Absorption, Metabolism, and Disposition of $[^{14}C]$SDZ ENA 713, an Acetylcholinesterase Inhibitor, in Minipigs Following Oral, Intravenous, and Dermal Administration

Francis L. S. Tse$^{1,3}$ and Robert Laplanche$^{2}$

Received April 23, 1998; accepted May 25, 1998

**Purpose.** SDZ ENA 713 (rivastigmine) is an acetylcholinesterase inhibitor intended for therapeutic use in Alzheimer’s disease. The present study compared the pharmacokinetics of $[^{14}C]$SDZ ENA 713 after intravenous, oral, and dermal administration to male minipigs, and also examined the effects of dose level and skin abrasion on transdermal absorption.

**Methods.** Four groups of 3 minipigs each received a single intravenous (0.1 mg/kg), single oral (1.0 mg/kg), or topical doses of 18 mg or 54 mg of $[^{14}C]$SDZ ENA 713. Topical doses were administered as dermal patches on two occasions 10 days apart. On Study Day 1, test patches were applied to a virgin skin site. Placebo patches were applied to a separate skin site and were replaced daily during Days 1–10. On Study Day 11, test patches were applied to the site on which the placebo patches had been previously applied. After each dose, serial blood and quantitative urine and feces were collected at designated intervals for 7 days. Concentrations of radioactivity, parent drug, and metabolite ZNS 114–666 were measured in whole blood. Radioactivity was also determined in excreta, skin application sites (at study termination), and on used dermal patches (at 24 hr after application).

**Results.** Oral doses of $[^{14}C]$SDZ ENA 713 were rapidly (t$_{1/2}$ = 0.83 hr) and efficiently (ca. 93%) absorbed, although the bioavailability of the parent drug was low, ca. 0.5%, apparently due to extensive first-pass metabolism. Radioactivity was excreted mainly in the urine (~90%) with a half-life of 56 hr, slightly longer than that observed after an intravenous dose, 46 hr. After dermal administration of $[^{14}C]$SDZ ENA 713 to a virgin skin site, absorption was 8% at both dose levels investigated. Following daily application of placebo patches for 10 days, absorption from a $[^{14}C]$SDZ ENA 713 dermal patch increased by approximately twofold, 17% and 19% of the 18 mg and 54 mg doses, respectively. The increase is possibly due to hydration or abrasion of the skin as a result of repeated application and removal of the adhesive patches. Whereas total absorption from the dermal dose was smaller than that from the oral dose, essentially all of the absorbed drug via the dermal route reached the systemic circulation intact, thus yielding a SDZ ENA 713 bioavailability of 20–40 times greater than that of the oral dose. Metabolite ZNS 114–666 was rapidly formed and accounted for <4% of total drug-related material in the systemic circulation.

**Conclusions.** Dermal administration in minipigs provided a markedly greater bioavailability of SDZ ENA 713 than the oral route. The extent of absorption was independent of dose within the range tested, and appeared to be enhanced by hydration or abrasion of the skin application site.

**KEY WORDS:** Alzheimer’s disease; rivastigmine; minipig; transdermal absorption; bioavailability; skin abrasion.

**INTRODUCTION**

SDZ ENA 713 (rivastigmine), (+)-(S)-N-ethyl-3-[(1-dimethylamino)ethyl]-N-methyl-phenylcarbamate hydrogen tartrate, is an acetylcholinesterase inhibitor (AChEI) of the carbamate type (1). It inhibits the enzymolysis of acetylcholine in the synaptic cleft, thus facilitating cholinergic transmission in uncompromised and partially compromised cholinergic neurons (2). These activities are anticipated to have an ameliorative effect on cholinergically-mediated cognitive deficits associated with Alzheimer’s disease (3) and, possibly, other dementias (4).

Oral doses of SDZ ENA 713 are rapidly and almost completely absorbed in humans and various animal models including the rat, dog (data on file at Novartis Pharma), and rabbit (5). However, the compound undergoes extensive first-pass metabolism resulting in relatively low bioavailability in all tested species. In order to improve the bioavailability of SDZ ENA 713, other means of drug delivery have been evaluated and a transdermal delivery system is being developed. The present study compared the systemic exposure of the parent drug and its metabolites after administering $[^{14}C]$-labeled SDZ ENA 713 as a dermal patch with that following an oral or intravenous dose in the minipig. The effects of dose level and skin abrasion, due to prolonged application of a dermal patch, on the transdermal absorption of the compound were also examined.

**MATERIALS AND METHODS**

**Test Compounds.**

$[^{14}C]$SDZ ENA 713 was synthesized with the $^{14}$C label at the benzylic carbon (figure 1). Radiochemical purity was >96% as determined by radio-thin-layer chromatography using three solvent systems and by radio-high-performance liquid chromatography. Dilution batches with specific activities of 12.8 and 62.8 $\mu$Ci/mg were used for preparing the oral and intravenous doses, respectively. Dermal patches (batch no. X095 0696) containing 18 mg $[^{14}C]$SDZ ENA 713 base per test patch (103.3 $\mu$Ci/10.51 cm²) and placebo patches (batch no. X099 0196) were prepared by Novartis Pharma, Basel, Switzerland and stored at 4°C until use.

**Animals.**

The study adhered to the “Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985), and was approved by the Novartis Animal Care and Use Committee. Twelve male minipigs (Sus scrofa) were obtained from Ellegaard Göttingen Minipigs ApS, Dalmore, Denmark. The minipigs were ca. 4 months old and weighed 7.2–8.3 kg on the day prior to initiation of dosing. During the study, the animals were housed in individual metabolism cages in a room with controlled temperature (19–26°C) and ventilated with about 15 air changes per hour. The animals were fed twice...
of 0.01 M eserine (physostigmine, Sigma-Aldrich, Dorset, UK), at predose and at designated times up to 96 h postdose. Each blood sample was divided into two portions which were stored in separate tubes at −20°C, one for total radioactivity analysis and the other for analysis of parent drug and a major metabolite, ZNS 114-666 (Figure 2).

Quantitative urine and feces were collected at 24-h intervals for 7 days after each dose administration, the former into containers cooled in dry ice. After collecting the final excreta samples, the insides of the cages were washed with distilled water and the washings were retained. At study termination, the minipigs were killed by intravenous injection of sodium pentobarbital and exsanguination. The skin application sites of all minipigs in the dermal dose groups were carefully excised to full dermal thickness and each site was placed in a separate plastic bag.

Sample Preparation and Analysis of Radioactivity

Test dermal patches were separately extracted three times with methanol (1 × 100 ml and 2 × 50 ml). The three extracts were pooled for each patch separately. The recovery of radioactivity from two unused test patches similarly extracted was complete (99.4%, 101%). Skin application sites were homogenized and digested in water:methanol:Triton X-405:sodium hydroxide (600 ml:300 ml:100 ml:80 g) at 55°C for 24 hr.

Radioactivity in all samples was measured in a liquid scintillation spectrometer (Model 1409 or 1410, Pharmacia-Wallac, Turku, Finland). Aliquots of blood (0.25 ml) and fecal homogenates (0.3 g) were air dried and combusted in a sample oxidizer (Model 307, Packard). The products of combustion were absorbed in Optisorb 1 (9 ml; Fisons, Loughborough, UK) and mixed with Optisorb S scintillator (12 ml; Fisons) for measurement of radioactivity. Urine (0.5 ml), cage wash (1 ml), test patch extracts (0.2 ml), skin application site digest (0.6 g), and dose checks (0.5 ml) were assayed by directly counting aliquots mixed with scintillation system MI31 (7 ml; Packard, Pangbourne, UK). All measurements were performed in duplicate or triplicate.

Analysis of SDZ ENA 713 and Metabolite ZNS 114-666

Blood concentrations of SDZ ENA 713 and ZNS 114-666 were determined using a gas-chromatographic/mass spectrometric (GC/MS) method as described previously (5). The lower limits of quantification in this study were 0.25 ng/ml for SDZ ENA 713 and 1.0 ng/ml for the phenolic metabolite.

Collection of Biologic Samples

After each administration of [14C]SDZ ENA 713, blood samples (ca. 2.5 ml) were obtained via the jugular vein and dispensed into individual heparinized tubes, containing 25 μl