Comparison of Metabolism of Donepezil in Rat, Mini-Pig and Human, Following Oral and Transdermal Administration, and in an In Vitro Model of Human Epidermis

S. R. Meier-Davis*, R. Murgasova2, C. Toole3, F. M. Arjmand1, L. Diehl4, B. Cayme1, J. Wen1, J. Shudo1 and T. Nagata1

1Teikoku Pharma USA, Inc., USA
2Charles River Preclinical Services, Montreal, Senneville (Quebec), Canada
3CeeTox, Inc., Kalamazoo, Michigan, USA
4Charles River Laboratories, Spencerville, OH, USA

Abstract

Donepezil hydrochloride is a centrally acting, reversible acetyl cholinesterase inhibitor approved for Alzheimer’s disease therapy. Donepezil is marketed as an oral tablet but a transdermal patch may have advantages as an alternate therapy. To determine whether the transdermal route alters known donepezil metabolism, this study compared plasma metabolites of donepezil hydrochloride with two routes of drug delivery, transdermal and oral, in three species; rat, mini-pig and human. The utility of a reconstituted human epidermis (RHE) in vitro model for transdermal drug biotransformation was also evaluated. In addition to donepezil, structures from three metabolic pathways were detectable in each matrix: 1) O demethylation (M1/M2; 2) N-dealkylation (M4); 3) N-oxidation (M6) utilizing LC/quadrupole–Ion trap MS/MS. In rats and humans, including RHE, the mean concentration levels of the donepezil and metabolites for both routes were found in the following order: donepezil=M4>M6>isomeric M1/M2 whilst in the pig, the order was the following: donepezil>M6>isomeric M1/M2>M4. Overall, there were no substantial differences in the plasma profiles between the rat and human. Donepezil metabolites were detectable in mini-pigs after both oral and transdermal administration, however, the concentrations varied from both rat and human. RHE showed qualitative and quantitative similarities between the metabolites produced in vitro with those found in human plasma after transdermal application. Thus, the data from the present study demonstrate that similar metabolites are found between the three species with rat most closely resembling humans and that the RHE model is a potentially valuable model to predict dermal metabolism.

Keywords: Donepezil; Transdermal; Metabolism; Reconstituted human epithelium; Rat; Mini-pig human

Abbreviations: AD: Alzheimer’s Disease; RHE: Reconstituted Human Epidermis; TDDS: Transdermal Drug Delivery System; ChEIs: Cholinesterase Inhibitors; CID: Collision Induced Dissociation; GI: Gastrointestinal Tract; MS/MS: Tandem Mass Spectrometry; LC: Liquid Chromatography; FDA: U.S. Food and Drug Administration

Introduction

Worldwide, it is estimated that one in 85 people will have Alzheimer’s disease (AD) by the year 2050. One of the primary pathological changes in AD is degeneration of cholinergic neurons in the brain, correlating with the severity of overt symptoms [1]. Currently, there is no cure for AD; i.e., reducing or halting the deterioration of affected brain cells, however, the U.S. Food and Drug Administration (FDA) has approved drugs that alleviate the cognitive symptoms associated with AD in some patients [2]. Among the approved drugs is donepezil hydrochloride (RS)-2-[1-(benzyl-4-piperidyl) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one monohydrochloride), also known as E2020 or Aricept, a reversible cholinesterase inhibitor (ChEI) with enhanced specificity for acetylcholinesterase over butylycholinesterase and less reported hepatic toxicity [3,4].

The approved therapies for the symptomatic treatment of AD are predominantly administered via the oral route [5]. Because of the cholinimimetic effects, however, the orally administered ChEIs, such as tacrine, rivastigmine, donepezil and galanthamine, may be associated with adverse reactions, including nausea, diarrhea, insomnia, vomiting, muscle cramps, fatigue and anorexia. Transdermal patch delivery provides a viable treatment option for patients with mild to moderate AD; with important potential benefits including avoidance of gastrointestinal (GI) tract and first pass metabolism, and may mitigate the common direct gastrointestinal affects of nausea, vomiting and diarrhea [6]. Additionally, in elderly patients, the transdermal route has several advantages over the oral route which include maintenance of sustained therapeutic plasma concentrations, alternate administration route for those patients with difficulty in swallowing, visual confirmation of patient compliance and reduced systemic adverse effects.

The first transdermal drug delivery system (TDDS), also known as a patch, developed for the treatment of mild to moderate AD contained the approved cholinesterase inhibitor rivastigmine [5]. This dosage form was designed to deliver a therapeutically effective amount of the drug across a patient’s skin in a controlled manner. The rivastigmine patch has shown an improved tolerability profile compared with rivastigmine capsule doses providing comparable drug exposure and efficacy. Several of the properties of donepezil make it chemically well-suited to transdermal patch delivery, e.g. a low molecular weight (less than 500 Da) and an amphiphilic nature that allows the drug to readily permeate the skin barrier to provide systemic exposure [7].
pass through the skin, enter the blood stream to cross the blood-brain barrier. The potency of donepezil (the highest recommended oral dose being less than 25 mg once daily) is also advantageous because the amount of drug required to cross the skin is less, also minimizing adverse effects associated with direct skin exposure.

A clinical metabolism and elimination study [7] has shown that donepezil undergoes first-pass hepatic metabolism following oral administration, and that the predominant route for elimination of both parent drug and its metabolites is renal. The metabolic pathway indicates the presence of five metabolic pathways: 1) O demethylation to metabolites M1 and M2; 2) mono hydroxylation to metabolite M3; 3) N-dealkylation to metabolite M4; 4) N-oxidation to metabolite M6; and 5) glucuronidation of O-demethylated metabolites (M1 and M2) to metabolites M11 and M12 [8]. O-demethylation in combination with glucuronidation, N-dealkylation, and N-oxidation are the major pathways for elimination of donepezil in humans [8]. The primary metabolites observed in plasma were the O-demethylated products and the N-oxy piperidine metabolite. One of the metabolites, 6-O-desmethyl donepezil, has similar potency to donepezil in inhibiting acetylcholinesterase activity [9].

Over the last ten years, metabolic activity of the skin has been reported, due in particular to the effects of enzymes which are located in the epidermal and dermal layers [10]. This skin metabolism may have an effect on the percutaneous penetration and delivered dose of xenobiotics administered by the transdermal route [10,11]. Skin absorption/permeation characteristics can be studied by using a reconstructed human epidermis (RHE), which is a normal (non-transformed), human cell-derived, metabolically active, 3-dimensional organotypic in vitro skin model. Also known as EpiDerm, RHE closely mimics human epidermis, both structurally and biochemically. In addition, recent studies indicate that the xenobiotic metabolizing capacity of the RHE model appears to be representative of human skin and, as such, it can provide a valuable in vitro approach for evaluation of metabolism and toxicity of cutaneous exposures to xenobiotics [12].

The objective of this study was to compare the effect of the two routes of drug delivery, transdermal and oral, on the metabolism of donepezil hydrochloride in rats, mini-pigs and humans. In addition, the utility of the reconstituted human epidermis (RHE) in vitro model for studying the metabolism of donepezil in skin was evaluated.

Materials and Methods

Chemicals

Donepezil hydrochloride (MW=415.96; Figure 1) and its metabolites, M1 (MW=365.5), M2 (MW=365.5), M4 (MW=289.4), and M6 (MW=395.5), with chemical purities greater than 98% (M1, M2, M4) to metabolites M11 and M12 [8]. O-demethylation in combination with glucuronidation, N-dealkylation, and N-oxidation are the major pathways for elimination of donepezil in humans [8]. The primary metabolites observed in plasma were the O-demethylated products and the N-oxy piperidine metabolite. One of the metabolites, 6-O-desmethyl donepezil, has similar potency to donepezil in inhibiting acetylcholinesterase activity [9].

The human 3D skin model, used for the in vitro percutaneous absorption study, was obtained from MatTek™ (Ashland, MA). The multilayer EPI-606-X tissues (22 mm diameter tissues) were equilibrated to the permeant solution temperature for 15 minutes, removed from their Millicell Cell Culture Inserts, cut to size, and mounted across a water-jacketed Franz static diffusion cell aperture (9 mm diameter, 0.64 cm²) with the epidermis facing upward into the donor compartment. The temperature of the cell was maintained at 32° ± 0.5°C.

The donepezil hydrochloride transdermal patch size was 2.5 mg/cm² placed directly onto the stratum corneum (donor side of the RHE model) in replicates of three, simulating topical exposure. A placebo patch containing the same formulation other than the donepezil hydrochloride was applied to the RHE and served as the negative control. Aliquots from the receptor compartment of the RHE model were taken at 1, 4, 8, 24 and 48 hours after application and analyzed for the parent compound and its metabolites by LC-MS/MS.

Cytotoxicity after donepezil or placebo patch application was determined using microscopic evaluation of the tissue and the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Tissue viability using the MTT assay was performed on the tissues after the 24- and 48-hour incubation periods. The tissue was rinsed twice with Dulbecco’s phosphate-buffered saline and incubated for three hours with media containing 1 mg/mL MTT in a humidified 37°C incubator containing 5% CO₂. The media was discarded and the tissue extracted in 250 mL isopropyl alcohol/well and 150 mL added to the apical side of the tissue. The tissue was extracted overnight; the tissue pierced to mix both sides of extracted tissue and the MTT solution was quantified by optical density (OD) measurement at 570 and 650 nm.

The tissue for histological evaluation was fixed in 10% buffered formalin, processed to slides and stained with hematoxylin and eosin.

Rat sample collection

Male and female Sprague-Dawley rats (Cr:CD(SD)) obtained from Charles River Laboratories (Kingston, NY) were administered vehicle or donepezil hydrochloride either via oral gavage or topical administration of a paste formulation for 13 weeks. The animals were ~8 weeks of age and male weights ranged from 223 to 294 grams and females 169 to 230 grams. The oral donepezil dose level was 10 mg/kg and the topical dose was 250 mg/kg administered at a volume of 10 mL/kg and 1 mL/kg, respectively. Blood samples for donepezil and metabolite analysis were collected at 24 hours after Day 1 and Day 86 of dosing, n=3/sex/dose group. Blood was placed into tubes containing K2EDTA, plasma collected after centrifugation at 1800 x g and stored at -70°C until analysis.

The procedures were reviewed by the responsible Institutional Animal and Use Committee. The study was performed in compliance with Good Laboratory Practice regulations (21 CFR, Part 58).

Mini-pig sample collection

Male and female Gottingen mini-pigs (Sus scrofa) from Marshall BioResources (North Rose, NY), aged 5-7 months and weighing 10-21 kg, were administered either an oral tablet or two donepezil HCl
transdermal patches, repeatedly, for nine months. Animals either received a 10 mg tablet with food, daily, or two transdermal patches (350 mg donepezil HCL/patch), weekly. Blood was collected on Days 1, 29 and 274 for metabolite identification in K2EDTA anti-coagulant. Plasma was processed by refrigerated centrifugation for at least ten minutes at 3000 rpm. After centrifugation, plasma was removed and stored frozen at or below -70°C until analysis. Samples (three animals/sex/group) either administered the oral tablet on Days 1 and 274 or administered two transdermal patches on Days 29 and 274 were analyzed for metabolites.

The procedures were reviewed by the responsible Institutional Animal and Use Committee. The study was performed in compliance with Good Laboratory Practice regulations (21 CFR, Part 58).

**Human subjects sample collection**

Healthy male and female subjects, age range 23-55 years, were randomized to receive either one 7-day patch or a 5 mg oral Aricept tablet for 7 days followed by 10 mg oral Aricept tablet for 14 days.

The study was conducted in accordance to Good Clinical Practice (21 CFR, Parts 50, 56). The study protocol was approved by an Institutional Review Board preceding the start of subject screening procedures. Prior to participation in the study, subjects were to provide written informed consent.

Venous blood samples were collected using evacuated tubes containing sodium (6 ml of blood). Sample tubes were placed into a covered ice water bath/Kryorack after collection and maintained in the covered ice water bath/Kryorack until centrifugation. Sample tubes were centrifuged for 15 minutes at 3400 rpm at 4°C. The time between sample collection and freezer storage did not exceed one hour. Samples for metabolite analysis were selected based upon a range of plasma concentrations for each subject.

**Analysis of donepezil and metabolites**

The stock solutions (~10 mM) of donepezil and metabolites M1, M2, M4 and M6 were prepared in methanol: ultrapure water (1:1, v/v) and stored at approximately -20°C, protected from light. The stock solutions were further diluted with ultrapure water to 0.25 mM as working solutions. For detection of donepezil and identified metabolites in the plasma and RHE samples, a positive ion full scan and a product ion scan acquisition were used for profiling and identification, respectively. The levels of donepezil, M1, M2, M4 and M6 in each sample were then calculated based on a one point calibration, and compared to the blank extract spiked with a known amount of the reference standards.

Plasma and RHE samples were extracted in acetonitrile:methanol (1:1, v/v) containing 0.1% formic acid, mixed and the precipitates separated by centrifugation. The acetonitrile-methanol-diluted supernatant samples were then evaporated to dryness under a stream of nitrogen and the residues reconstituted using 100 µL of water:acetonitrile:formic acid (80:20:0.1, v/v/v). The residues were reconstituted using 100 µL of water:acetonitrile:formic acid (80:20:0.1, v/v/v). Aliquots of 10 µL were injected into the LC-MS system. The extraction efficiency was determined by comparing the peak areas of known amounts of donepezil, M1, M2, M4 and M6 in plasma after extraction. Recovery was determined at ~1.7 µM (n=3) for mini-pig and human samples and 667 ng/mL for rat samples (n=3).

Extracts were separated at ambient temperature on a Hallo 2.7 µm C18 (150×3 mm) column (Advanced Technologies, Wilmington, DE), with a gradient of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). An initial isocratic elution of 2% solvent B (3 min) was followed by a linear gradient elution of 2-98% solvent B for 20 min and then switched in 0.1 min to the initial gradient conditions containing 2% of solvent B for remainder of the 30 min run. The column was equilibrated for 10 min prior to each injection. The flow rate of the HPLC effluent was kept at 0.2 ml/min. Samples were analyzed under the following MS experimental conditions in positive ion mode: capillary voltage of 5.5 kV, declustering potentials of 60 V, nebulizer gas (GS1) and turbo gas (GS2) with a value of 45 and 60, respectively, curtain gas (CUR) of 25, ion source temperature (TEM) of 650°C and collision energy of 30 and 35 eV.

The initial method used to identify metabolites in mini-pig and human plasma samples did not differentiate between M1 and M2 peaks. Subsequently, the HPLC method was modified to separate the two peaks in rat plasma samples. The HPLC modifications included an initial isocratic elution of 2% solvent B (3 min) followed by a 65% solvent B for 20 min and then switched in 0.1 min to the initial gradient conditions containing 2% of solvent B for remainder of the 30 min run. The column was equilibrated for 10 min prior to each injection. The flow rate of the HPLC effluent was kept at 0.2 ml/min. Samples were analyzed under the following MS experimental conditions in positive ion mode: capillary voltage of 5.5 kV, declustering potentials of 60 V, nebulizer gas (GS1) and turbo gas (GS2) with a value of 45 and 60, respectively, curtain gas (CUR) of 25, ion source temperature (TEM) of 650°C and collision energy of 30 and 35 eV.

![Figure 2](image_url)
conditions containing 98% of solvent B for five minutes followed by a 2% solvent B for the remainder of the 38 min run. Samples were analyzed with the LC/MS/MS system comprised an Agilent 1100 LC binary pump and a Shimadzu SIL-HTC auto sampler coupled to a Sciex API 4000 mass spectrometer (MDS Sciex, Framingham, MA) under the following experimental conditions in the positive ion mode: capillary voltage of 5.5 kV, declustering potentials of 60 V, nebulizer gas (GS1) and auxiliary gas (GS2) with a value of 45 and 60, respectively, curtain gas (CUR) of 25, ion source temperature (TEM) of 600°C and collision energies of 30 to 36 eV.

Samples were analyzed using an Agilent 1100 HPLC binary pump and a Shimadzu SIL-HTC auto sampler coupled to a Sciex Q Trap 4000 LC-MS/MS mass spectrometer (Applied Biosystems/MDS Sciex, Framingham, MA) under the following experimental conditions in the positive ion mode: capillary voltage of 5.5 kV, declustering potentials of 60 V, nebulizer gas (GS1) and turbo gas (GS2) with a value of 45 and 60, respectively, curtain gas (CUR) of 25, ion source temperature (TEM) of 700°C and collision energies of 30 to 35 eV.

**Results**

**Metabolite identification**

The proposed metabolic pathways of donepezil are shown in Figure 2. The structures of the metabolites were postulated based on the masses of protonated molecules and their diagnostic product ions. Representative LC-MS/MS characteristics of the donepezil metabolites derived from human plasma are shown in Figures 3-7. The characteristic fragment ions in the tandem mass spectra of the parent and its metabolites are compiled in Table 1.

**The nomenclature for metabolites [8]**

**Parent (Donepezil):** The total ion chromatogram and the corresponding MS/MS spectrum of donepezil are shown in (Figures 3a and 3b), respectively. The fragmentation pattern of the protonated molecular ion at m/z 380 was determined from the MS/MS spectrum as shown in the adjoining fragment structure in Figure 3. It can be seen that the precursor ion [M+H]+ at m/z 380 can lose the toluene moiety (m/z 91) to form a daughter ion at m/z 288, which further fragments to yield the carbocations at m/z 273 and 243, likely by successive cleavage of the piperidine ring.

Additionally, a less prominent fragment at m/z 205 resulting from the removal of the benzyl piperidine moiety was also observed in the product ion spectrum of donepezil. The m/z 205 daughter ion further fragments, likely via the cleavage of the dimethoxyindanone moiety, to form ions at m/z 189 and 151.

**O-Demethylated Metabolites, M1 and M2:** Using the above described standard LC-MS/MS setup, the positional isomers M1 and M2 with O-demethylation on the 6’(M1)-or 5’(M2)-position, showed the same chromatographic (Figure 4a) and mass spectral characteristics (Figure 4b) for mini-pig and human plasma samples. The two metabolites M1 and M2 co-eluted at a relative retention time of 0.96. The fragmentation pattern of these isobaric ions [M+H]+ with m/z 366 showed diagnostic fragment ions at m/z 348, 274, 259,
229, 175, and 137. These daughter ions, which were 14 amu less than the fragments at m/z 362, 288, 273, 243, 189 and 151 in the product ion spectrum of chemically unchanged donepezil (Figure 3b), were indicative of O-demethylation (loss of 14 amu). The fragment ion at m/z 91, generated by the cleavage of the toluene moiety, was also observed in the product ion spectra of the M1 and M2 isobaric ions. Due to dealkylation, the fragment ion at m/z 91, corresponding to the mass of the toluene moiety, was not observed in the product ion spectrum of the M4 metabolite. For rat samples using modification described above, the metabolite M4 relative retention time was 17.4 with MRM transitions of m/z 290 to 189.

N-oxidated metabolite, M6: The representative total ion chromatogram (a) and the corresponding product ion spectrum (c) of the N-oxidated donepezil metabolite M6 are shown in Figure 6. The M6 metabolite was characterized by its protonated molecule at m/z 396 and a relative retention time of 1.02. The CID product ion mass spectrum of M6 gave ions at m/z 288, 273, 243, 205, 189, 151 and 91, which corresponded to those of parent (Figure 3b). The daughter ion detected at m/z 378 was due to loss of water from the hydroxylated parent molecule. For rat samples using modifications described above, the metabolite M6 relative retention time was 20.6 with MRM transitions of m/z 396 to 288.

Monohydroxylated Metabolite, M3: The total ion chromatogram...
and the corresponding product ion spectrum of the M3 metabolite, obtained by CID of the [M+H]+ ion at m/z 396, is shown in Figures 6a and 6b, respectively. The CID product ion mass spectrum of M3, with a relative retention time of 0.95, gave ions at m/z 378, 290, 286, 243, 204, 107, and 91. The fragment ions at m/z 290 and 241 occurred from the cleavages of the intact dimethoxyindanone methylpiperidine moiety. The presence of the fragments at m/z 204 and 290, corresponding to the hydroxylated benzyl methylpiperidine and the intact dimethoxyindanone methylpiperidine, respectively, indicated the site of biotransformation on the toluene moiety.

O-Demethylated Glucuronides, M11 and M12: the total ion chromatogram and the corresponding product ion spectrum of a metabolite eluting at a relative retention time of 0.9 are shown in Figures 7a and 7b. This metabolite was characterized by its protonated molecule at m/z 542. Its diagnostic fragmentation pattern was the loss of the glucuronic acid (176 amu) to m/z 366, which corresponded to those of the reference standards, confirmed the identity of the m/z 366, 396 and 290 major components present in vivo and in vitro samples. The relative retention times and the fragmentation patterns (Figures 3-7) of the components at m/z 362, 348, 396, and 290, which corresponded to those of the reference standards, confirmed the presence of parent drug (donepezil), O demethylated metabolites (M1 and M2) or the O-demethylated glucuronides (M11 and M12). Nuclear magnetic resonance (NMR) spectroscopy would be necessary to determine whether the biotransformation occurred on the 6’- or 5’-position of the donepezil structure.

**Extraction**

The mean extraction efficiencies (n=3) from human plasma, using a concentration of 1.7 µM, were approximately 96% (donepezil), 99% (M1 and M2), 92.3% (M4) and 97.1% (M6), with a coefficient of variation less than 10%. The results indicated that the extraction procedure performed well with respect to reproducibility and recovery and therefore was suitable for the further metabolite profiling of the plasma samples. The relative retention times and the fragmentation patterns (Figures 3-7) of the components at m/z 362, 348, 396, and 290, which corresponded to those of the reference standards, confirmed the presence of parent drug (donepezil), O demethylated metabolites (M1 and M2), N-oxidized metabolite (M6) and N-dealkylated metabolite (M4), respectively, in plasma profiles from all three species following both oral and topical/transdermal administration of donepezil hydrochloride. Two additional metabolites at m/z 396 and 542, which were assigned putative structures of monohydroxylated metabolite M3 and isomeric monoglucuronide metabolites M11 and M12 derived from the O-demethylated metabolites (M1 and M2), respectively, were also detected in the human plasma samples from subjects administered
both the oral tablet and the transdermal patch. The same major human circulating metabolites were previously reported by [7].

**RHE profiles**

The placebo and donepezil HCl transdermal patches overlaid over RHE for up to 48 hours did not induce cytotoxicity of the samples, as evidenced by >90% cell viability using the MTT assay (data not shown). Additionally, microscopic examination of the RHE samples showed normal histology after 48 hours of the placebo and donepezil patch application (Figures 8a and 8b). Therefore, the cell viability and architecture were adequate to assess the flux of donepezil and formation of metabolites over a 48-hour period.

As reported for human plasma [7], the major metabolites, M1, M2, M3, M4, and M6, were identified in the samples collected from the receiver compartment after application of the donepezil HCl transdermal patch to RHE (Table 2). The yields of the parent drug and its metabolites increased over the incubation period and, at 48 h after application, the parent appeared to be the most abundant component in the receiver compartment of the RHE model. The yields of the main donepezil metabolites M1, M2, M4, and M6 represented less than 8% of the parent drug concentration at the end of the incubation period. The mean (± SD) concentration of donepezil was 1860.3 ± 459.0 ng/mL, followed in descending order by M4 (114.5 ± 91.7 ng/mL), M6 (53.1 ± 26.4 ng/mL, 134.5 ± 66.8 nmol/L), and the isomeric M1/M2 glucuronides of O-demethylated donepezil, were not detected in any of the samples from the RHE model, likely due to the lower expression of these Phase II metabolizing enzymes in this tissue culture-derived skin equivalent compared to normal human epidermis.

The concentration ratio of the demethylated isomeric metabolites (M1/M2) to the parent compound (Cm/CP) in the RHE model, 48 h after test article application, was comparable to the ratio obtained from the transdermal treatment group, while the metabolite to parent drug ratios for the N-oxidated metabolite M6 and the dealkylated metabolite M4 in the RHE model. The yields of the main donepezil metabolites M1, M2, M4, and M6 represented less than 8% of the parent drug concentration at the end of the incubation period. The mean (± SD) concentration of donepezil was 1860.3 ± 459.0 ng/mL, followed in descending order by M4 (114.5 ± 91.7 ng/mL), M6 (53.1 ± 26.4 ng/mL, 134.5 ± 66.8 nmol/L), and the isomeric M1/M2 glucuronides of O-demethylated donepezil, were not detected in any of the samples from the RHE model, likely due to the lower expression of these Phase II metabolizing enzymes in this tissue culture-derived skin equivalent compared to normal human epidermis.

The concentration ratio of the demethylated isomeric metabolites (M1/M2) to the parent compound (Cm/CP) in the RHE model, 48 h after test article application, was comparable to the ratio obtained from the transdermal treatment group, while the metabolite to parent drug ratios for the N-oxidated metabolite M6 and the dealkylated metabolite M4 in the RHE in vitro model were greater compared with the corresponding values determined in human plasma after
transdermal administration of donepezil (Table 3). Although the above results indicate some differences in the expression of drug metabolizing enzymes between the RHE model and human skin, the presence of the same major metabolic pathways suggests their commonality in function.

**Rat sample analysis**

Oral administration of donepezil HCl (10 mg/kg) to Sprague-Dawley rats gave detectable plasma levels 24 hours after dosing, i.e., 14 ng/mL, but levels were lower relative to those observed in humans after oral dosing. Female Sprague-Dawley rats administered donepezil HCl topically (250 mg/kg), however, achieved similar donepezil plasma levels to the human oral dose, i.e., ~300 ng/mL (Table 4). Similar to human metabolism, the predominant metabolite found after both dosing routes was M4, followed by M6, M2 and M1. The proportion of M4 relative to the parent was increased relative to humans, ~3 to 15%. Lower levels of M6 were observed, i.e., ~1 to 3%, a similar percentage to that of humans. Both M2 and M1 were found in levels that were ~1 to 2% that of the parent concentration, also similar to the human concentrations (Table 3). The percentages of the metabolites relative to parent were similar between oral and transdermal, i.e., parent

> M4 > M6 > M2 > M1. The duration of dosing did not change the relative metabolite expression, although the absolute levels tended to increase with repeated dosing, especially for the oral route (Table 4, Figure 9).

**Mini-pig sample analysis**

The donepezil plasma levels observed in the mini-pig were substantially lower compared to human subjects regardless of administration route (Table 5, Figure 9). In mini-pigs, the mean donepezil plasma concentrations after oral administration of the tablet ranged from 5.1 to 52.5 ng/mL whereas the transdermal administration ranged from 23.8 to 113.3 ng/mL (Table 5). Unlike the human plasma, the predominant metabolite was M6 followed by M4 and no M2 was detectable after oral administration. Similar to the human plasma analysis, M3, M11/M12 were not detectable. Plasma collected from animals administered the transdermal patch had detectable levels of M6, M4 and M1/M2 in decreasing order. The proportion of M6 relative to the parent after oral administration was relatively high, ranging from ~38 to 490%, whilst the percentage of M4 compared to parent ranged from ~0.1 to 9% with no detectable levels of M1/M2. The plasma samples derived from animals administered the transdermal patch showed differing levels from that of oral administration. The percentage of M6
relative to parent ranged from ~30 to 114%, M1/M2 were detectable and ranged from ~1 to 7% of parent concentrations and M4 ranged from 0.02 to 0.6% of parent. The mean maximum concentrations of the M1, M2, M4 and M6 metabolites in plasma from animals administered the transdermal patch had higher values relative to the animals administered the oral tablet (Table 5). Furthermore, with repeated dosing (nine months), the levels of parent, M1/M2 (detectable with patch only) and M6 decreased markedly. The levels of M4 remained comparable over the dosing duration; however, the levels were close to the limit of detection (Figure 9). Although the concentrations were different between the two routes of administration and decreased with repeated administration, the relative levels were consistent between oral and transdermal, i.e., parent>M6>M4> M1/M2.

Human sample analysis

In human subjects, the steady-state donepezil plasma concentrations after oral administration ranged from 59.3 to 456.3 ng/mL and after transdermal administration, concentrations ranged from 45.6 to 410.7 ng/mL (Table 3). A range of concentrations was chosen from the available human plasma samples to assess the impact of initial concentrations on metabolite formation. The yields of the major donepezil metabolites M1, M2, M4, and M6, in both treatment groups, were low, representing less than 2% of the parent drug concentration and M3, M11/12 were not detectable. The mean maximum concentrations of the M1, M2, M4 and M6 metabolites in plasma from subjects following oral administration were slightly greater compared to the corresponding metabolites in the transdermal group (Table 3). Because the samples were selected to obtain a range of plasma concentrations to study potential effect on metabolite concentrations, individual variability was high but comparable between the treatment groups. The mean minimum plasma concentration values of the main metabolites were similar between the two treatment groups; however the range of plasma concentrations appeared to affect the metabolite concentrations of M4 and M6 more than M1/M2 (Figure 9). Overall, the contribution of each metabolic path in the donepezil biotransformation produced no significant difference between the two routes of administration. The mean concentration levels of the parent and its metabolites with both routes of administration decreased in a similar manner, as follows: parent>M4>M6>M1/M2.

Discussion and Conclusions

Determination of a xenobiotic metabolic pathway is an integral part of the drug development process and required by regulatory agencies as a portion of submission packages. Although the metabolism of drugs administered by the oral route is well-studied, significantly less is known about drugs delivered by the transdermal route. The purpose of these studies was to directly compare the oral and transdermal routes of administration of an approved drug, donepezil, where the human metabolism is known. An in vitro-in vivo comparison was made between metabolites formed in vitro using reconstituted human epithelium (RHE) and those found in human plasma after donepezil administration. The metabolic potential for the species used for nonclinical safety testing, i.e., the rat and mini-pig was also assessed. The evaluation of the metabolite formation by two routes in three species, compared to in vitro metabolism allowed a comprehensive review of donepezil HCl biotransformation. This comparison serves as a model for the future development of reformulated products by the dermal route.

Donepezil HCl is metabolized by cytochrome P450 subtypes 3A4 (predominantly) and 2D6, forming four metabolites, M4, M6, M1 and M2.
Species differences were most notable between the mini-pigs and human in that the plasma concentrations of both the parent and major metabolites in the two treatment groups decreased in the same order, as follows: parent > metabolites. Whereas humans showed a relatively low proportion of circulating metabolites were significantly lower in the mini-pig relative to human. Qualitative differences were also noted between mini-pig and human. Whereas humans showed a relatively low proportion of circulating metabolites, the mini-pig showed the largest proportion of M6, reaching similar levels as the parent and levels tended to decrease with repeated administration. By contrast, the rat showed similar plasma levels of the parent to that of human by the topical route. Although the proportion of metabolites in relation to parent was higher in the rat, the metabolites were found in similar order: parent > M4 > M6 > M2 > M1, as in humans. Therefore, the rat is a relevant nonclinical species to study human safety because of the qualitative similarity in metabolite formation. Additionally, since donepezil has a lower protein binding capacity in rats compared to humans, the circulating levels of donepezil and its metabolites are likely, higher, allowing a better safety margin.

The RHE model's potential utility is to screen dermal metabolism in vitro for compound and/or formulation screening. However, without the establishment of an in vitro/in vivo correlation, the utility of this approach is unknown. Furthermore, the use of a transdermal patch in the RHE system has not been previously reported. In this study, (i) percutaneous transport of donepezil HCI via a transdermal patch was technically feasible, (ii) donepezil HCI exposed to the RHE system was qualitatively and quantitatively correlated, the utility of this approach is unknown. Furthermore, the use of a transdermal patch in the RHE system has not been previously reported. In this study, (i) percutaneous transport of donepezil HCI via a transdermal patch was technically feasible, (ii) donepezil HCI exposed to the RHE system was qualitatively similar to those determined in human plasma after transdermal application.

### Table 4: Donepezil and Metabolite(s) Concentrations from Sprague-Dawley Rats Administered Donepezil HCl Orally (10 mg/kg) and Topically (250 mg/kg) for Three Months.

| Collection Day (24-hr timepoint) | Donepezil (ng/mL) | M1 / M2 (ng/mL) | M4 (ng/mL) | M6 (ng/mL) |
|---------------------------------|-------------------|-----------------|------------|------------|
|                                 | Oral Tablet       | Patch           | Oral Tablet| Patch      |
| Day 1 (Oral) or Day 29 (Patch)  |                   |                 |            |            |
| 68.8                            | 93.4              | ND              | 1.1        | ND         |
| 43.7                            | 79.4              | ND              | 2.1        | 0.1        |
| 45.2                            | 91.4              | ND              | 0.8        | 0.1        |
| 62.5                            | 127.6             | ND              | 2.1        | 0.1        |
| 32.5                            | 136.1             | ND              | 2.0        | 0.2        |
| 62.5                            | 151.7             | ND              | 7.9        | 0.1        |
| Mean ± SD                       | 52.5 ± 12.9       | 113.3 ± 26.5    | 2.7 ± 2.4  | 0.1 ± 0    | 0.2 ± 0.1  | 27.6 ± 18.7 | 53.9 ± 28.9 |
|                                 |                   |                 |            |            |
| Day 274                         |                   |                 |            |            |
| 5.0                             | 12.8              | ND              | 0.9        | 0.1        |
| 3.6                             | 20.0              | ND              | 0.9        | 0.1        |
| 1.3                             | 39.2              | ND              | 0.5        | 0.1        |
| 3.7                             | 13.1              | ND              | 0.5        | 0.2        |
| 11.3                            | 19.6              | ND              | 0.2        | 0.1        |
| 5.5                             | 38.2              | ND              | 0.8        | 0.1        |
| Mean ± SD                       | 5.1 ± 3.1         | 23.8 ± 10.9     | 0.6 ± 0.3  | 0.1 ± 0    | 10.5 ± 6.5 | 13.8 ± 4.7 |

NA = Not applicable; ND = not detectable; SD standard deviation

### Table 5: Donepezil and Metabolite(s) Concentrations from Gottingen Mini-pigs Administered Donepezil HCI Tablet (10 mg) and Transdermal Patch (2-350 mg patches) for Nine Months.

| Collection Day (24-hr timepoint) | Donepezil (ng/mL) | Parent / Metabolite (ng/mL) |
|---------------------------------|-------------------|-----------------------------|
|                                 | Oral              | Topical                     |
| Day 7                           |                   |                             |
| 1.78                            | 104.69            | ND                          |
| 4.80                            | 111.99            | ND                          |
| 5.98                            | 131.11            | ND                          |
| 7.86                            | 183.52            | ND                          |
| 18.02                           | 219.99            | ND                          |
| 9.76                            | 121.02            | ND                          |
| Mean ± SD                       | 8.03 ± 5.60       | 145.39 ± 46.02              |
| Day 86                           |                   |                             |
| 1.56                            | 82.10             | ND                          |
| 11.32                           | 120.67            | 0.04                        |
| 30.19                           | 120.39            | 0.06                        |
| 13.16                           | 151.37            | 0.07                        |
| 18.30                           | 169.53            | 0.12                        |
| 12.09                           | 128.41            | 0.08                        |
| Mean ± SD                       | 14.44 ± 9.44      | 128.75 ± 29.95              |

NA = Not applicable; ND = not detectable; SD standard deviation

M2 [13]. Two of the metabolites, M1 and M2 are further conjugated to form M11 and M12. After donepezil administration by the oral and transdermal routes, (1) plasma metabolite profiles of donepezil in the two treatment groups were similar and involved O-demethylation, N-oxidation, monohydroxylation of the phenyl moiety, N-dealkylation, and O-demethylation followed by glucuronidation; (2) the mean concentration levels of the parent and its metabolites in the two treatment groups decreased in the same order, as follows: parent > M4 > M6 > M2 > M1, as in humans. Therefore, the rat is a relevant nonclinical species to study human safety because of the qualitative similarity in metabolite formation. Additionally, since donepezil has a lower protein binding capacity in rats compared to humans, the circulating levels of donepezil and its metabolites are likely, higher, allowing a better safety margin [14].

The RHE model's potential utility is to screen dermal metabolism in vitro for compound and/or formulation screening. However, without the establishment of an in vitro/in vivo correlation, the utility of this approach is unknown. Furthermore, the use of a transdermal patch in the RHE system has not been previously reported. In this study, (i) percutaneous transport of donepezil HCI via a transdermal patch was technically feasible, (ii) donepezil HCI exposed to the RHE system was qualitatively similar to those determined in human plasma after transdermal administration.

---

Citation: Meier-Davis SR, Murgasova R, Toole C, Arjmand FM, Diehl L, et al. (2012) Comparison of Metabolism of Donepezil in Rat, Mini-Pig and Human, Following Oral and Transdermal Administration, and in an In Vitro Model of Human Epidermis. J Drug Metab Toxicol 3:129. doi:10.4172/2157-7609.1000129
The RHE model is, therefore, a relevant model for human skin to evaluate xenobiotic metabolism with transdermal patch formulations and as an alternate experimental system for prediction of transdermal drug biotransformation.

Acknowledgement

Standards for M1, M2, M4 and M6 were kindly supplied by Eisai Ltd, Tokyo, Japan.

References

1. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, et al. (1982) Alzheimer’s disease and senile dementia: loss of neurons in the basal forebrain. Science 215: 1237-1239.

2. Alzheimer’s Association, Thies W, Bleiler L (2011) 2011 Alzheimer’s disease Facts and Figures.阿尔茨海默病研究会, 贝利尔 L (2011) 2011年阿尔茨海默病事实和数据.

3. Liston DR, Nielsen JA, Villalobos A, Chapin D, Jones SB, et al. (2004) Biotransformation of Drugs in Human Skin. Drug Metab Dispos 32: 1083-1088.

4. Heyneman CA, Lawless-Liday C, Wall GC (2000) Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs 60: 555-574.

5. Tiseo PJ, Perdomo CA, Friedhoff LT (1998) Metabolism and elimination of 14C-donepezil in healthy volunteers: a single-dose study. Br J Clin Pharmacol 1: 19-24.

6. Matsui K, Mishima M, Nagai Y, Yuzuriha T, Yoshimura T (1999) Correlation of the intrinsic clearance of donepezil (Aricept) after a single oral administration to Rat. Drug Metab Dispos 27: 1406-1414.

7. Seltzer B (2005) Donepezil: a review. Expert Opin Drug Metab Toxicol 1: 527-536.

8. Schaefer H, Filaquier C (1992) [Skin metabolism] Pathol Biol (Paris) 40: 196-204.

9. Svensson CK (2009) Biotransformation of Drugs in Human Skin. Drug Metab Dispos 37: 247-253.

10. Patel D, Patel N, Parmar M, Kaul N (2011) Transdermal drug delivery system: review. Int J Biopharm & Tox Research 1: 61-80.

11. Tiseo PJ, Perdomo CA, Friedhoff LT (1998) Concurrent administration of donepezil HCl and ketoconazole: assessment of pharmacokinetic changes following single and multiple doses. Br J Clin Pharmacol 46: 30-34.

12. Matsui K, Taniguchi S, Yoshimura T (1999) Correlation of the intrinsic clearance of donepezil (Aricept) between in vivo and in vitro studies in rat, dog and human. Xenobiotica 29: 1059-1072.