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

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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_{max} = 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 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 μCi/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 μCi/10.51 cm^2) and placebo patches (batch no. X009 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 miniature pigs (*Sus scrofa*) were obtained from Ellegaard Göttingen Minipigs ApS, Dalmose, 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

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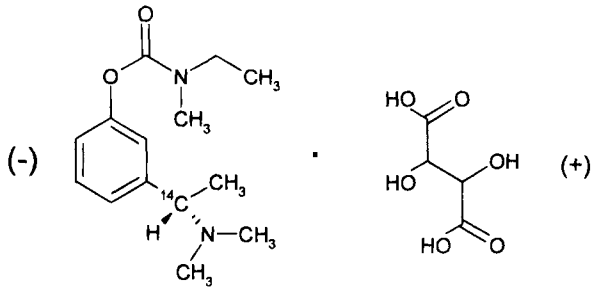

Fig. 1. The chemical structure of [^{14}C]SDZ ENA 713.

daily (Modified Sow Minipig diet, Special Diets Services Ltd., Witham, Essex, UK) and had free access to water.

Study Design

The 12 minipigs were divided into 4 groups of equal size, each receiving a single intravenous (0.1 mg/kg), single oral (1.0 mg/kg), or dermal doses of 18 mg or 54 mg of [^{14}C]SDZ ENA 713 (base equivalents). Animals in the dermal groups received test patches on two occasions 10 days apart. On Study Day 1, [^{14}C]SDZ ENA 713 dermal 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, [^{14}C]SDZ ENA 713 dermal patches were applied to the site on which the placebo patches had been previously applied.

Dose Preparation and Administration

The intravenous and oral doses were freshly prepared on the morning of dosing, both as a 0.64 mg [^{14}C]SDZ ENA 713 hydrogen tartrate salt/ml (equivalent to 0.4 mg free base/ml) solution in sterile water for injection. The intravenous dose (0.25 ml/kg) was administered by bolus injection into an ear vein, whereas the oral dose (2.5 ml/kg) was given by gastric intubation. For dermal dosing on Study Day 1, 1 or 3 self-adhesive patches each containing 18 mg [^{14}C]SDZ ENA 713 were applied to a shaved area on the left flank of each pig (Site A1 or Sites A1-A3), and their positions marked on the skin with an indelible marker. One or 3 placebo patches were similarly applied and marked on the right flank (Site B1 or Sites B1-B3). An elasticized tubular bandage was used to prevent the patches from being dislodged. On Study Day 2, 24 h after application, all patches were removed. The placebo patches were discarded and test patches were retained for analysis. No additional patches were applied to Site A1 or Sites A1-A3 and the area was occluded with a non-porous plastic covering. Fresh placebo patches were applied to Site B1 or Sites B1-B3, and replaced with fresh placebo patches at 24-h intervals until Study Day 11, when 1 or 3 test patches each containing 18 mg [^{14}C]SDZ ENA 713 were applied. The test patches were removed at 24 h after application and retained for analysis. Site B1 or Sites B1-B3 were then occluded with a non-porous plastic covering until study termination.

Collection of Biologic Samples

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

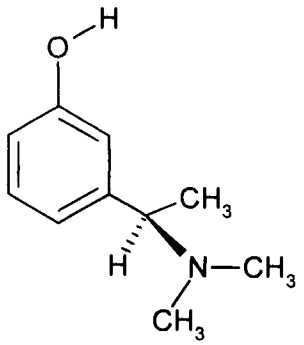

Fig. 2. The chemical structure of metabolite ZNS 114-666.

Pharmacokinetic Interpretation

Whole-blood concentration data of total radioactivity, unchanged SDZ ENA 713, and metabolite ZNS 114-666 were analyzed in terms of maximum measured concentration (C_{max}), time to peak (t_{max}), and area under the concentration-time curve (AUC) determined by the linear trapezoidal rule to the last measurable point and, where possible, extrapolated to infinite time (6). Terminal half-life ($t_{1/2}$) was calculated as 0.693/mean k_{el}, where k_{el} represents the elimination rate constant determined by log-linear regression analysis of those points which constitute the final, linear phase of the concentration-time curve. The extent of absorption was estimated as the dermal or oral:intravenous ratio of dose-normalized AUC values of radioactivity, and also using the relative recoveries of radioactivity in urine (7). Similarly, the bioavailability of SDZ ENA 713 was calculated using the AUC ratios of the parent drug. Using the intravenous data, the total body clearance (CL) of SDZ ENA 713 was calculated as Dose/AUC and the volume of distribution (V_β) as CL/k_{el}.

RESULTS AND DISCUSSION

Blood Concentrations

After a single intravenous dose (0.1 mg/kg) of [^{14}C]SDZ ENA 713, the highest concentration of radioactivity averaged 74.0 ng equiv./ml and was achieved at a mean t_{max} of 0.36 hr (Table 1). Blood radioactivity subsequently declined in a biexponential manner to a mean of 2.4 ng equiv./ml at 12 hr and 0.9 ng equiv./ml at 96 hr postdose. The mean terminal half-life was 46 hr. Unchanged SDZ ENA 713 concentrations were highest at the first sampling time (5 min, mean C_{max} = 27.0 ng/ml). At this time, parent drug accounted for approximately 42% of total blood radioactivity, which indicates that the drug was metabolized relatively rapidly. Concentrations of SDZ ENA 713 in blood declined biexponentially to a mean of 0.67 ng/ml at 2 hr, at which time it represented <2% of the total radioactivity concentration in blood. SDZ ENA 713 concentrations were below the limit of quantification in two of the three animals at 3 hr postdose. Pharmacokinetic analysis of the parent drug concentration-time data indicated that SDZ ENA 713 exhibited high clearance (CL = 116 ± 30.4 ml/min/kg) and a large volume of distribution (V_β = 5.52 ± 3.55 L/kg). The mean cardiac output for 3-kg minipigs is 239 ml/min/kg and if hepatic blood flow is assumed to be 25% of cardiac output, then mean hepatic blood flow is estimated at *ca* 60 ml/min/kg. Thus the total body clearance of SDZ ENA 713 is approximately 2-fold greater than the hepatic blood flow, indicating extrahepatic clearance of the compound in minipigs. The mean volume of distribution appeared to be in excess of the probable total body water volume in this species, which suggests binding of SDZ ENA 713 to plasma and/or tissue components. The mean terminal half-life of SDZ ENA 713 was 0.5 hr. With respect to metabolite ZNS 114-666, peak blood concentrations were attained at 0.25 hr postdose, which indicates rapid formation of this metabolite. At this time, the mean blood concentration of ZNS 114-666 was 3.6 ng/ml, equivalent to 5.5% of the total drug-related material in blood. Blood concentrations of ZNS 114-666 declined rapidly and were below the limit of quantification in all animals by 2 hr postdose.

Following oral administration (1 mg/kg) of [^{14}C]SDZ ENA 713 in solution form, absorption was relatively rapid yielding a mean radioactivity concentration of 247 ng equiv./ml in blood at 0.25 hr, the first sampling time. Peak radioactivity concentrations averaged 516 ng equiv./ml and the t_{max} was 0.83 hr (Table 2). Thereafter, blood radioactivity concentrations declined to

Table 1. Blood Concentrations and Relevant Pharmacokinetic Parameters (mean ± SD, N = 3) After a Single Intravenous Dose of [^{14}C]SDZ ENA 713 (0.1 mg/kg)

Parameter[a]	Radioactivity	SDZ ENA 713	ZNS 114-666
Concentration (ng/ml)[b] at (hr)			
Predose	BQL[c]	BQL	BQL
0.083	64.4 ± 22.2	27.0 ± 12.7	1.7 ± 1.7
0.25	64.9 ± 6.8	15.7 ± 5.44	3.6 ± 1.2
0.5	65.5 ± 6.4	8.03 ± 1.23	2.6 ± 0.5
1	61.1 ± 9.8	3.38 ± 1.14	1.7 ± 0.2
2	41.8 ± 7.4	0.67 ± 0.12	BQL
3	25.5 ± 1.9	0.12 ± 0.21	BQL
5	10.0 ± 0.8	BQL	BQL
8	4.3 ± 0.7	BQL	BQL
12	2.4 ± 1.0	BQL	BQL
24	2.3 ± 1.2	BQL	BQL
48	1.2 ± 0.8	BQL	BQL
72	1.0 ± 0.8	BQL	BQL
96	0.9 ± 0.2	BQL	BQL
C_{max} (ng/ml)	74.0 ± 14.7	27.0 ± 12.7	3.6 ± 1.2
t_{max}(hr)	0.36 ± 0.24	0.08 ± 0	0.25 ± 0
AUC (ng·hr/ml)	372 ± 100	14.7 ± 3.7	3.2 ± 0.6
$t_{1/2}$ (hr)	46	0.5	—

[a] Parameters are defined in text.
[b] Radioactivity levels are given as ng equiv. SDZ ENA 713/ml.
[c] Below quantification limit.

Table 2. Blood Concentrations and Relevant Pharmacokinetic Parameters (mean ± SD, N = 3) after a Single Oral Dose of [^{14}C]SDZ ENA 713 (1.0 mg/kg)

Parameter[a]	Radioactivity	SDZ ENA 713	ZNS 114-666
Concentration (ng/ml)[b] at (hr)			
Predose	BQL[c]	BQL	BQL
0.25	247 ± 172	0.48 ± 0.28	46.1 ± 35.8
0.5	383 ± 144	0.63 ± 0.26	49.6 ± 24.1
1	515 ± 20.9	0.28 ± 0.29	50.8 ± 4.1
2	448 ± 83.2	0.09 ± 0.16	28.7 ± 7.4
3	288 ± 67.3	BQL	14.6 ± 4.3
5	104 ± 18.9	BQL	3.7 ± 1.0
8	35.8 ± 7.6	BQL	1.0 ± 0.9
12	19.6 ± 4.7	BQL	BQL
24	13.0 ± 5.2	BQL	BQL
48	10.1 ± 4.7	BQL	BQL
72	8.0 ± 4.0	BQL	BQL
96	10.2 ± 3.1	BQL	BQL
C_{max} (ng/ml)	516 ± 22.3	0.67 ± 0.21	62.8 ± 20.8
t_{max} (hr)	0.83 ± 0.29	0.67 ± 0.29	0.75 ± 0.43
AUC (ng·hr/ml)	3450 ± 994	0.7 ± 0.2	131 ± 8.0
$t_{1/2}$ (hr)	56	—	1.2

[a] Parameters are defined in text.
[b] Radioactivity levels are given as ng equiv. SDZ ENA 713/ml.
[c] Below quantification limit.

19.6 ng equiv./ml at 12 hr and approximately 10 ng equiv./ml during 48–96 hr. The mean terminal half-life of total ^{14}C was 56 hr. Comparison of the mean AUC values after oral and intravenous doses, corrected for the dose levels administered, showed that the extent of oral absorption was *ca.* 93%. Blood concentrations of unchanged SDZ ENA 713 were low, the mean peak value being 0.67 ng/ml, <0.2% of the peak radioactivity concentration and thus suggesting rapid metabolism. No measurable blood levels of SDZ ENA 713 were observed after 3 hr so that an elimination half-life could not be accurately determined. The bioavailability of SDZ ENA 713 was approximately 0.5% of the dose. Metabolite ZNS 114-666 concentrations also appeared rapidly, reaching a mean C_{max} of 62.8 ng/ml (~12% of peak radioactivity concentration) with a t_{max} of 0.75 hr. Blood concentrations subsequently declined in a monoexponential manner to a mean of 1.0 ng/ml at 8 hr postdose, after which time they were below the limit of quantification in all animals. The mean terminal half-life of ZNS 114-666 was 1.2 hr.

The blood concentration data after topical administration of [^{14}C]SDZ ENA 713 to a virgin skin site on Study Day 1 are given in Table 3. Following the 18 mg dose, the rate of absorption of radioactivity from the dermal patch was slow and no measurable blood radioactivity was observed until 8 hr postdose. Subsequent radioactivity concentrations were low and near the limit of accurate determination throughout the sampling period, with a mean C_{max} of 17.4 ng equiv./ml and t_{max} of 72 hr. Including one animal that showed no detectable concentrations of SDZ ENA 713, peak blood levels of the parent drug averaged 1.03 ng/ml and the mean t_{max} was 18 hr. Dose-normalized, dermal:intravenous AUC ratios were 0.075 for total radioactivity and 0.079 for SDZ ENA 713, suggesting that approximately 8% of the topical dose was absorbed and the absorbed drug was completely bioavailable, without metabolic loss during transit across the skin. Metabolite ZNS 114-666 was detected in only one minipig, slightly above the minimum detection limit and only at 12 and 24 hr postdose. The 54 mg dose yielded blood level profiles similar in shape to those obtained after the 18 mg dose, and the concentrations showed approximate dose-proportionality (radioactivity) or slight overproportionality (SDZ ENA 713 and ZNS 114-666). The dose-normalized, dermal:intravenous AUC ratio was 0.084 for total radioactivity and 0.13 for the parent drug. The similar extent of absorption at both dose levels investigated may be expected considering that the dose per unit area of skin was kept constant.

Upon administration of [^{14}C]SDZ ENA 713 on Study Day 11 to a site that had received daily applications of placebo patches during Study Days 1–10, the rate of absorption of radioactivity from the dermal patch (Table 4) was faster than that observed after drug application to a virgin skin site on Study Day 1. Following the 18 mg dose, maximal blood radioactivity concentrations averaging 30 ng equiv./ml were reached at a mean t_{max} of 20 hr. Blood radioactivity subsequently declined in an apparently biexponential manner to reach a mean concentration of 7.9 ng equiv./ml at 96 hr postdose. Compared with Study Day 1, the increased blood radioactivity concentrations indicated enhanced transdermal absorption of [^{14}C]SDZ ENA 713, which most likely has been caused by prolonged occlusion and ensuing hydration of the skin (8). In addition, skin abrasion due to repeated application and removal of the adhesive patches also may have exerted a positive effect on drug penetration. Similarly, peak concentrations of unchanged drug also were higher and reached faster on Study Day 11 (C_{max} = 2.20 ng/ml, t_{max} = 9.3 hr) than on Study Day 1. Dose-normalized, dermal:intravenous AUC ratios were 0.17 and 0.15 for radioactivity and SDZ ENA 713, respectively, suggesting that 17% of the dose was absorbed and there was minimal loss by metabolism in the skin. Metabolite ZNS 114-666 concentrations again were low, detectable only in one or two minipigs during 5–24 hr postdose. The 54 mg dose also yielded blood concentrations

Table 3. Blood Concentrations and Relevant Pharmacokinetic Parameters (mean ± SD, N = 3) After Dermal Administration of [^{14}C]SDZ ENA 713 to Virgin Skin Site on Study Day 1

Dose parameter[a]	Radioactivity	18 mg SDZ ENA 713	ZNS 114-666	Radioactivity	54 mg SDZ ENA 713	ZNS 114-666
Concentration (ng/ml)[b] at (hr)						
Predose	BQL[c]	BQL	BQL	BQL	BQL	BQL
0.25	BQL	BQL	BQL	BQL	BQL	BQL
0.5	BQL	BQL	BQL	BQL	BQL	BQL
1	BQL	BQL	BQL	BQL	BQL	BQL
2	BQL	BQL	BQL	BQL	BQL	BQL
3	BQL	BQL	BQL	BQL	BQL	BQL
5	BQL	0.23 ± 0.39	BQL	BQL	0.29 ± 0.26	BQL
8	5.7 ± 9.8	0.56 ± 0.98	BQL	11.0 ± 9.5	1.86 ± 0.50	BQL
12	9.2 ± 15.9	1.01 ± 1.33	0.4 ± 0.7	30.7 ± 3.3	3.53 ± 0.20	2.0 ± 0.4
24	9.5 ± 16.4	0.85 ± 1.02	0.4 ± 0.7	44.6 ± 4.7	4.06 ± 0.40	3.0 ± 0.9
48	3.2 ± 5.6	0.11 ± 0.18	BQL	20.8 ± 1.2	0.90 ± 0.27	BQL
72	5.9 ± 2.6	BQL	BQL	13.3 ± 2.9	0.44 ± 0.16	BQL
96	12.4 ± 1.0	BQL	BQL	18.4 ± 1.5	BQL	BQL
C_{max} (ng/ml)	17.4 ± 9.5	1.03 ± 1.32	0.4 ± 0.7	44.6 ± 4.7	4.06 ± 0.40	3.0 ± 0.9
t_{max}(hr)	72 ± 42	18 ± 8.5	12	24 ± 0	24 ± 0	20 ± 6.9
AUC (ng·hr/ml)	632 ± 662	28.6 ± 32.5	10.4 ± 18.0	2130 ± 105	141 ± 4.98	69.4 ± 14.1

[a] Parameters are defined in text.
[b] Radioactivity levels are given as ng equiv. SDZ ENA 713/ml.
[c] Below quantification limit.

that increased considerably compared with Study Day 1. Despite relatively large variability in the data (e.g. parent drug concentration at 3 hr), the concentrations were approximately proportional (radioactivity) or slightly overproportional (SDZ ENA 713 and ZNS 114-666) to the 18 mg dose results. The dose-normalized, dermal:intravenous AUC ratio was 0.19 for total radioactivity and 0.33 for SDZ ENA 713, indicating a *ca.* twofold increase in absorption and bioavailability compared to the results after drug application to a virgin skin site on Study Day 1. Judging by the mean AUC of ZNS 114–666, this metabolite accounted for < 4% of the total drug-related material in the systemic circulation.

Excretion and Mass Balance

The excretion of radioactivity in urine and feces after a single intravenous or oral dose of [^{14}C]SDZ ENA 713 is shown in Table 5. For both dose routes, excretion was rapid with *ca.*

Table 4. Blood Concentrations and Relevant Pharmacokinetic Parameters (mean ± SD, N = 3) After Dermal Administration of [^{14}C]SDZ ENA 713 to Placebo Patch Skin Site on Study Day 11

Dose parameter[a]	Radioactivity	18 mg SDZ ENA 713	ZNS 114-666	Radioactivity	54 mg SDZ ENA 713	ZNS 114-666
Concentration (ng/ml)[b] at (hr)						
Predose	BQL[c]	BQL	BQL	3.3 ± 5.7	BQL	BQL
0.25	BQL	BQL	BQL	6.0 ± 5.2	BQL	BQL
0.5	2.6 ± 4.4	BQL	BQL	9.5 ± 0.7	0.60 ± 0.52	BQL
1	BQL	BQL	BQL	16.2 ± 7.4	2.38 ± 2.27	0.7 ± 0.6
2	6.8 ± 6.4	0.41 ± 0.51	BQL	36.1 ± 23.5	4.91 ± 4.49	1.6 ± 1.5
3	8.9 ± 9.8	0.94 ± 0.78	BQL	74.9 ± 68.7	30.9 ± 43.9	3.2 ± 3.3
5	17.3 ± 13.8	1.59 ± 1.09	0.4 ± 0.8	82.9 ± 58.9	10.1 ± 7.71	4.9 ± 3.9
8	24.9 ± 18.7	2.13 ± 1.52	0.5 ± 0.9	105 ± 65.2	12.6 ± 8.60	6.7 ± 4.2
12	29.7 ± 22.3	2.14 ± 1.37	1.0 ± 1.1	121 ± 69.5	12.3 ± 6.28	7.8 ± 4.1
24	22.6 ± 9.3	1.13 ± 0.16	0.3 ± 0.6	79.7 ± 38.6	6.18 ± 2.71	4.6 ± 1.6
48	8.4 ± 8.8	0.11 ± 0.18	BQL	33.8 ± 9.7	0.70 ± 0.10	BQL
72	9.0 ± 5.2	BQL	BQL	21.9 ± 9.9	0.19 ± 0.17	BQL
96	7.9 ± 5.5	BQL	BQL	21.4 ± 9.8	BQL	BQL
C_{max} (ng/ml)	30.0 ± 22.0	2.20 ± 1.47	1.0 ± 1.1	122 ± 68.8	33.2 ± 41.8	7.8 ± 4.1
t_{max}(hr)	20 ± 6.9	9.3 ± 2.3	12 ± 0	11 ± 2.3	6.0 ± 5.2	12 ± 0
AUC (ng·hr/ml)	1390 ± 763	53.4 ± 28.0	17.1 ± 23.0	4730 ± 2300	354 ± 213	189 ± 90.5

[a] Parameters are defined in text.
[b] Radioactivity levels are given as ng equiv. SDZ ENA 713/ml.
[c] Below quantification limit.

Table 5. Excretion of Radioactivity (mean ± SD, N = 3) After a Single Intravenous or Oral Dose of [^{14}C]SDZ ENA 713

Sample	Radioactivity excreted (% dose)	
	0.1 mg/kg intravenous	1.0 mg/kg oral
Urine at (hr)		
0–24	84.4 ± 0.8	87.1 ± 2.2
24–48	2.3 ± 1.1	2.3 ± 0.3
48–72	0.6 ± 0.3	0.8 ± 0.1
72–96	0.5 ± 0.3	0.3 ± 0.2
96–168	1.0 ± 0.9	0.7 ± 0.4
0–168	88.8 ± 2.2	91.3 ± 1.5
Feces at (hr)		
0–24	1.5 ± 0.7	1.0 ± 0.7
24–48	0.6 ± 0.4	0.9 ± 0.1
48–72	0.3 ± 0.2	0.1 ± 0.1
72–96	0.1 ± 0.01	0.1 ± 0.1
96–168	0.2 ± 0.1	0.1 ± 0.1
0–168	2.7 ± 1.4	2.3 ± 0.6
Cage wash, 168 hr	1.1 ± 0.3	0.4 ± 0.3
Total recovery, 0–168 hr	92.6 ± 0.9	94.0 ± 1.7

85% of the administered radioactivity recovered in urine during the first 24 hr, increasing to *ca.* 90% after 168 hr. Therefore, the urinary recovery data indicated an oral absorption of >90% which is in good agreement with that deduced from the blood radioactivity data, *ca.* 93%. During 0–168 hr, 2–3% of the dose was excreted in feces. The relatively small fecal recovery of radioactivity indicated that excretion in the bile or secretion into the gut lumen via the gastrointestinal tract mucosa are minor routes of elimination for SDZ ENA 713 and its metabolites. Including radioactivity in cage washes, the total recovery was 93–94% of the intravenous or oral dose.

The excretion of radioactivity in urine and feces and the retention of radioactivity on test patches and in skin application sites following the dermal doses are given in Table 6. After dermal application of 18 mg or 54 mg [^{14}C]SDZ ENA 713 to a virgin skin site on Study Day 1, absorbed radioactivity was excreted predominantly via the renal route. For both doses, cumulative recovery during 0–168 hr averaged 9–10% in urine but only 0.1–0.2% in feces. The total radioactivity excreted in urine and feces indicated an absorption of approximately 9% from the 18 mg dose and 10% from the 54 mg dose. These results compare favorably with the blood radioactivity data from which the extent of transdermal absorption was estimated

Table 6. Excretion and Total Recovery of Radioactivity (mean ± SD, N = 3) After Dermal Administration of [^{14}C]SDZ ENA 713

Sample	Radioactivity (% dose)			
	Study Day 1 18 mg	Study Day 1 54 mg	Study Day 11 18 mg	Study Day 11 54 mg
Urine at (hr)				
0–24	2.2 ± 2.8	3.0 ± 0.7	4.1 ± 1.6	10.4 ± 4.7
24–48	3.5 ± 1.6	3.5 ± 0.4	4.2 ± 0.6	4.6 ± 0.1
48–72	1.3 ± 0.2	1.0 ± 0.4	1.5 ± 0.7	1.2 ± 0.2
72–96	0.9 ± 0.2	1.0 ± 0.3	0.7 ± 0.2	0.7 ± 0.1
96–168	1.1 ± 0.3	1.0 ± 0.2	3.8 ± 5.1	1.1 ± 0.5
0–168	9.0 ± 4.2	9.5 ± 1.9	14.2 ± 5.5	17.8 ± 5.3
Feces at (hr)				
0–24	0.04 ± 0.1	0.1 ± 0.02	0.1 ± 0.1	0.2 ± 0.3
24–48	0.01 ± 0.01	0.04 ± 0.02	0.1 ± 0.1	0.1 ± 0.2
48–72	0.01 ± 0.02	0.03 ± 0.02	0.1 ± 0.1	0.1 ± 0.1
72–96	0.01 ± 0.01	0.02 ± 0.01	0.1 ± 0.1	0.1 ± 0.04
96–168	0.02 ± 0.03	0.04 ± 0.03	0.1 ± 0.1	0.1 ± 0.1
0–168	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.5	0.5 ± 0.6
Cage wash, 168 hr	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.04	0.4 ± 0.5
Test patches[a]				
1	66.4 ± 4.2	23.5 ± 2.1	62.1 ± 7.8	22.0 ± 5.4
2	—	24.1 ± 0.7	—	22.3 ± 2.3
3	—	22.4 ± 2.0	—	19.3 ± 3.5
total	66.4 ± 4.2	69.9 ± 3.5	62.1 ± 7.8	63.6 ± 11.0
Application sites[b]				
1	1.7 ± 0.5	0.6 ± 0.2	4.1 ± 0.5	1.4 ± 0.3
2	—	0.6 ± 0.1	—	1.4 ± 0.6
3	—	0.6 ± 0.2	—	1.2 ± 0.6
total	1.7 ± 0.5	1.8 ± 0.1	4.1 ± 0.5	4.0 ± 1.4
Total recovery	77.3 ± 1.2	81.5 ± 1.9	80.9 ± 1.3	86.3 ± 6.8

[a] 24 hr after application.
[b] At study termination, i.e. 408 hr after patch application on Study Day 1 and 168 hr after patch application on Study Day 11.

to be 8% from both doses. Approximately 66–70% of either dose remained on the dermal patches removed at 24 hr. Application (on Study Day 11) of the 18 mg or 54 mg dermal patches to a skin site which had previously had daily applications of placebo patches resulted in an increased extent of drug absorption. The 0–168 hr excretion in urine was 14–18% whereas that in feces was 0.4–0.5%, the combined values indicating an extent of absorption approximately 15% from the 18 mg dose and 19% from the 54 mg dose. These results again are in good agreement with the absorption estimates using blood radioactivity data, 17% and 19%, respectively, and showed a *ca.* twofold increase in SDZ ENA 713 absorption due apparently to abrasion of the skin application site. Enhanced absorption on Study Day 11 was reflected in a slightly lower retention of radioactivity on the dermal patches at 24 hr after application, 62–64% compared with 66–70% for Study Day 1. At 168 hr after application of the 18 mg or 54 mg dermal dose on Study Day 11, approximately 4% of the applied radioactivity was retained at the skin application site. At this time (408 hr after application of the Day 1 dose), the mean amount of dose remaining at the skin application site from a dose applied on Study Day 1 was 1.7–1.8%. These data essentially represent the dispersion of the dose from the dose site over a period 7–17 days after application, which appears to occur at a similar rate at both dose levels.

CONCLUSIONS

Oral doses of [^{14}C]SDZ ENA 713 were rapidly (t_{max} = 0.83 hr) and efficiently (*ca.* 93%) absorbed in the minipig, although the bioavailability of the parent drug was low, *ca.* 0.5%, apparently due to extensive first-pass metabolism. Radioactivity was excreted predominantly in the urine (~90%) with an elimination 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 application site, absorption was 8% at both dose levels investigated (18 mg and 54 mg). 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 application site as a result of repeated application and removal of the adhesive dermal 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 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.

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