T. Batty, School of Pharmacy, Curtin University of Technology, GPO Box U1987, Perth 6845, Western Australia. Kenneth F. Ilett, Department of Pharmacology, University of Western Australia, Nedlands 6907, Western Australia. Shane M. Powell, School of Agricultural Science, University of Tasmania, GPO Box 252–54, Hobart 7001, Tasmania, Australia. Jaye Martin, Powell, School of Agricultural Science, University of Tasmania, Western Australia, Nedlands 6907, Western Australia. Timothy M. E. Davis, Department of Medicine, University of Western Australia, PO Box 673, Armadale 6112, Western Australia. Timothy M. E. Davis, Department of Medicine, University of Western Australia, Fremantle Hospital, PO Box 480, Fremantle 6959, Western Australia.

Reprint requests: Kevin T. Batty, School of Pharmacy, Curtin University of Technology, GPO Box U1987, Perth 6845, Western Australia.

REFERENCES


APPENDIX 1
EQUATIONS FOR PHARMACOKINETIC ANALYSIS

Intrinsic clearance (CL_{int}), from the venous equilibrium model of hepatic elimination, was determined from

\[ F = \frac{Q_C}{(f_U \times CL_{int}) + Q_C} \]  \hspace{1cm} (1)

where F is bioavailability (determined from F = C_{OUT} + C_{IN}, where C_{OUT} and C_{IN} are outflow and inflow concentrations of the drug, respectively), Q_C is perfusate flow rate, CL_{int} is intrinsic clearance, and f_U is fraction unbound of drug. The mean f_U for dihydroartemisinin (DHA) at 35 μM in the isolated rat liver perfusate containing 1% albumin was 0.47 ± 0.02 (n = 4) and the mean f_U for artesunate (ARTS) at 41 μM was 0.35 ± 0.03 (n = 7).

The fraction of the dose (f_m) of precursor (ARTS), which forms the metabolite (DHA), according to

(i) the venous equilibrium model of hepatic elimination:

\[ f_m = \frac{C_{OUT(M)}}{C_{IN(P)} \times E(P) \times F(M)} \]  \hspace{1cm} (2)

(ii) the parallel tube model of hepatic elimination:

\[ f_m = \frac{C_{OUT(M)} \times \ln \left(\frac{F(M)}{F(P)}\right)}{C_{IN(P)} \times (F(M) - F(M)) \times \ln F(P)} \]  \hspace{1cm} (3)

where C_{OUT(M)} is the concentration of the metabolite (DHA) in the perfusate leaving the liver, C_{IN(P)} is the concentration of the precursor (ARTS) in the perfusate entering the liver, E(P) is the extraction ratio for the precursor (ARTS), F(M) is the availability of exogenously delivered metabolite (DHA), and F(P) is the availability of precursor (ARTS).

For the volunteer studies,

\[ F = \frac{AUC_{DHA(DHA)}}{AUC_{DHA(ARTS)} \times \text{Dose}_{ARTS}} \times \frac{\text{Dose}_{DHA}}{\text{Dose}_{DHA}} \]  \hspace{1cm} (4)

where AUC_{DHA(DHA)} is the AUC (area under the curve) of DHA after administration of DHA tablets and AUC_{DHA(ARTS)} is the AUC of DHA after oral ARTS. With correction for tablet potency, the doses of DHA (Dose_{DHA}) and ARTS (Dose_{ARTS}) were 422 and 352 μmole, respectively.