

Absorption, Distribution and Excretion Pattern of Oral and Transdermal Donepezil Hydrochloride after Single and Repeated Administration to the Rat

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Abstract

Donepezil hydrochloride formulated in a transdermal patch for weekly administration is an alternative to the approved daily oral tablet. Transdermal delivery has a locally high concentration at the application site causing potential safety issues. To assess the skin exposure for safety evaluation, studies were performed comparing the donepezil transdermal patch with an oral donepezil dose previously tested in a carcinogenicity study, in which no test article-induced tumors were found. Quantitative Whole Body Autoradiography (QWBA) comparison in Long-Evans rats administered ¹⁴C-donepezil either as single oral dose (30 mg/kg) or a 24-hour transdermal patch (~60 mg/kg) revealed similar organ distribution, including skin. Pigmented tissue, namely, skin and eye showed sustained labeling over 7-10 days, suggesting melanin binding. Subsequently, a ¹⁴C-donepezil study in Long-Evans rats comparing seven daily oral doses (10-30 mg/kg) with a seven-day transdermal patch (~40 mg/kg) again revealed similar tissue distributions between the two delivery methods, however, skin not directly exposed to the transdermal patch had less label as compared to both unpigmented and pigmented skin after oral dosing. Similarly, eye concentrations generally had higher label after oral dosing as compared to the transdermal administration. Upon patch removal, the non-viable stratum corneum under the dose-site contained the majority of the label with the underlying skin layers returning to baseline levels within 5 and 7 days for unpigmented and pigmented skin, respectively, illustrating dermal clearance. Skin structures were comparably labeled after oral and transdermal administration, as evidenced by microautoradiography.

Keywords: Donepezil; Transdermal; Absorption; Distribution; Excretion; Microautoradiography

Introduction

Donepezil hydrochloride, an acetylcholinesterase inhibitor, is approved for the symptomatic treatment of mild, moderate to severe cases of Alzheimer's disease. Currently, the approved treatment regimen is a daily oral tablet (5 mg, 10 mg and 23 mg). Given that memory deficits are one of the symptoms of Alzheimer's disease, a multi-day transdermal patch could enhance patient compliance. Transdermal patches have additional advantages in that the treatment compliance can be visualized, there is circumvention of gastrointestinal absorption and hepatic first-pass metabolism and a minimization of adverse effects secondary to peak plasma drug concentrations. Although the majority of Alzheimer's disease-affected patients are elderly, percutaneous absorption is unlikely affected relative to the younger population [1]. Furthermore, the common adverse effects of transdermal patches, namely, irritation and sensitization, tend to decrease in severity and incidence with age [2,3]. Therefore, a donepezil transdermal patch was evaluated for absorption, distribution and excretion patterns relative to the approved oral route to determine potential differences and feasibility of the transdermal route of administration.

The primary objective of this study was to determine whether differences exist in the quantity and extent of tissue distribution of donepezil when administered transdermally relative to the currently approved oral route. The secondary objective was to determine what skin structures were specifically labeled with radioactive transdermal administration.

Materials and Methods

Chemicals

¹⁴C-labeled donepezil (¹⁴Cdonepezil) was synthesized at

Amersham Co., Ltd. (Amersham, UK). Its specific activity was 4.53 MBq (122.31 mCi)/mg donepezil. The chemical structure and the site of labeling are identical to that referenced elsewhere [4]. The radiochemical purity estimated by HPLC was 96.5%.

Unlabeled donepezil was purchased from Ranbaxy Laboratories Ltd. Additional chemicals were either USP or analytical grade available commercially.

Animals

Male Sprague-Dawley and Long-Evans rats (*Rattus norvegicus*) were received from Charles River Canada Inc. (St. Constant, Quebec, Canada) and ranged in age from 9-11 weeks. Animals had access to certified diet and water *ad libitum*. The oral dose was formulated in deionized water at a concentration of 3 mg/mL. Animals received an oral dose volume of 10 mL/kg with a radiochemical dose of approximately 20 μ Ci/ animal by gavage. The transdermal patch was applied dorsally and secured with Vetrap™. For the seven-day administration, the patch was retained at the dosing site by applying

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a Tegaderm overlay followed by Vetwrap and secured with tape and jacket. The mean radiochemical dose administered transdermally was ~7 $\mu\text{Ci}/\text{animal}$. The dosing schedule for the seven-day application is summarized in Table 1. The protocol and animal procedures were reviewed and approved by the Charles River Montreal Institutional Animal Care and Use Committee. This study was in compliance with Good Laboratory Practices (21 CFR, Part 58).

Radioactivity and dose determination

The stability of the dose formulations for the dosing period of the radiolabelled test article in the oral dose formulation and from a patch was analyzed by High Performance Liquid Chromatography (HPLC) with on-line radiometric detection. For the oral dose formulation, two sets of samples were collected (both in duplicate), from the vessel, before administration of the dose and following completion of dose administration. For the transdermal administration, the test article was extracted from one unused patch (predose samples) and from one patch after the 24 hour-period and 168 hours (repeated dose only) administration (post dose samples). To calculate the actual oral dose administered to the animals, the oral dose formulation was analyzed by liquid scintillation spectroscopy to determine the radioactivity concentration before and after treatment. The radioactivity concentration was determined by preparing appropriate dilutions of the dose formulation in vehicle and duplicate aliquots of each dilution were analyzed.

The ^{14}C -donepezil remaining in the patches following a maximum of 24-hour (single dose) and 168-hour (repeated dose) wear time was extracted and analyzed by liquid scintillation spectroscopy in order to determine the residual radioactivity level. The actual dose administered to each animal was calculated by subtracting the amount of radioactivity remaining in each patch from the amount calculated to be in the patch prior to dosing (obtained by oxidation of one patch). Then, the dose administered was corrected by the amount of radioactivity removed from the skin with washing following the patch removal. From this correction, the dose administered to the animal was calculated based upon the calculated specific activity. The ^{14}C -donepezil dose was corrected for the animal's body weight measured on the day prior dose administration.

Specific activity

The specific activity of the test article in the oral dose formulation was calculated on the basis of the mean (pre- and post dose) measured levels of radioactivity and total mass of test articles (^{14}C -donepezil + donepezil HCl) in the dose formulation.

The specific activity of the test articles in the patch was calculated based on the specific activity of the radiolabelled test article and the total mass of test articles (^{14}C -donepezil + donepezil HCl) used to prepare the patches.

Group	Route	Rat Strain	Dose (mg/kg/day)
1	Oral	Sprague Dawley	30 on Days 1 and 2 10 on Days 3-7
2		Long Evans	
3		Long Evans	
4	Topical	Sprague Dawley	30 on Days 1-7
5		Long Evans	
6		Long Evans	

Table 1: Study Design and Dosing Schedule.

Sample collection

For animals administered a single oral dose or a 24-hour patch application, approximately one mL of blood was collected from the jugular vein at 2, 8, 24, 48, 72, 120, 168, 240 and 360 hours post dose or post-application of patch, transferred to tubes containing K2-EDTA anticoagulant. Background radioactivity levels were collected from an untreated animal. Following euthanasia, the dosed skin area (no tape-stripping) and a non-dosed skin area were collected at 2, 8 and 24 hours.

Urine and feces were collected at room temperature from animals (3 animals/group; oral and transdermal at each timepoint) administered either the single oral dose or 24-hour patch application at the following time intervals: 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours. One animal was placed into a metabolism cage overnight prior to dosing to provide samples for the determination of background radioactivity levels.

Expired air was collected for radioactivity analysis ($^{14}\text{CO}_2$) by drawing the cage air through a single collection tower containing *circa* 300 mL of 4M KOH at a target flow rate of 500-600 mL/min. The $^{14}\text{CO}_2$ trapping solutions was collected at the following time intervals 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours post dose or post-application of patch. Moisture and CO_2 was removed from the air drawn through the cage by columns of anhydrous calcium sulphate. Since radioactivity was not detected in the expired air after the single-dose study, expired air was not evaluated with repeated applications.

Radioactivity of the stratum corneum was determined by tape stripping on the transdermal dosing site. Repeated application and removal of twenty tape strips using adhesive tape (scotch tape or equivalent) was performed on the dosing site and the level of radioactivity determined. Twenty tape strips were used to remove the non-viable stratum corneum from the underlying skin layers (data not shown). The animals were clipped, as required, prior to the tape stripping procedure. The underlying dosing site was collected and analyzed separately for radioactivity content. Tetraethylammonium hydroxide (TEAH) was used to determine the background radioactivity level for the tape strippings.

For animals administered seven daily oral doses, blood samples, urine, feces and tissues were collected at various timepoints including 146, 152, 168, 192, 216, 264, 312, 384 and 504 hours post-first dose. Animals administered the seven-day transdermal patch had blood, urine, feces and tissues collected at various timepoints including 24, 96, 168, 192, 216, 264, 312, 384 and 504 hours post-application of patch. Prior to blood collection and while under isoflurane anesthesia, tape-stripping was performed on the dosing area.

Sample processing and sample determination of radioactivity

Duplicate 100 μl weighed aliquots of whole blood were taken for analysis of radioactivity. The remaining blood was kept on wet ice before being centrifuged refrigerated at approximately 4°C at 2700 rpm (1250 rcf) for approximately 10 minutes to separate plasma (within 1 h of blood collection). Plasma samples were then kept on wet ice pending aliquotting for radioactivity analysis (2 x 100 μl weighed aliquots). All remaining plasma samples were stored deep frozen (-55°C to -80°C).

Duplicate weighed aliquots of dose formulation, plasma, urine, $^{14}\text{CO}_2$ trapping solution and cage wash samples were mixed directly with liquid scintillation fluid for radioactivity measurement. The transdermal dosing sites were isolated from the remaining carcass and

counted separately to differentiate the radioactivity from the dosing site and from the carcass. All tissues and carcasses were solubilized *in toto* in 35% TEAH. Duplicate aliquots were then mixed with liquid scintillation fluid prior to radioactivity measurement.

Fecal samples were homogenized in water (4x w/v of water, minimum added volume 2 mL). Duplicate weighed aliquots of whole blood and fecal homogenates were solubilized (Solucene-350), decolorized with hydrogen peroxide (30% w/v) and mixed with liquid scintillation fluid for radioactivity measurement. The tape strips were solubilized for at least 48 hours in a weighed volume of 35% TEAH before weighed aliquots were mixed with liquid scintillation fluid for radioactivity measurement. The tape strips were stored at room temperature (18°C to 24°C) until completion of radioactivity analysis (including repeat analysis) and then discarded. The ¹⁴C-donepezil content remaining in the patches following dosing were extracted (based on the method supplied by the Sponsor) and analyzed by liquid scintillation spectroscopy.

The weight of one unused patch, as indicated on the pouch label, was recorded. Then the patch was cut into approximately 35 pieces, each piece (without the release liner) was combusted (PerkinElmer Model 307 Sample Oxidizer) and the products of combustion, trapped as ¹⁴CO₂, were mixed with liquid scintillation fluid for radioactivity measurement. All pieces were combusted one after the other. Then, sufficient washes of the system were performed and counted. Radioactivity measurements were conducted by liquid scintillation spectroscopy, each sample was counted for 5 minutes or to a two-sigma error of 0.1%, whichever occurred first. All counts were converted to absolute radioactivity (DPM) by automatic quench correction based on the shift of the spectrum for the external standard. The appropriate background DPM values were subtracted from all sample DPM values. Following background subtraction, samples that exhibited radioactivity less than or equal to the background values were considered as zero for all subsequent calculations.

Radioactivity concentration

All radioactivity measurements were entered into a standard computer database program (Debra Version 5.2) for the calculation of concentrations of radioactivity (dpm/g and mass eq/g) and percentage-administered radioactivity in sample. Blood, plasma, tissues, urine, feces and cage wash concentrations of radioactivity in dpm/g and mass eq/g were calculated based on the measured specific activity (dpm/mg or appropriate mass unit) of radiolabelled test article in the dose solution. The radioactivity concentrations in blood and plasma samples were converted to mass eq/mL using the density of rat blood and plasma. Total tissue content was calculated for the total organ weights. Total dosing site was calculated for total tissue weight. Skin other than the dosing site, was not corrected for total skin content but expressed as µg eq/g of sample weight. Total carcass content was calculated for the total carcass weights and total excreted mass equivalents were calculated for the total urine and total feces weight (for each designated interval).

Quantitative whole body autoradiography

At specified timepoints, Animals were euthanized by intravenous injection of Euthanyl®. Following euthanasia, animals administered the transdermal patch, had tape stripping of the dosing site as demarcated upon patch removal. Immediately following euthanasia or tape stripping, animals were deep frozen in a mixture of hexane and dry ice for 20 minutes. Animals were embedded, lying on their right side, in a 2% Carboxy Methyl Cellulose (CMC) medium using a freezing frame

to collect sagittal whole-body sections. Twelve holes were made in the frozen CMC block in order to incorporate ten ¹⁴C-glucose standard solutions and the two quality control solutions. Ink spiked with ¹⁴C-glucose was used as reference points for identification of structures presenting low radioactivity level or low contrast. Each animal specimen block was sectioned using the Leica CM 3600 cryomicrotome to collect 30 µm sections.

Microautoradiography

Following euthanasia, skin samples were collected from animals administered both oral and transdermal donepezil. For transdermal administration, both the dosing site and non-dosing site area were collected from the right side of the animals. Skin samples including both pigmented and non-pigmented skins were collected. The skin samples were preserved in 10% neutral buffered formalin pending the paraffin wax embedding and sectioning with the exception that no cover slip was applied. Deparaffinated glass slide sections were dipped in Kodak photographic emulsion NTB and then exposed for an appropriate period of time in light proof plastic boxes. Glass slide sections were then developed using Kodak developer D-19 and Kodak fixer. Procedures were carried out under reduced safety light conditions whenever necessary. Glass slide sections were stained following development and then examined using light microscopy by a pathologist. The localization and relative concentration of silver grains observed at the cellular surface was used to evaluate the distribution and the extent of radioactivity (semi-quantitative) in the skin. Pigmented adnexae were examined under epi-illumination with an immuno-gold staining cube to distinguish silver grain (fluorescent green) from melanin.

Results

Clinical observations

Animals administered the single oral dose received 30 mg/kg (20 µCi/animal) as confirmed by analysis (data not shown). The calculated mean dose of ¹⁴C-donepezil administered via the transdermal route was between 61.3 ± 14.7 mg/kg and 66.7 ± 19.6 mg/kg, equivalent to a radiochemical dose of 6.74 ± 1.60 µCi/animal and 7.30 ± 2.19 µCi/animal and, respectively. Animals administered a single dose of donepezil by either route did not exhibit significant changes in body weights or clinical observations.

With repeated oral administration of 30 mg/kg, clinical observations including tremors and brief convulsions were observed; therefore, the oral dose was decreased to 10 mg/kg from the third to seventh dose. The mean oral dose for these days ranged from 9.84 to 10.4 mg/kg/day which was equivalent to a radiochemical dose of 1.56 to 1.78 µCi/animal/day. The calculated mean dose of ¹⁴C-donepezil administered by the transdermal route was estimated from the residual radioactivity from the worn patch and ranged from 19.1 ± 9.26 mg/kg to 65.4 ± 26.4 mg/kg, equivalent to a radiochemical dose of 2.08 ± 1.03 µCi/animal to 7.00 ± 2.72 µCi/animal, respectively.

Blood levels of radioactivity

The highest observed concentration of radiolabelled donepezil in plasma of male Long-Evans rats following a single oral dose was 1.302 µg eq/mL, observed at 8 hours post dose. Thereafter, plasma radioactivity concentrations declined rapidly and were below the Limit Of Quantitation (LOQ) by 48 hours post dose. In whole blood from the same animals, the highest concentration of radioactivity was 0.831 µg eq/mL, observed at 8 hours post dose. Thereafter, concentrations declined rapidly and were below the LOQ by 48 hours post dose.

Blood-to-plasma ratios for these animals ranged from 0.644 to 0.741. The percentage of the dose found in the blood was estimated, using a standard blood volume/body weight (64.0 mL/kg). The percentage of the dose in the blood of male rats following the oral dose was estimated as 0.171% at 8 hours post dose, reducing to 0.105% by 24 hours post dose. Plasma and blood concentration values were below the LOQ at all time points analyzed following transdermal patch administration over a maximum of 24-hour wear duration to male Long-Evans rats.

The highest observed concentration of radiolabelled donepezil in plasma of male Long-Evans rats following a repeated oral dose was $0.887 \pm 0.308 \mu\text{g eq/mL}$, observed at 146 hours post first-dose (or 2 hours after the final dose). Similar to the single dose, plasma radioactivity concentrations declined rapidly and were below the limit of quantitation (LOQ) by 168 hours post first-dose. In whole blood from the same animals, the highest concentration of radioactivity was $0.683 \pm 0.101 \mu\text{g eq/mL}$, observed at 146 hours post-first dose (*i.e.* the first timepoint analyzed) and concentrations declined rapidly and were below the LOQ by 168 hours post-first dose (*i.e.* 24 hours after the final dose). Blood-to-plasma ratios for these animals ranged from 0.756 to 0.818. The percentage of the dose found in the blood was estimated, using a standard blood volume/body weight (64.0 mL/kg). The percentage of the dose in the blood of male rats following the oral dose was estimated as 0.042% at 146 hours post first-dose, reducing to 0.032% by 152 hours post-first dose. Plasma and blood concentration values were below the LOQ at all timepoints analyzed following transdermal patch administration over 168-hour wear duration to male Long-Evans rats.

Tissue distribution of radioactivity

The highest concentration of ^{14}C -labelled donepezil in rats administered a single oral dose was observed in stomach wall ($63.746 \mu\text{g eq/g}$ at 2 hours post dose), followed by eye ($48.753 \mu\text{g eq/g}$; at 24 hours post dose) and pigmented skin ($20.093 \mu\text{g eq/g}$; 72 hours post dose). The C_{max} values for the majority of tissues occurred at 2 hours post dose (*i.e.* 21 of 30 tissues). The lowest radioactivity C_{max} values were observed in bone ($0.861 \mu\text{g eq/g}$; at 24 hours post dose) followed

by brain ($0.952 \mu\text{g eq/g}$, at 2 hours post dose) and skeletal muscle ($0.993 \mu\text{g eq/g}$, at 2 hours post dose). Radioactivity concentrations decreased rapidly and were below the LOQ (LOQ values ranged from $0.196 \mu\text{g eq/g}$ to $0.440 \mu\text{g eq/g}$) by 72 hours post dose in the majority of tissues, the exception being eye and pigmented skin, where concentrations remained high at 72 hours post dose (Table 2a). At 360 hours post dose, radioactivity was still detected in eye ($19.912 \mu\text{g eq/g}$, 40.8% of C_{max}), small intestine wall ($0.424 \mu\text{g eq/g}$, 2.2% of C_{max}), large intestine wall ($0.352 \mu\text{g eq/g}$, 5.7% of C_{max}) and urinary bladder wall ($0.265 \mu\text{g eq/g}$, 6.1% of C_{max}). Maximum radioactivity concentrations in the gastrointestinal tract contents were observed in stomach contents ($473.727 \mu\text{g eq/g}$, at 2 hours post dose), followed by small intestine contents ($378.895 \mu\text{g eq/g}$, at 2 hours post dose) and large intestine contents ($161.538 \mu\text{g eq/g}$, at 24 hours post dose) (Table 2a).

Tissue-to-plasma ratios were higher than 1.0 for all tissues at 2 hours post dose.

The highest tissue-to-plasma ratios were observed in stomach wall (85.609 at 2 hours post dose), eye (77.072 at 24 hours post dose) and small intestine wall (29.281 at 24 hours post dose).

Following repeated oral dosing over the seven days, the highest tissue radioactivity was observed in eyes ($192.305 \pm 47.126 \mu\text{g eq/g}$ at 146 hours post-first dose), followed by pigmented skin ($33.397 \pm 10.921 \mu\text{g eq/g}$; at 146 hours post-first dose) and Harderian glands ($18.634 \pm 9.166 \mu\text{g eq/g}$; 146 hours post-first dose) (Figure 2a). The C_{max} values for the majority of tissues occurred at 146 hours post-first dose (*i.e.* 2 hours after the final dose) with the only exception being the large intestine for which the C_{max} value was observed at 152 hours post-first dose (*i.e.* 8 hours after the final dose). The lowest mean radioactivity C_{max} values were observed in brain ($1.024 \pm 0.370 \mu\text{g eq/g}$, at 146 hours post-first dose), followed by muscle ($1.317 \pm 0.460 \mu\text{g eq/g}$, at 146 hours post-first dose) and bone ($1.941 \pm 0.895 \mu\text{g eq/g}$, at 146 hours post-first dose). Radioactivity concentrations decreased rapidly and were below the LOQ by 192 hours post-first dose (*i.e.* 48 hours after the final dose) in the majority of tissues. In the remainder, mean concentrations of radioactivity were low (less than $2 \mu\text{g eq/g}$), the exceptions being eyes

Tissue ($\mu\text{g eq/g}$) (n=3)	Oral (30 mg/kg)						Transdermal (~61.3 mg/kg ³)					
	2h	8h	24h	72h	168h	360h	2h	8h	24h	72h	168h	360h
Blood/Plasma	0.75	1.23	0.63	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Adrenal Gland	7.32	6.96	4.98	0.26	LOQ	LOQ	LOQ	2.55	6.10	2.76	LOQ	LOQ
Eye	20.55	31.85	48.75	37.13	18.93	19.91	LOQ	1.70	37.77	47.54	13.69	28.29
Liver	9.80	10.07	8.27	0.30	LOQ	LOQ	LOQ	1.96	4.58	3.39	LOQ	LOQ
Pituitary	10.32	9.13	5.91	0.44	1.10	LOQ	LOQ	LOQ	6.98	LOQ	LOQ	LOQ
Skin-Pigmented	7.27	13.29	10.62	20.09	LOQ	LOQ	LOQ	LOQ	1.68	17.19	LOQ	LOQ
Skin-Unpigmented	2.15	2.14	1.31	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Thyroid /Parathyroid	8.32	12.68	2.93	0.96	0.31	LOQ	LOQ	1.86	3.32	1.42	1.54	LOQ
Bone	0.76	0.68	0.86	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	2.10
Stomach	473.73	422.16	59.74	LOQ	LOQ	LOQ	LOQ	2.40	LOQ	48.18	4.16	LOQ
Small Intestine	378.90	46.60	322.26	0.76	LOQ	LOQ	LOQ	8.71	38.82	37.77	1.99	LOQ
Large Intestine	1.31	96.26	161.54	4.07	LOQ	LOQ	LOQ	1.94	42.51	21.06	LOQ	LOQ
Dose Site ⁴	NA						LOQ	35.28	91.71	24.64	LOQ	LOQ
Stratum Corneum ⁵	NA						NA	109.81	17.78	13.12	1.33	1.20

LOQ = below limit of quantitation

NA = not applicable to route of administration

³dose estimated based upon residual patch analysis

⁴ pigmented skin + unpigmented skin at the dose site

⁵ expressed as per cent of dose (mean dose of 61.3 mg/kg based upon residual patch analysis)

Table 2a: Mean Radioactivity in Blood, Plasma, Selected Tissues and Stratum Corneum (Transdermal only) from Long-Evans Rats Administered a Single Oral ^{14}C -Donepezil or 24-h Transdermal Patch Containing ^{14}C -Donepezil.

and pigmented skin, where mean concentrations remained high at this timepoint. At 504 hours post-first dose, radioactivity was still detected in eyes ($92.136 \pm 19.990 \mu\text{g eq/g}$, 47.9% of C_{max}), pigmented skin ($8.317 \pm 5.679 \mu\text{g eq/g}$, 24.9% of C_{max}), non-pigmented skin ($0.750 \pm 1.300 \mu\text{g eq/g}$, 11.3% of C_{max}) and urinary bladder ($0.072 \pm 0.124 \mu\text{g eq/g}$, 1.2% of C_{max}).

Mean tissue-to-plasma ratios were higher than 1.0 for all tissues at 146 hours post-first oral dose. The highest mean tissue-to-plasma ratios were observed in eyes (294 at 152 hours post-first dose), pigmented skin (40.6 at 146 hours post-first dose) and Harderian glands (23.8 at 146 hours post-first dose). In terms of proportion of the radioactivity administered to male Long-Evans rats, the highest mean values in tissues were observed in the liver (0.443% at 146 hours), followed by small intestine (0.274% at 146 h), eyes (0.137% at 146 hours) and kidneys (0.061% at 146 hours). In gastro-intestinal (GI) tract contents, the highest mean values were 3.291% of the administered dose in small intestine contents (at 146 hours), 2.765% in the large intestine contents (at 152 hours) and 2.573% in stomach contents (146 hours). By 504 hours post dosing, the highest proportion of the administered dose was detected in eyes (0.066%).

Radioactivity concentrations were below the LOQ for all tissues at 2 hours post application of the 24-hour transdermal patch. Following this timepoint, the concentrations in tissues increased and the C_{max} values for the majority of tissues occurred at 24 hours post-application of patch (i.e. 21 of 32 tissues). The highest concentrations of radiolabelled material in tissues were observed in pigmented skin from the dosing site ($62.458 \mu\text{g eq/g}$; at 24 hours post-application of patch), eye ($47.541 \mu\text{g eq/g}$; 72 hours post-application of patch), non-pigmented skin from the dosing site ($29.252 \mu\text{g eq/g}$; 24 hours post-application of patch), and pigmented skin from a site other than the dosing site ($20.322 \mu\text{g eq/g}$; 48 hours post-application of patch). The radioactivity concentrations were below the LOQ (LOQ values ranged from $1.238 \mu\text{g eq/g}$ to $2.356 \mu\text{g eq/g}$) for some tissues (brain, lung, myocardium, rectum wall, skeletal muscle, non-pigmented skin from site other than dosing site and stomach wall) at all timepoints analyzed. Radioactivity concentrations decreased rapidly in the majority of tissues and were below the LOQ by 120 hours post-application of patch, with the exception of eye, where

concentrations remained high at this timepoint. At 360 hours post-application of patch, radioactivity was still detected in eye ($28.286 \mu\text{g eq/g}$, 59.5% of C_{max}) and bone ($2.102 \mu\text{g eq/g}$) (Table 2a).

The highest mean concentrations of radiolabelled material in tissues from animals administered the transdermal patch for seven days were observed in eyes ($118.611 \pm 16.835 \mu\text{g eq/g}$; at 192 hours post-application of patch), non-pigmented skin from the dosing site ($100.905 \pm 33.617 \mu\text{g eq/g}$; 96 hours post-application of patch), pigmented skin from the dosing site ($73.283 \pm 71.559 \mu\text{g eq/g}$; 96 hours post-application of patch), pigmented skin from a site other than the dosing site ($14.872 \pm 12.109 \mu\text{g eq/g}$; 168 hours post-application of patch) and pancreas ($10.798 \pm 2.893 \mu\text{g eq/g}$; 24 hours post-application of patch) (Table 2b, Figure 3, Figure 4). The radioactivity concentrations were below the LOQ for some tissues (muscle and pituitary gland) at all timepoints analyzed. The lowest mean radioactivity C_{max} values were observed in bone ($0.206 \pm 0.357 \mu\text{g eq/g}$, at 24 hours post dose), followed by brain ($0.302 \pm 0.264 \mu\text{g eq/g}$, at 24 hours post dose) and thyroid/parathyroid glands ($0.585 \pm 1.014 \mu\text{g eq/g}$, at 24 hours post dose).

Radioactivity concentrations decreased rapidly in the majority of tissues and were below the LOQ by 216 hours post-application of patch (i.e. 48 hours following removal of the patch), with the exception of eyes, and pigmented and non-pigmented skin from the dosing site, where concentrations remained high at this timepoint. At 504 hours post-application of patch, radioactivity was still detected in eyes ($21.489 \pm 6.827 \mu\text{g eq/g}$; 18.1% of C_{max}), pigmented skin other than dosing site ($2.763 \pm 2.998 \mu\text{g eq/g}$; 18.6% of C_{max}), non-pigmented skin from dosing site ($1.863 \pm 1.667 \mu\text{g eq/g}$; 1.8% of C_{max}) and adrenal glands ($0.613 \pm 1.062 \mu\text{g eq/g}$; 12.3% of C_{max}). In terms of proportion of the radioactivity administered to male Long-Evans rats, the highest mean values in tissues were observed in the non-pigmented skin from the dosing site (4.817% at 96 hours), followed by pigmented skin from the dosing site (2.038% at 96 hours), the liver (1.181% at 24 hours), small intestine (0.329% at 24 hours) and the eyes (0.184% at 192 hours). In GI tract contents, the highest mean values were 3.426% of the administered dose in small intestine contents (at 24 hours), 3.351% in the large intestine contents (at 24 hours) and 0.101% in stomach contents (24 hours). By 504 hours post dosing, the highest proportion

Tissue ($\mu\text{g eq/g}$) n=3	Oral (time after first dose) ⁶					Transdermal (time after application)				
	168h	192h	216h	312h	504h	168h	192h	216h	312h	504h
Blood/Plasma	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Adrenal Gland	0.47	0.42	0.15	0.26	LOQ	1.75	1.54	LOQ	LOQ	0.61
Aorta	0.65	0.20	LOQ	0.22	LOQ	LOQ	LOQ	LOQ	13.74	LOQ
Eye	163.40	157.21	172.44	112.49	92.14	76.23	118.61	67.65	42.56	21.49
Liver	1.30	0.36	0.11	LOQ	LOQ	1.98	2.08	0.60	LOQ	LOQ
Pituitary	LOQ	1.96	LOQ	0.86	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Stomach	0.28	LOQ	LOQ	LOQ	LOQ	0.25	0.15	LOQ	LOQ	LOQ
Small Intestine	0.86	LOQ	LOQ	LOQ	LOQ	1.10	0.66	0.14	LOQ	LOQ
Large Intestine	1.32	LOQ	LOQ	LOQ	LOQ	0.87	0.75	0.16	LOQ	LOQ
Skin-Pigmented	11.44	25.68	31.05	9.64	8.32	14.87	7.76	4.51	4.36	2.76
Skin-Unpigmented	0.52	LOQ	LOQ	LOQ	0.75	0.39	0.58	LOQ	LOQ	LOQ
Thyroid/Parathyroid	LOQ	0.64	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Dose Site	Pigmented	NA				72.04	8.69	23.77	8.49	LOQ
	Unpigmented	NA				88.91	8.37	10.31	4.99	1.86
Stratum Corneum ⁷	NA				12.70	2.92	2.49	0.65	0.49	

LOQ = below limit of quantitation; NA = not applicable to route of administration

⁶ Days 1 and 2 – 30 mg/kg; days 3-7, 10 mg/kg; oral gavage

⁷ Expressed as per cent of dose (mean dose of 42.4 mg/kg)

Table 2b: Mean Radioactivity in Blood, Plasma, Selected Tissues and Stratum Corneum (Transdermal only) from Long-Evans Rats Administered a Repeated Oral ¹⁴C-Donepezil or 7-Day Transdermal Patch Containing ¹⁴C-Donepezil.

of the administered dose was detected in the non-pigmented skin from the dosing site (0.057%) and the eyes (0.048%) (Table 2b).

Transdermal patch dosing site radioactivity

A significant portion of radioactivity in the dosing site was found in the stratum corneum, as evidenced from the tape-stripping results. Tape stripping was performed at 312 hours post-application of patch and the percentages of dose ranged from 0.474% to 4.222%. At early timepoints post-application of the patch (from 24 hours to 168 hours), the mean percentage of the administered dose in the tapes was high, ranging from 35.292% at 24 hours to 12.698% at 168 hours post-application of the patch. By 192 hours post-application of the patch the percentage was lower and remained relatively similar up to 504 hours (from 2.919% to 0.487%). In general, the highest proportion of the administered radioactivity was recovered in the first tapes collected (Figure 4).

Cellular radioactivity distribution by microautoradiography

After oral administration, ¹⁴C-donepezil related material also distributed to the dermis. Small amounts of ¹⁴C-donepezil related material were present within the lumen of blood vessels of the lower dermis and subcutis after both oral and transdermal patch administration (Figure 5a). Following transdermal patch administration, ¹⁴C-donepezil related material distributed to the

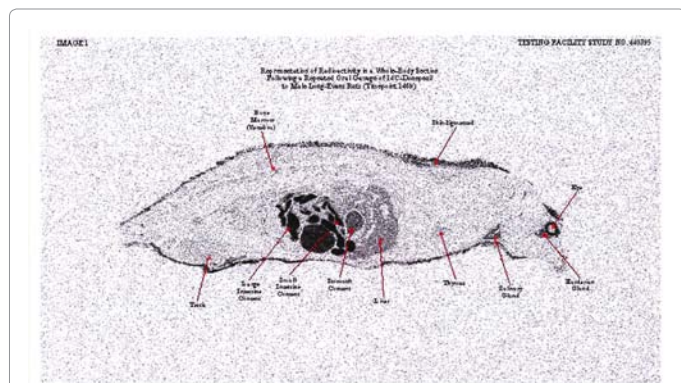


Figure 1a: Representative autoradiogram of whole body section from a Long-Evans rat orally administered ¹⁴C-donepezil daily for seven days. Arrows indicate pigmented skin, eye and portions of the gastrointestinal tract.

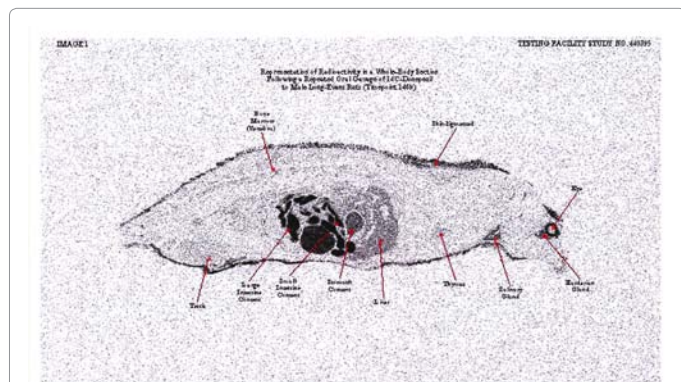


Figure 1b: Representative autoradiogram of whole body section from a Long-Evans rat administered ¹⁴C-donepezil via a transdermal patch applied for seven days. Arrows indicate dosing site, pigmented skin, eye, Harderian gland, salivary gland, liver, testis and portions of the gastrointestinal tract.

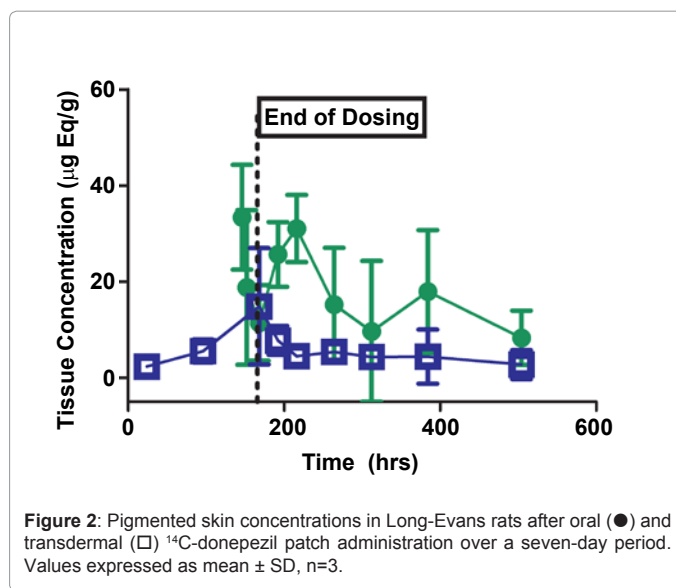


Figure 2: Pigmented skin concentrations in Long-Evans rats after oral (●) and transdermal (□) ¹⁴C-donepezil patch administration over a seven-day period. Values expressed as mean ± SD, n=3.

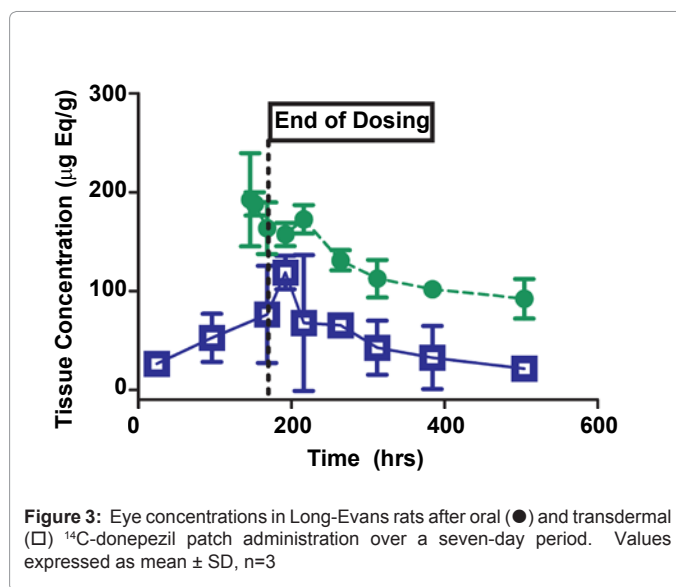


Figure 3: Eye concentrations in Long-Evans rats after oral (●) and transdermal (□) ¹⁴C-donepezil patch administration over a seven-day period. Values expressed as mean ± SD, n=3

superficial, mid and deep dermis and subcutis with a concentration gradient slightly higher on the surface and diminishing deeper within the skin (Figures 5b and 5c). Compared to the untreated skin, the dosing site had additional labeling within the keratin layer of the treated skin (Figures 5b and 5c). In both treatment groups (oral gavage and transdermal patch administration) (Figures 5a-5c), ¹⁴C-donepezil related material was found within pigmented hair shafts and in pigmented cells from the hair bulb (Figure 5d). Radioactivity appeared to reduce as time post-exposure increased in both treatment groups. Other than the stratum corneum, the radioactive labeling of the deeper skin structures was similar between oral and transdermal delivery (Table 4). Additionally, radioactivity appeared to reduce after dosing completion for both oral and transdermal administration.

Excretion

As summarized in Table 3, the majority of the orally administered radiolabelled material was recovered in the feces over the period 0 to 312 hours post-first dose (mean of 51.087% of the administered dose)

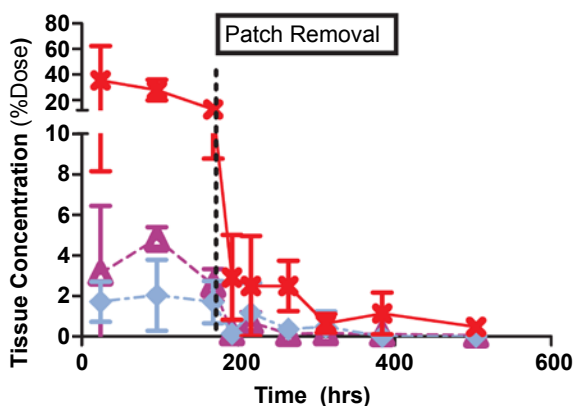


Figure 4: Dose-site concentrations expressed as a percentage of the total dose in Long-Evans rats after a seven-day transdermal ¹⁴C-donepezil patch administration. Stratum corneum (×) removed by 20 sequential tape strips and underlying skin divided into pigmented (◆) and unpigmented (○) sections and counted separately. Values expressed as mean ± SD, n=3.

with 43.685% recovered in the urine over the same time period. Of the material in feces, approximately 89% was excreted in the period 0 to 144 hours post-first dose, where 144 h corresponded to the last dose administered. Approximately 89% of the material excreted in urine was recovered in the period 0 to 144 hours post-first dose. A further 2.561% of the administered radiolabelled material was recovered in the cage wash over the period 0 to 312 hours post-first dose.

The overall mean recovery in excreta from the male Sprague-Dawley rats was 97.334%. The residual carcass accounted for a mean

Sample (n=3)	Oral (% of dose)	Transdermal (% of dose)
Carcass	0.12	0.72
Dosing Site (Transdermal)	NA	1.71
Stratum Corneum ⁸	NA	1.95
Urine	43.69	38.13
Feces	51.09	66.83
Cage Wash	2.56	21.27

⁸Collected with 20 Tapes

NA = Not Applicable to Route of Administration

Table 3: Mean Recovered Radioactivity after Oral and Transdermal Administration to the Rat.

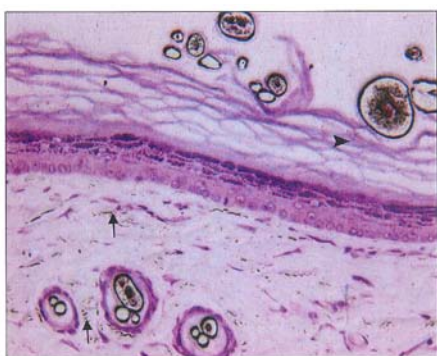


Figure 5a: Representative microautoradiograph of a pigmented skin section from a Long-Evans rat orally administered ¹⁴C-donepezil daily for seven days. Silver grains are observed adjacent to hair shafts (black arrowhead) and are present in collagen fibers of the superficial and mid dermis (black arrows). Magnification = 200X.

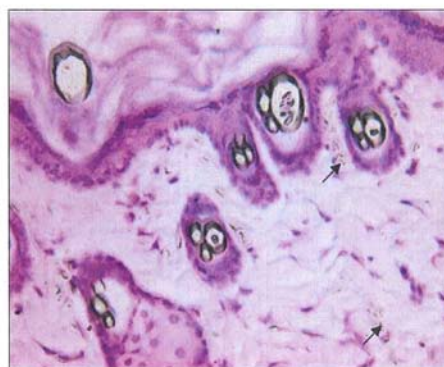


Figure 5c: Representative microautoradiograph of an area of untreated pigmented skin from a Long-Evans rat administered ¹⁴C-donepezil via a transdermal patch applied for seven days. Note that no silver grains are found in the keratin layer of untreated skin, however, arrows indicate silver staining of collagen fibrils of the superficial and mid dermis. Magnification = 200X.

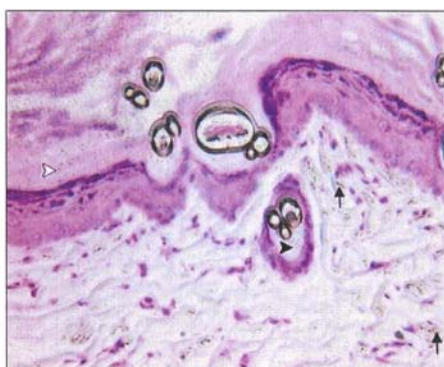


Figure 5b: Representative microautoradiograph of a treated pigmented skin section from a Long-Evans rat administered ¹⁴C-donepezil via a transdermal patch applied for seven days. Silver grains can be seen within the keratin layer of treated skin (white arrowhead). Arrows indicate silver staining of collagen fibrils, pigmented hair shaft and blood vessel lumen. Magnification = 200X.

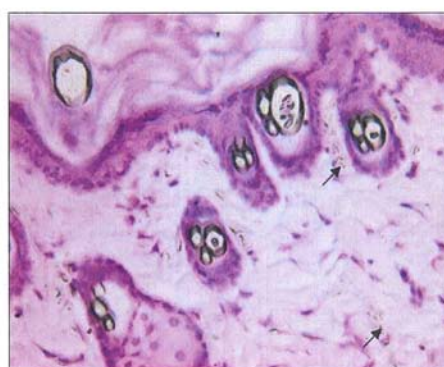


Figure 5d: Higher magnification of a treated pigmented skin section from a Long-Evans rat administered ¹⁴C-donepezil via a transdermal patch applied for seven days. Visualization with an immuno-gold staining cube indicates silver grains (fluorescent green dots, black arrow) overlying melanin. Magnification = 400X.

of 0.118% of the administered radioactivity; therefore, the overall mean mass balance of radioactivity for male Sprague-Dawley rats, at 312 hours following repeated daily oral doses of ¹⁴C-donepezil, was 97.451 ± 1.745%.

Although variability was observed, the majority of the recovered radiolabelled material was recovered in the feces over the period 0 to 312 hours post-application of patch (mean of 66.831% of the administered dose) with 38.129% recovered in the urine over the same time period (Table 3). Of the material in feces, approximately 68% was excreted in the period 0 to 168 hours post dosing, which corresponds to the time of removal of the patch. Approximately 72% of the material excreted in urine was recovered in the period 0 to 168 hours post-application of patch. A further 21.271% of the administered radiolabelled material was recovered in the cage wash over the period 0 to 312 hours post-application of patch (Table 3).

The overall mean recovery in excreta from the male rats administered the transdermal patch was 126.232%. Tape stripping was performed on the dosing site and a mean of 1.948% of the administered dose was recovered on the tapes. A further 1.706% was recovered in the dosing site and the residual carcass accounted for a mean of 0.721% of the administered radioactivity. Therefore, the overall mean mass balance of radioactivity for male Sprague-Dawley rats, at 312 hours following a transdermal patch administration of ¹⁴C-donepezil, was 130.608 ± 24.809%.

Discussion

Reformulation of drug products for delivery by another route is a means to extend the product life-cycle; however, there are potential

safety issues that must be addressed prior to market approval [5]. One way to evaluate the similarity of exposure between different routes of administration is through nonclinical ADME data, as this is usually included as part of the Investigational New Drug application [6]. Drugs reformulated for transdermal delivery are also limited by their physicochemical properties and ability to penetrate the stratum corneum [7,8]. ADME studies allow regulatory agencies to evaluate distribution patterns between delivery routes and potential drug product accumulation with repeated applications [9]. ADME data is also valuable as a correlate to nonclinical safety findings, biomarker expression and relevance to human exposure [10-12].

Despite the value of ADME studies, distribution studies with transdermal patches are not commonly done due to the complexity of manufacturing a patch containing radiolabel. Furthermore, the delivered dose is difficult to ascertain. However, this data is critical to understand potential differences in tissue distribution with alternate routes of administration. Additionally, accumulation at the transdermal dosing site must be monitored to determine potential safety risks. In this study, the distribution of donepezil administered either as a single (30 mg/kg) or repeated oral dose (10 and 30 mg/kg) was compared to a 24-hour and 168-hour transdermal administration of ¹⁴C-donepezil to male Long-Evans or Sprague-Dawley rats and concentrations of radioactivity in blood, plasma, tissues and excreta were determined. The delivered dose from the transdermal patch was estimated from radioactivity extraction of the worn patch.

The highest concentrations of radioactivity in plasma and whole blood following the oral dose were observed at 146 hours after the first dose (i.e. 2 hours following final dose) and were below the limit of

Route of Administration Location	Transdermal						Oral		
	Dose Site			Untreated skin			Untreated skin		
Time after administration (h)	168	192	312	168	192	312	168	192	312
Animal Identification	6003	6004	6007	6003	6004	6007	3003	3004	3007
Skin layer / Compartment ⁹									
Epidermis									
Pigmented hair shaft ¹⁰									
Silver grain density score	3	2	2	2	2	0	1	1	0
Keratin									
Silver grain density score	3	0	0	0	0	0	0	0	0
Upper and Mid-Dermis									
Collagen									
Silver grain density score	3	2	2	2	1	1	2	2	2
Deep Dermis									
Collagen									
Silver grain density score	2	2	2	2	1	1	1	2	1
Hair bulb									
Silver grain density score	3	2	1	2	1	1	1	2	1
Blood vessel lumen									
Silver grain density score	2	2	2	2	1	0	1	0	2
Subcutis									
Collagen									
Silver grain density score	2	1	2	1	1	¹¹	1	1	1
Blood vessel lumen									
Silver grain density score	1	1	1	1	1	¹¹	1	1	0

⁹Autoradiography scored on a 5-level scale for silver grain density

¹⁰Refers to silver grains observed within and/or adjacent to hair shafts

¹¹Subcutis not present on section

Table 4: Microautoradiographic Analysis of Skin Structures after ¹⁴C-donepezil Administration to Long-Evans Rats.

quantitation by 168 hours (24 hours after final dose) suggesting that the oral dose-related donepezil was cleared quite rapidly from the systemic circulation. The concentrations of radioactivity in plasma and in whole blood were below the limit of quantitation at all timepoints analyzed following the maximum 168-hour transdermal patch administration, suggesting that absorption through the skin was low, slow and limited consistent with the patch formulation designed to administer a steady-state amount over a seven-day period. The distribution of radioactivity into tissues following the oral administration was general and widespread by the first timepoint analyzed, but limited at 24 hours following the transdermal administration. Following both repeated oral and transdermal dose routes, the distribution was generally similar and high concentrations were associated with eyes and pigmented skin, suggesting that melanin binding had occurred.

Excretion recoveries following the oral doses were complete, by 312 hours post-first dose, with the major route of excretion occurring *via* the feces. For the animals administered the transdermal dose, the administered dose was excreted mainly *via* feces, suggesting that biliary excretion had occurred. Excretion was generally slow as compared to the oral dose, consistent with the design of a transdermal formulation. Also, consistent with transdermal administration, an increased percentage of the administered radiolabelled donepezil was found in the dosing site, the stratum corneum (tape stripping) and underlying skin, in addition to the remaining carcass. The dosing site did not have significant accumulation over the seven-day dosing period and cleared rapidly from the dosing site upon patch removal. The skin structures that were labeled after both oral and transdermal delivery were comparable. Microautoradiography revealed similar labeling of the deeper skin structure after both oral and transdermal delivery.

Other than the topical dosing site where most radioactivity was associated with the stratum corneum, consistent tissues were radiolabelled and sustained for a similar duration after transdermal administration with that of the oral route. In other words, potential concern regarding the locally high concentration at the transdermal dosing site is limited to non-viable tissue. In the deeper viable tissues, these radiolabel studies clearly demonstrate that the skin is labeled similarly between the daily oral and the seven-day transdermal patch. The skin labeling after oral dosing is consistent with previous

reports [4] suggesting that humans also have skin exposure after orally administered donepezil hydrochloride, which is more likely given the lower clearance rate relative to the rodent. Despite the likely skin exposure in humans and apparent melanin-binding potential of donepezil, significant adverse clinical findings related to skin or melanin have not been reported for Aricept®.

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