CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-083

PHARMACOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-083**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**08 SEP 2006**
PRODUCT:	**Exelon® Patch**
INTENDED CLINICAL POPULATION:	**Patients with mild to moderated Alzheimer's disease and mild to moderate Parkinson's dementia**
SPONSOR:	**Novartis Pharmaceuticals Corporation**
DOCUMENTS REVIEWED:	**Electronic NDA submission of 08 SEP 2006**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**David B. Hawver, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Melina Griffis**

Date of review submission to Division File System (DFS): **06 JUL 2007**

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b(4)

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

From a Pharmacology/Toxicology perspective, this NDA is adequate to be **approved**, provided appropriate changes are made to the proposed labeling.

B. Recommendation for nonclinical studies: None.

C. Recommendations on labeling:

8.1 Pregnancy

Pregnancy Category B: There are no adequate or well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, the Exelon Patch should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus. No dermal reproduction studies in animals have been conducted. Oral reproduction studies conducted in pregnant rats at doses up to 2.3 mg-base/kg/day and in pregnant rabbits at doses up to 2.3 mg-base/kg/day revealed no evidence of teratogenicity. Studies in rats showed slightly decreased fetal/pup weights, usually at doses causing some maternal toxicity.

8.3 Nursing Mothers

Milk transfer studies in animals have not been conducted with dermal rivastigmine. In rats given rivastigmine orally, concentrations of rivastigmine plus metabolites were approximately two times higher in milk than in plasma. It is not known whether rivastigmine is excreted in human breast milk. Exelon Patch (rivastigmine transdermal system) has no indication for use in nursing mothers.

12.1 Mechanism of Action

Pathological changes in Dementia of the Alzheimer's type and Dementia associated with Parkinson's disease involve cholinergic neuronal pathways that project from the basal forebrain to the cerebral cortex and hippocampus. These pathways are thought to be intricately involved in memory, attention, learning, and other cognitive processes. While the precise mechanism of rivastigmine's action is unknown, it is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by cholinesterase. If this proposed mechanism **is correct, rivastigmine's effect may lessen as** the disease process advances and fewer cholinergic neurons remain functionally intact. There is no evidence that rivastigmine alters the course of the underlying dementing process.

12.2 Pharmacodynamics

After a 6-mg oral dose of rivastigmine in humans, anticholinesterase activity is present in CSF for about 10 hours, with a maximum inhibition of about 60% 5 hours after dosing.
In-vitro and *in-vivo* studies demonstrate that the inhibition of cholinesterase by rivastigmine is not affected by the concomitant administration of memantine, an N-methyl-D-aspartate receptor antagonist.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In oral carcinogenicity studies conducted at doses up to 1.1 mg-base/kg/day in rats and 1.6 mg-base/kg/day in mice, rivastigmine was not carcinogenic.
In a dermal carcinogenicity study conducted at doses up to 0.75 mg-base/kg/day in mice, rivastigmine was not carcinogenic. The mean rivastigmine plasma exposure (AUC) at this dose was 0.3-0.4 times **that observed in Alzheimer's disease patients at the** recommended clinical dose (one Exelon Patch 9.5 mg/24 hours).

Rivastigmine was clastogenic in two in vitro assays in the presence, but not the absence, of metabolic activation. It caused structural chromosomal aberrations in V79 Chinese hamster lung cells and both structural and numerical (polyploidy) chromosomal aberrations in human peripheral blood lymphocytes. Rivastigmine was not genotoxic in three in vitro assays: the Ames test, the unscheduled DNA synthesis (UDS) test in rat hepatocytes (a test for induction of DNA repair synthesis), and the HGPRT test in V79 Chinese hamster cells. Rivastigmine was not clastogenic in the in vivo mouse micronucleus test.

No fertility or reproduction studies have been conducted in animals treated with dermal rivastigmine. Rivastigmine had no effect on fertility or reproductive performance in rats at oral doses up to 1.1 mg-base/kg/day.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

It is unfortunate that the minipig study comparing oral and dermal with [^{14}C]-rivastigmine did not include evaluation of metabolites other than ZNS 114-666 (NAP 226-90) and that a comprehensive metabolism study was not conducted in humans at the maximum recommended clinical doses. However, the similarity of the ZNS 114-666:rivastigmine ratios in humans at steady state following treatment at the maximum recommended oral and dermal doses, and the substantially lower exposures after dermal vs. oral administration at these doses, provide reasonable assurance that dermal administration of rivastigmine in humans is not likely to result in important changes in the plasma metabolite profile *(see table below)*.

The pivotal 26-week dermal minipig study did not evaluate a maximum tolerated or maximum feasible dose of rivastigmine. At the highest dose tested, two 10 cm^2 patches per day, no systemic toxicity and only mild skin irritation was observed. However, the similarity between the rivastigmine and ZNS 114-666 exposures in humans after oral and dermal administration noted above, combined with the lack of systemic toxicity noted in minipigs treated with twelve 10 cm^2 rivastigmine patches per day for four weeks despite exposures ≥ 15-fold higher than those at the highest recommended clinical dermal dose, provide a compelling argument that no new toxicities are likely to emerge from a chronic toxicity study in minipigs at a maximum tolerated or maximum feasible dose.

The studies submitted demonstrate that administration of Exelon® Patch is likely to cause mild local skin irritation that could progress to severe irritation if the same skin sites are used more often than once every sixth day. The evidence also suggests that systemic toxicity may be lower with dermal administration than with oral administration at comparable doses.

Rivastigmine and ZNS 114-666[1] Exposures after Oral vs. Dermal Rivastigmine Administration in Minipigs and Humans

Duration, Species and Dose	Rivastigmine Cmax (ng/mL)	ZNS 114-666[1] Cmax (ng/mL)	Rivastigmine AUC $_{0\text{-}24\ hr}$ (ng*hr/mL)	ZNS 114-666[1] AUC $_{0\text{-}24\ hr}$ (ng*hr/mL)
4-Wk Minipig 6 mg/kg/day oral gavage	27.8 (M) 14.4 (F)	392 (M) 457 (F)	69.2 (M) 24.4 (F)	1682 (M) 1483 (F)
4-Wk Minipig 12 patches/day (Exelon® Patch 9.5 mg/24 hours)	117 (M) 137 (F)	169 (M) 155 (F)	2033 (M) 2138 (F)	2826 (M) 2471 (F)
26-Wk Minipig 2 patches/day (Exelon® Patch 9.5 mg/24 hours)	2.6 (M) 3.2 (F)	2.8 (M) 2.7 (F)	44 (M) 52 (F)	Not Calculated[3]
2-Week AD Patients[2] 1 patch/day (Exelon® Patch 9.5 mg/24 hours)	7.9	4.0	127	75.5
2-Week AD Patients[2] 6 mg bid oral capsule	29.3	12.5	191	142

(Reviewer's Table; 1-ZNS 114-666 is also referred to as NAP226-90; 2-AD Patients were titrated up from lower doses of 1.5, 3, and 4.5 mg bid oral rivastigmine, or from Exelon® Patch 4.6 mg/24 hours, for two weeks each; 3-AUCs were not calculated for ZNS 114-666 because the concentration of ZNS 114-666 was below the limit of quantification at several time-points)

B. Pharmacologic activity:

Rivastigmine is a cholinesterase inhibitor; no new studies were submitted.

C. Nonclinical safety issues relevant to clinical use:

Mild irritation occasionally became severe when the same skin site was used every day or every other day for at least 9 days.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

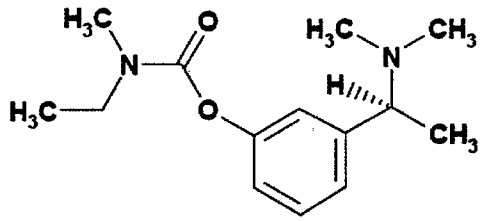
NDA number: 22-083
Review number: 1
Sequence number/date/type of submission: 000 08 SEP 2006 New Formulation
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Novartis Pharmaceuticals Corporation, East Hanover, NJ
Manufacturer for drug substance: Novartis Pharma AG, Basel, Switzerland

Reviewer name: David B. Hawver, Ph.D.
Division name: Division of Neurology Products (DNP)
HFD #: 120
Review completion date: 06 JUL 2007

Drug:

Trade name: Exelon Patch (rivastigmine transdermal system)
Generic name: rivastigmine
Code name: ENA 713 base
Chemical name: (S)-3-[1-(Dimethylamino)ethyl]phenyl ethylmethylcarbamate

CAS registry number: 123441-03-2
Molecular formula/molecular weight: $C_{14}H_{22}N_2O_2$ 250.34
Structure:



Relevant INDs/NDAs/DMFs:
IND 61,392 Exelon (rivastigmine tartrate) for dementia of the Alzheimer's type, Novartis
NDA 20823 Exelon (rivastigmine tartrate) for treatment of mild to moderate dementia of the Alzheimer's type, oral capsule 0.5, 1.0, 1.5 mg, Novartis, approved 21 APR 2000; extended to Parkinson's dementia in 2006
NDA 20823 Exelon (rivastigmine tartrate) for treatment of Alzheimer's disease, oral solution 2 mg/mL, Novartis, approved 21 APR 2000

Drug class: Cholinesterase inhibitor

Intended clinical population: Patients with dementia of the Alzheimer's type or Parkinson's disease dementia

Clinical formulation: Transdermal patch

Route of administration: Transdermal

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Absorption

Study 303-363 (543/963921): Absorption, distribution and excretion of 14C-labelled SDZ ENA713 in minipigs following oral, intravenous and dermal administration

Metabolism

Study DMPK 1997/229: Metabolism in a Human In Vitro Dermal Model.

Repeat-Dose Toxicity

Study Report 96/SPM106/0739: Pharmacokinetic study by dermal administration to CD-1 mice for two weeks.

Study Report 95/SPM058/0945: Preliminary toxicity study by dermal administration to CD-1 mice for 24 days.

Study Report 96/SPM069/0337: Preliminary toxicity study by dermal administration to CD-1 mice for 13 weeks followed by a 4 week reversibility period

Study Report NV97262: A 2-week percutaneous dose toxicity study of SDZ ENA 713 base in rats.

Study Report NV98084: A 4-week percutaneous dose toxicity study of SDZ ENA 713 base in rats with a 2-week recovery period.

Study report 282058: 5-day repeated dose dermal toxicity study with SDZ ENA 713 TDS in rabbits (range finding).

Study report 282857: Subacute 28-day repeat-dose dermal toxicity study with SDZ ENA 713 TDS in rabbits.

Study report 643285: Subacute 28-day repeat-dose dermal toxicity in rabbits.

Study report 645985: 4-week oral (gavage) toxicity study in minipigs.

Study report 60DEMP: A dermal dose-escalating study in minipigs.

Study report 208DFP: A 2-week dermal dose-range-finding study in minipig.

Study report 442P: A 4-week dermal toxicity study in minipigs.

Study report 445P: A 4-week dermal tolerability study in minipigs.

Study Report 17727: A 26-week dermal toxicity study in minipigs.

Carcinogenicity

Study Report 15151: TCS. SDZ ENA 713 Carcinogenicity study by dermal administration in mice.

Local Tolerance

Study Report 95/SPM080/1248: Acute dermal irritation in the rabbit.

Study Report 0320061: Acute dermal irritation in rabbits.

Study Report 282060: Primary skin irritation with SDZ ENA 713 TDS in rabbits (24 hour semi-occlusive application).

Study report 640686: Contact hypersensitivity in albino guinea pigs modified buehler method.

Study Report 640697: Primary skin irritation study in rabbits (4-hour semi-occlusive application)

Study Report 605777: Primary eye irritation study in rabbits.

Study Report 613135: Primary eye irritation study in rabbits (low volume procedure).

Study Report 0420009: Assessment of contact hypersensitivity to ENA713 in the albino guinea pig (Buehler test).

Study Report 282071: Contact hypersensitivity to SDZ ENA 713 TDS in albino Guinea pigs. Modified Buehler method.

Study Report 605891: Contact hypersensitivity in albino Guinea pigs modified Buehler method.

Study Report 622631: Contact hypersensitivity in albino Guinea pigs maximization-test.

Study Report 214380: Delayed contact hypersensitivity to SDZ212-713 hta in albino guinea pigs. The Maurer optimization test.

Study Report 646740: Primary skin irritation study in rabbits (4-hour semi-occlusive application).

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_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

No pharmacology studies were submitted for review. See Pharmacologist Review of NDA 20-823 for oral Exelon, dated 26 OCT 1997.

2.6.2.2 Primary pharmacodynamics

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

A minipig study demonstrated that administration of rivastigmine via dermal application of ENA713D essentially avoided the strong hepatic first-pass metabolism observed with oral dosing, resulting in much higher bioavailability of parent drug, much higher parent drug AUCs, and much lower metabolite to parent drug ratios compared to oral administration. This study also showed that rivastigmine is excreted primarily via the urine following dermal administration, and that ~65% of the drug remained in each patch after the 24-hr treatment.

A rat milk transfer study demonstrated that after oral [^{14}C]-rivastigmine, radioactivity rapidly appeared in the milk (Tmax = 1 hr in both plasma and milk), achieving Cmax and AUC exposures ~2-fold higher in milk compared to plasma.

A rat embryo-fetal transfer study showed that administration of oral [^{14}C]-rivastigmine on Gestation Day 13 or 17 resulted in levels of radioactivity in the placenta greater than those in the maternal blood on both days, and in levels of radioactivity that were quantifiable in fetal liver on GD 17 only.

An in vitro human skin model study demonstrated that the absorption and metabolism of rivastigmine was relatively low; only one metabolite was formed in the cells (the N-oxide product) and the rate of formation was low.

In vitro metabolism studies provided evidence suggesting that the liver is probably the major organ involved in the metabolism of oral rivastigmine, but the intestines my also play a significant role; metabolism by butyrylcholinesterase in plasma was considered to be comparatively negligible.

The comparison of plasma metabolite profiles in rat, dog, and human after oral administration revealed that human was more similar to rat, both qualitatively and quantitatively.

A human metabolism study showed that a single oral dose of [^{14}C]-rivastigmine hydrogen tartrate parent drug was rapidly metabolized into ZNS 114-666 (NAP 226-90) and its sulfate conjugate, resulting in a ratio in plasma of ~1:5.6 at 1.0 mg and ~1:2.3 at 2.5 mg. Parent drug was not detected in the plasma or urine.

In vitro enzyme inhibition assays suggested that rivastigmine is neither likely to inhibit the metabolism of other drugs, nor to have its metabolism be affected by concomitant medications.

2.6.4.2 Methods of Analysis

[not reviewed]

2.6.4.3 Absorption

Absorption, distribution and excretion of 14C-labelled SDZ ENA713 in minipigs following oral, intravenous and dermal administration

[Study 303-363 (543/963921): ——————————————————— b(4)
GLP UK, 1997, FDA, 1978; QA; Dosing commenced 12 AUG 1996; ^{14}C-SDZ ENA 713 hydrogen tartrate Batch #1697-260-36, specific activity 12.8 uCi/mg for oral dosing; Batch #1697-261-44, specific activity 62.8 uCi/mg for IV dosing; Dermal patch Batch #X095 0696 containing nominally 18 mg ^{14}C-SDZ ENA 713 (assay 19.2 mg/patch), specific activity 103.3 uCi/patch (10.51 cm^2); Placebo patch Batch X009 0196]

Methods

Gottingen minipigs (N=3M/group; ~4 months old; 7.2-8.3 kg) were treated with single IV (0.1 mg/kg), oral (1.0 mg/kg), or dermal (1x 18 mg/10 cm^2 and 3x 18 mg/10 cm^2 = 54 mg/30 cm^2 patches) doses of ^{14}C-SDZ ENA 713. The dermal patches were applied for 24 hrs to virgin sites in Phase 1, and (dosing the same animals again on Day 11) to sites previously exposed to daily placebo patches for 10 days in Phase 2. Whole blood was collected at various intervals and whole excreta were collected from 0-168 hrs after each dose. Concentrations of total radioactivity, parent drug, and metabolite ZNS 114-666 were measured in whole blood. Radioactivity was also measured in whole excreta, skin application sites, and in used patches.

Results

As shown in the tables below, oral absorption of radiolabeled SDZ ENA 713 was relatively rapid (Tmax = 0.83 hr) and complete (~93%). In contrast, dermal absorption was relatively slow (Tmax = 24-72hrs at the virgin sites, and 10.7-20 hrs at the previously used sites) and incomplete (~7.5-8.4% at the virgin sites and 16.5-18.7% at the previously used sites)

Whole Blood Radioactivity Pharmacokinetic Parameters

Pharmacokinetic parameter	Intravenous dose (0.1 mg/kg)	Oral dose (1.0 mg/kg)
C_{max} (ng equiv./g)	74.0 ± 14.7	515.9 ± 22.3
T_{max} (hours)	0.36 ± 0.24	0.83 ± 0.29
AUC_{96} (ng equiv.h/g)	340.4 ± 76.1	2806 ± 417
AUC (ng equiv.h/g)	372.3 ± 100.4	3450 ± 994
t½ (hours)	46.2	55.9
F	-	0.93

Pharmacokinetic parameter	Dermal dose (18 mg)		Dermal dose (54 mg)	
	Day 1	Day 11	Day 1	Day 11
C_{max} (ng equiv./g)	17.4 ± 9.5	30.0 ± 22.0	44.6 ± 4.7	121.7 ± 68.8
T_{max} (hours)	72.0 ± 41.6	20.0 ± 6.9	24.0 ± 0	10.7 ± 2.3
AUC_{96} (ng equiv.h/g)	632.4 ± 661.9	1392 ± 763	2126 ± 105	4732 ± 2304
F	0.075	0.165	0.084	0.187

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The table below reveals that, despite rapid and complete oral absorption, the systemic bioavailability of the parent drug was very low (~0.05%). Dermal administration of SDZ ENA 713 resulted in much greater systemic bioavailability of parent drug (~11.9-13.0% at virgin sites, and ~14.8-32.7% at previously used sites) than oral administration.

Whole Blood SDZ ENA 713 Pharmacokinetic Parameters

Pharmacokinetic parameter	Intravenous dose (0.1 mg/kg)	Oral dose (1.0 mg/kg)
C_{max} (ng/ml)	27.04 ± 12.69	0.67 ± 0.21
T_{max} (hours)	0.083	0.5
AUC_{96} (ng.h/ml)	14.6 ± 3.7	0.7 ± 0.2
AUC (ng.h/ml)	14.7 ± 3.7	-
t½ (hours)	0.5	-
F	-	0.005

Pharmacokinetic parameter	Dermal dose (18 mg)		Dermal dose (54 mg)	
	Day 1	Day 11	Day 1	Day 11
C_{max} (ng/ml)	1.03 ± 1.32	2.20 ± 1.47	4.06 ± 0.40	33.18 ± 41.75
T_{max} (hours)	18	8	24	3
AUC_{96} (ng.h/ml)	43.0	53.4 ± 28.0	140.8 ± 5.0	353.8 ± 212.7
F	0.119	0.148	0.130	0.327

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The tables above also shows that patch application to previously used skin sites (Day 11) increased the maximum blood levels, reduced the time to peak, and improved the systemic availability of SDZ ENA 713 compared to patch application to virgin skin sites.

The data in the tables below indicate that the major metabolite ZNS 114-666 was rapidly formed after oral administration of SDZ ENA 713 and was present in the blood at concentrations greatly exceeding those of the parent drug (Cmax was 94-fold greater, AUC_{96} was 188-fold greater). In contrast, the ZNS 114-666 AUC_{96} exposures after dermal administration of SDZ ENA 713 were only about 50% as great as parent drug AUC_{96} exposures.

Whole Blood ZNS 114-666 Pharmacokinetic Parameters

Pharmacokinetic parameter	Intravenous dose (0.1 mg/kg)	Oral dose (1.0 mg/kg)
C_{max} (ng/ml)	3.6 ± 1.2	62.8 ± 20.8
T_{max} (hours)	0.25	1.0
AUC_{96} (ng.h/ml)	3.2 ± 0.6	131.6 ± 8.0
AUC (ng.h/ml)	-	131.0 ± 8.0
t½ (hours)	-	1.2

Pharmacokinetic parameter	Dermal dose (18 mg)		Dermal dose (54 mg)	
	Day 1	Day 11	Day 1	Day 11
C_{max} (ng/ml)	-	1.03 ± 1.05	3.0 ± 0.9	7.8 ± 4.1
T_{max} (hours)	-	12	24	12
AUC_{96} (ng.h/ml)	-	25.7	69.4 ± 14.1	188.7 ± 90.5

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The data above are consistent with the conclusion that dermal administration avoids the strong first-pass metabolism of SDZ ENA 713 into ZNS 114-666 observed after oral administration.

Excretion of radioactivity was predominantly via the urine (~90%) after both oral and IV dosing of ^{14}C-SDZ ENA 713, with ≥ 86% of the dose excreted during the first 24 hrs. The table below shows that the urine was also the primary route of excretion of radioactivity after dermal application of ^{14}C-SDZ ENA 713 (~95-97% of the total radioactivity excreted). Furthermore, since the total excretion represents the extent of systemic absorption of the radioactivity from the patch, ~9-10% of the total radiolabeled dose was absorbed transdermally from both the 18 mg and the 54 mg patch treatments at the virgin site, and ~15-18% was absorbed at the site that had been treated with placebo patches for the previous 10 days. The increase in absorption at the non-virgin sites was attributed to abrasion of the skin site by repeated application and removal of the strongly adhesive patches.

Approximately 65% of the radioactivity remained in the patches after the 24 hr treatment period, ~4% of the total dose was recovered at the skin site 168 hrs after the Day 11 dosing, and ~2% of the first dose was recovered at the original skin site 408 hrs after the Day 1 dosing was complete.

2.6.4.4 Distribution

[redacted]

This study was not reviewed due to time constraints.

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[redacted]

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_____ Deliberative Process (b5)

2.6.4.5 Metabolism

Metabolism in a Human In Vitro Dermal Model b(4)

(Study DMPK 1997/229; Report released 13 DEC 2005; location conducted not clear; document prepared by R. Fluckiger, Novartis, Basel, Switzerland, [redacted] and A. Vickers, Novartis, E. Hanover, NJ)

Sponsor's Materials and Methods:

2 Materials and Methods

Chemicals. [^{3}H]CSA was obtained from [redacted] at a specific activity of 7.93 mCi/mg. [^{14}C]SDZ ENA (S)-(-) N-ethyl-N-methyl-3-[1'-(dimethylamino)-ethyl]-phenyl-carbamate base) with a specific activity of 225.8 µCi/mg was synthesized by the [redacted] b(4)
(Figure 1). Unlabeled SDZ ENA 713 and CSA were prepared at [redacted] The purity of the compounds was checked by HPLC and found to be greater than 98%. The human dermal model skin$^{2™}$ ZK 1100 as well as tissue culture media, the MTT and IL-6 assay kits were obtained from [redacted] All other reagents were of the highest grade available and were purchased from commercial sources. b(4)

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b(4)

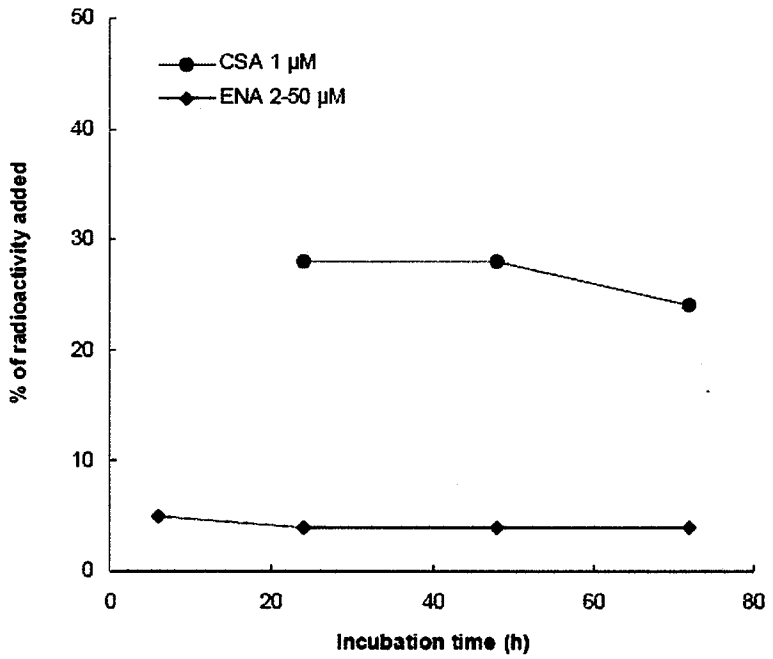
(Pages 5-7 of Study Report)

Results

Pooled human foreskin fibroblasts cultured on nylon mesh squares showed little absorption of the radiolabeled SDZ ENA 713 when incubated for 6-72 hrs at concentrations of 2, 10, or 50 uM. Only ~5% of the radioactivity was associated with the cells, while ~95% remained in the medium throughout the experiment. In contrast, ~30% of the positive control, radiolabeled Cyclosporin A at 1 uM, was associated with the cell fraction *(see Figure 2 below)*.

Figure 2 Percentage of radioactivity associated with human dermal cells

Human dermal cells were cultured in the presence of ENA (2, 10 and 50 µM) and CSA (1 µM). At the indicated time points, aliquots from the skin fraction were taken for radioactivity determination and calculated as the percentage of total radioactivity recovered in cells plus medium.



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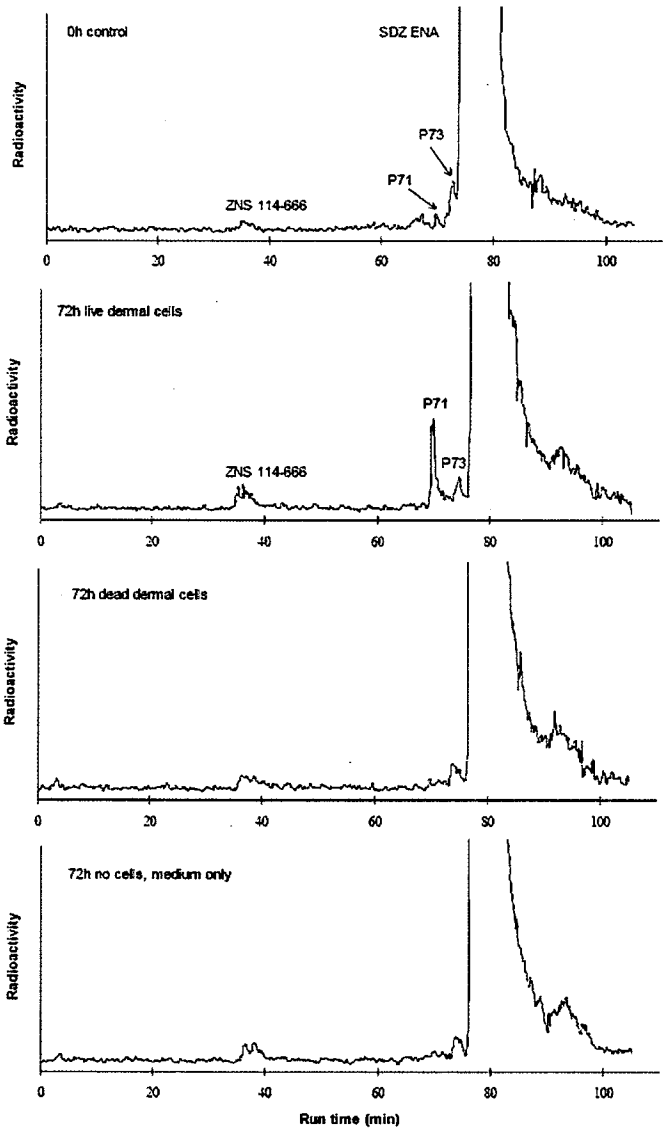
Comparison of the HPLC radiochromatograms after incubation of radiolabeled SDZ ENA 713 with live cells for 0 hrs vs. 72 hrs, with dead cells, or with medium alone *(see Figure 3 below)* revealed very little metabolism occurring in the skin cells; only peak 71 appeared to be increased with time in the live cell fraction, and the parent drug peak was by far the largest peak present in all radiochromatograms.

Figure 3 HPLC chromatograms of [^{14}C]SDZ ENA 713 samples (2 µM)

800 µl of sample extracts (~2 x 10^5 dpm) were separated by HPLC.

The scale of the radiochromatograms represents 40 mV with a full scale corresponding to 5: mV.



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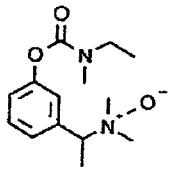
Peak 71 (P71) was identified as the N-oxide of SDZ ENA 713, and the mean rate of formation of this metabolite was calculated to be 0.39, 1.93, and 7.71 pmol/mg/hr in the presence of 1, 10, and 50 uM SDZ ENA 713, respectively. In contrast, the rate of formation of ZNS 114-66 from rivastigmine in human liver slices was 164 and 504 pmol/mg/hr at 10 and 50 uM rivastigmine *(page 16 of Study Report #303-302).* The P71 N-oxide metabolite was also identified in preparations of human liver slices incubated with rivastigmine, but the rate of formation of P71 was not provided.

P71
human, rat



The peak corresponding to the esterase mediated decarbamylation product, ZNS 114-666, was present at about the same level in the presence or absence of live cells, indicating that it was probably formed by the action of esterases present in the culture medium rather than via metabolism in the skin cells.

Peak 73, identified as the N-demethylation product of SDZ ENA 713, was present in the stock solution, and did not increase with time in the presence of live cells, indicating that it was not formed by enzymes in the skin cells.

Direct comparison of the amount and rate of total metabolite formation between SDZ ENA 713 and Cyclosporin A revealed that biotransformation of SDZ ENA 713 was 16 to 31 fold lower than that of CSA, after normalizing for the differences in parent drug concentration *(see Table 1 below).*

Table 1 SDZ ENA 713 and Cyclosporin A: Comparison of total metabolit formation

by human dermal cells during 72 h of cultures

Compound		Amount (pmol/skin square)	Amount (pmol/mg protein)	Rate (pmol/mg protein per h)
SDZ ENA 713[a]	2 µM	34	43	0.60
	10 µM	120	157	2.18
	50 µM	395	554	7.69
CSA[b]	*1 µM*	*243*	*348*	*4.83*

[a]Total metabolites of SDZ ENA 713: Peak 71, ZNS 114-666, peak 73
[b]Total metabolites of Cyclosporin A: AM1
Metabolites were calculated from live cells minus stability samples (dead cells)

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10 Page(s) Withheld

X Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:
Acute, subchronic, and/or chronic oral toxicity studies with rivastigmine in mice, rats, dogs, and monkeys were completed in support of NDA 20-823 for oral rivastigmine tartrate. General toxicology studies submitted in support of the current NDA for rivastigmine transdermal system (ENA713D) include dermal studies in mice, rats, rabbits, and minipigs, and one oral gavage study in minipigs for comparison, since this species had not been evaluated previously with oral rivastigmine.

Mice and rats treated dermally with rivastigmine in 50% ethanol once daily for up to 13 weeks (mice) or 4 weeks (rats) showed only clinical signs expected for a cholinergic drug: underactivity, piloerection, lacrimation, twitching, body tremors, irregular breathing, unusual posture, salivation, and/or yawning. No local toxicity was noted.

In rabbits treated for up to 4 weeks with placebo patches or ENA713D (SDZ ENA 713 TDS patches) applied to the same skin site each day, very slight to well-defined erythema was observed, sometimes with edema, scaling, bruising, and scabbing. These effects were attributed to the mechanical injury caused by repeated removal of the strongly adhesive patches from the skin.

In dermal minipig studies of up to 4 weeks, placebo patches and ENA713D both were observed to induce severe irritation in occasional individual animals when applied to the same skin site every day or every other day. However, when patches were rotated among 6-12 distinct skin sites in the 26-week minipig study, skin irritation was less severe (slight to well-defined) and was not observed at all with placebo patches. No systemic toxicity was observed in the dermal minipig studies.

Minipigs given SDZ ENA 713 via oral gavage for 4 weeks showed typical cholinergic signs: frequent, transient, slight to moderate tremors, slight lateral recumbency, and slight occasional decreased activity and salivation. No other toxicities were noted.

Genetic toxicology: No new genetic toxicology studies were submitted.

Carcinogenicity:
No treatment-related findings were observed in a 98-99-week dermal carcinogenicity study in mice given SDZ ENA 713 in 100% ethanol at 0.25, 0.50, and 0.75 mg/kg/day, except for reduced body weight gain (16-17%) in high dose females.

Reproductive toxicology: No new reproductive toxicology studies were submitted.

Local tolerance:
Rat primary irritation studies generally showed that 4-hr application of ENA713D induced very slight to well-defined erythema and very slight edema. Contact hypersensitivity studies in guinea pigs generally showed that ENA713D did not cause sensitization; however, mild irritation was observed at the test site during the induction phase, and spontaneous deaths observed after the second or third induction treatment were attributed to treatment-related cholinergic toxicity. Rivastigmine applied directly to the eye caused miosis, moderate to marked lacrimation, loss of reactivity to light in the iris, reddening and swelling of the conjunctivae, and transient corneal opacities, but the eyes returned to normal by seven days after dosing.

Special toxicology:
ENA713D was found to be non-phototoxic in guinea pigs treated for 30 minutes followed by UV-A irradiation for 90 minutes.

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were submitted.

2.6.6.3 Repeat-dose toxicity

Pharmacokinetic study by dermal administration to CD-1 mice for two weeks
(Study Report 96/SPM106/0739; [redacted], Dosing commenced 09 MAY 1996; GLP, QA; SDZ ENA 713 Lot #95703, Purity by HPLC 101.1%, by titration 99.5%) b(4)

Key Points

- Dermal application of SDZ ENA 713 at the HD of 37.5 ug/mouse/day (1.2 mg/kg/day M, 1.5 mg/kg/day F; in 50:50 ethanol:water) induced transient underactivity and body tremors during the first few days of treatment in up to 8/21 M and 17/21 F. Only one or two MD mice showed these signs.
- Parent drug mean HD Cmax was 7.1 ng/mL (Day 1 M), 7.8 ng/mL (Day 14 M), 5.5 ng/mL (Day 1 F), and 6.8 ng/mL (Day 14 F).
- Metabolite ZNS 114-666 level was much higher than parent drug level: mean HD Cmax was 66.6 ng/mL (Day 1 M), 20.5 ng/mL (Day 14 M), 111.7 ng/mL (Day 1 F), and 21.8 ng/mL (Day 14 F).
- Parent drug mean HD $AUC_{0\text{-}24\ hr}$ was 25.2 ng*hr/mL (Day 14 M), and 26.8 ng*hr/mL (Day 14 F).
- ZNS 114-666 mean HD $AUC_{0\text{-}24\ hr}$ was 44.3 ng*hr/mL (Day 14 M), and 78.3 ng*hr/mL (Day 14 F).

Methods

CD-1 mice (N=21/sex/group; 8 wks old; 22.0-34.1 g) received 0.25, 0.6, or 0.75 mg/mL SDZ ENA 713 (50 uL/animal) in a 50:50 ethanol:water mixture applied dermally (onto dorsum, between limb girdles, ~10% of body surface clipped free of hair) once daily for 14 days. Low Dose (LD) = 12.5 ug/animal, or ~0.4 mg/kg for M, ~0.5 mg/kg for F; Mid Dose (MD) = 30 ug/animal, or ~1.0 mg/kg for M, ~1.2 mg/kg for F; High Dose (HD) = 37.5 ug/animal, or ~1.2 mg/kg for M, ~1.5 mg/kg for F. Parameters measured included clinical signs, body weights, food consumption, food conversion efficiency, and pharmacokinetics (Days 1 and 14; 0.25, 0.5, 1, 2, 4, 8, and 24 hrs postdose; 3/sex/dose/timepoint). Clinical labs, necropsy, and histopathology were not performed.

Results

All animals survived. Observations included underactivity (1/21 MDM, 1/21 MDF, 8/21 HDM, and 10/21 HDF), and body tremors (2/21 MDM, 1/21 MDF, 8/21 HDM, and 17/21 HDF), predominantly during the first few days of treatment, generally between 1 and 2 hrs postdose. No changes were noted in body weight, food consumption, or food conversion efficiency.

The tables below demonstrate that exposures to the primary metabolite, ZNS 114-666, were much higher than exposures to the parent drug.

Toxicokinetic characteristics tmax, Cmax and AUC(0-24h) of SDZ ENA713 in mouse blood after the first and 14th dermal administration of SDZ ENA 713

Day 1

Dose		Gender	tmax h	Cmax ng/ml	Cmax / dose	AUC(0-24h) h*ng/ml	AUC / dose
mg/animal/day	mg/kg/day						
0.0125	0.41	M	0.25	2.1	5.1		
	0.53	F	0.25	2.9	5.5		
		M + F	**0.25**	**2.5**	**5.3**		
0.0300	0.98	M	0.25	3.6	3.7	insufficient data	
	1.24	F	0.25	5.7	4.6		
		M + F	**0.25**	**4.7**	**4.2**		
0.0375	1.21	M	0.25	7.1	5.9		
	1.56	F	0.25	5.5	3.5		
		M + F	**0.25**	**6.3**	**4.7**		

Day 14

Dose		Gender	tmax h	Cmax ng/ml	Cmax / dose	AUC(0-24h) h*ng/ml	AUC / dose
mg/animal/day	mg/kg/day						
0.0125	0.39	M	0.25	1.2	3.1	insufficient data	
	0.48	F	0.25	3.3	6.9		
		M + F	**0.25**	**2.3**	**5.0**		
0.0300	0.93	M	0.25	6.3	6.8	12.5	13.4
	1.16	F	0.50	8.3	7.2	22.0	19.0
		M + F	**0.38**	**7.3**	**7.0**	**17.3**	**16.2**
0.0375	1.14	M	0.25	7.8	6.8	25.2	22.1
	1.45	F	1.00	11.2	7.7	26.8	18.5
		M + F	**0.63**	**9.5**	**7.3**	**26.0**	**20.3**

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Toxicokinetic characteristics tmax, Cmax and AUC(0-24h) of ZNS 114-666 in mouse blood after the first and 14th dermal administration of SDZ ENA 713

Day 1

Dose		Gender	tmax	Cmax	Cmax /	AUC(0-24h)	AUC /
mg/animal/day	mg/kg/day		h	ng/ml	dose	h*ng/ml	dose
0.0125	0.41	M	0.25	19.6	47.8	18.7	45.6
	0.53	F	0.25	30.1	56.8	28.7	54.2
		M + F	**0.25**	**24.9**	**52.3**	**23.7**	**49.9**
0.0300	0.98	M	0.25	37.2	38.0	39.2	40.0
	1.24	F	0.50	45.6	36.8	72.8	58.7
		M + F	**0.38**	**41.4**	**37.4**	**56.0**	**49.4**
0.0375	1.21	M	0.25	66.6	55.0	55.1	45.5
	1.56	F	0.25	111.7	71.6	128.6	82.4
		M + F	**0.25**	**89.2**	**63.3**	**91.9**	**64.0**

Day 14

Dose		Gender	tmax	Cmax	Cmax /	AUC(0-24h)	AUC /
mg/animal/day	mg/kg/day		h	ng/ml	dose	h*ng/ml	dose
0.0125	0.39	M	0.25	19.0	48.7	17.3	44.4
	0.48	F	0.25	35.4	73.8	24.6	51.3
		M + F	**0.25**	**27.2**	**61.3**	**21.0**	**47.9**
0.0300	0.93	M	0.50	26.7	28.7	47.5	51.1
	1.16	F	1.00	19.5	16.8	72.4	62.4
		M + F	**0.75**	**23.1**	**22.8**	**60.0**	**56.8**
0.0375	1.14	M	0.50	20.5	18.0	44.3	38.9
	1.45	F	0.50	21.8	15.0	78.3	54.0
		M + F	**0.50**	**21.2**	**16.5**	**61.3**	**46.5**

Note: Cmax/dose and AUC/dose are normalized to a dose of 1 mg/kg/day

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Preliminary toxicity study by dermal administration to CD-1 mice for 24 days
(Study Report 95/SPM058/0945; [redacted], Dosing commenced 28 JUN 1995; GLP; not QA; SDZ ENA 713 Lot #94702, Purity by HPLC 101.1%, by titration 101.0%) b(4)

Key Points

- Dermal application of a single dose of SDZ ENA 713 at the HD of 20 ug/mouse (0.6 mg/kg M, 0.8 mg/kg F; in 50:50 ethanol:water) induced transient underactivity 20-40 min postdose (M/F), irregular breathing (M), unusual posture, and yawning.
- Repeated dosing at 15 ug/animal/day (0.45 mg/kg/day M; 0.6 mg/kg/day F) for 9 days, after 14 days at 5 ug/animal/day, resulted in no changes in the application site appearance or histology, or in clinical signs, body weight, food consumption, food conversion efficiency, or plasma cholinesterase activity.

Methods

CD-1 mice (N=5/sex/group; 7-8 wks old; ~33 g M, ~25 g F) received No Treatment, Vehicle, LD (0.1 mg/mL) or HD (0.2 mg/mL) SDZ ENA 713 (50 uL/animal) in a 50:50 ethanol:water mixture applied dermally (onto dorsum, between limb girdles, ~10% of body surface clipped free of hair) once daily for 14 days; Days 15-23 the LD group received 0.3 mg/mL (15 ug/animal; ~0.45 mg/kg M; ~0.6 mg/kg F), and the other groups continue as before; on the 24th day, the Vehicle and HD groups were given 0.4 mg/mL (20 ug/animal; ~0.6 mg/kg M; ~0.8 mg/kg F), the LD group continued at 0.3 mg/mL and the No Treatment group also received 0.3 mg/mL. Animals were killed 5-6 hrs after this last dose on Day 24. Parameters measured included application site inspection, clinical signs, body weights, food consumption, food conversion efficiency, plasma cholinesterase activity and detailed necropsy and histopathological evaluation of the test site skin. Clinical labs were not performed. In a preliminary study, mice receiving a single dermal dose of 0.4, 1.0, 10, or 100 mg/mL showed ataxia, severe tremors, underactivity, and fast respiration, followed by spontaneous death or moribund sacrifice.

Results

All animals survived. No clinical signs were associated with treatment at 0.1, 0.2, or 0. 3 mg/mL; at 0.4 mg/mL, underactivity was observed 20-40 min postdose in all animals of both the Vehicle and HD groups, irregular respiration was seen in HDM but not the Vehicle M (that received 0.4 mg/mL on Day 24 only); and several animals at this dose had unusual posture and yawning. No changes were noted in application site appearance, body weight, food consumption, or food conversion efficiency.

Preliminary toxicity study by dermal administration to CD-1 mice for 13 weeks followed by a 4 week reversibility period

(Study Report 96/SPM069/0337; _____ Dosing commenced 05 SEP 1995; GLP; QA; SDZ ENA 713 Lot #94702, Purity by HPLC 101.1%, by titration 101.0%) b(4)

Key Points

- Dermal application of a single dose of SDZ ENA 713 at the HD of 50 ug/mouse (1.6 mg/kg M, 2.0 mg/kg F; in 50:50 ethanol:water) induced severe signs (severe tremors, underactivity, piloerection, and prostate posture) necessitating humane sacrifice in 1/22 M and 2/22 F.
- Repeated dosing at 25 or 37.5 ug/animal/day (0.8 or 1.2 mg/kg/day M; 1.0 or 1.5 mg/kg/day F) for 13 weeks was tolerated, though transient signs appeared during the first week: underactivity, piloerection, prostrate or hunched posture, and exaggerated yawning. The higher reduced plasma cholinesterase activity by 14-16% during Wk 12.
- No important treatment-related changes were observed in the application site appearance, body weights, food consumption, food conversion efficiency, hematology, blood chemistry, urinalysis, detailed necropsy, or histopathological evaluation.

Methods

CD-1 mice (N=10/sex/group; 8-9 wks old; ~32-40 g M, ~25-30 g F) received No Treatment, Vehicle, LD (0.1 mg/mL), LMD (0.25 mg/mL), HMD (0.5 mg/mL), HD (1.0 mg/mL Day 1, 3 days off drug, then 0.75 mg/mL) SDZ ENA 713 (50 uL/animal) in a 50:50 ethanol:water mixture applied dermally (onto dorsum, between limb girdles, ~10% of body surface clipped free of hair) once daily for 13 weeks. Recovery groups included 6/sex/group No Treatment, Vehicle, LMD, HMD, and HD, sacrificed 4 weeks after the last dose. Toxicokinetic (TK) groups included 6/sex/group Vehicle, LD, LMD, HMD, and HD evaluated at 2 and 24 hrs postdose during Wk 13. Parameters measured included application site inspection, clinical signs, body weights, food consumption, food conversion efficiency, plasma cholinesterase activity, hematology, blood chemistry, urinalysis, detailed necropsy, and histopathological evaluation (Vehicle and HD only; 13 tissues, including test site).

Results

One LDM was killed in extremis in Wk 8, with splenomegaly and severe acute broncho-pneumonia not considered to be treatment-related (by the sponsor or the reviewer). 1/22 HDM and 2/22 HDF were killed after the single dose of 1.0 mg/mL (50 ug/animal; 1.6 mg/kg M; 2.0 mg/kg F) due to the severity of signs: severe tremors, underactivity, piloerection, and prostate posture; 2 F at this dose had involuntary muscle spasms in the back, in the region of the test site. At 0.75 mg/mL (37.5 ug/animal; 1.2 mg/kg M; 1.5 mg/kg F) and 0.5 mg/mL (25 ug/animal; 0.8 mg/kg M; 1.0 mg/kg F), signs were mainly observed during the first week of treatment, including transient underactivity, piloerection, prostrate or hunched posture, and exaggerated yawning.

Plasma cholinesterase activity was decreased 14-16% in HD vs Vehicle during Wk 12, demonstrating the expected pharmacological effect.

No important treatment-related changes were observed in body weights, food consumption, food conversion efficiency, hematology, blood chemistry, urinalysis, detailed necropsy, or histopathological evaluation.

Mean Wk 13 2-hr blood levels of SDZ ENA 713 were 7.8 ng/mL (HDM) and 6.6 ng/mL (HDF), whereas those of metabolite ZNS 114-666 were 9.9 ng/mL (HDM) and 10.5 ng/mL (HDF).

A 2-week percutaneous dose toxicity study of SDZ ENA 713 base in rats
(Study Report NV97262: [redacted] Dosing commenced 03-04 FEB 1998; GLP; QA; SDZ ENA 713 Lot #96912, Purity by HPLC 99.9%, by titration 100.6%) b(4)

Key Points

- Dermal application of 15, 30, or 50 mg/kg/day in 50:50 ethanol:water resulted in reduced feces in all animals from Day 2 onward; systemic twitching in 5/6 MDF, 2/6 HDM, and 6/6 HDF from Day 3 onward; and similarly reduced body weight and food intake in all 3 groups on Day 4 and Day 7 compared to Day 1.
- Day 1 mean Cmax values associated with doses of 15, 30, and 50 mg/kg/day were 14, 24, and 46 ng/mL, respectively, for M; and 9, 34, and 57 ng/mL, respectively, for F.
- Dermal application of 0.375, 1.125, 1.5, or 3.0 mg/kg/day in 50:50 ethanol:water for 2 weeks did not induce treatment-related changes in clinical signs, body weight, food intake, hematology, blood chemistry, gross pathology, organ weights, or histopathology.

Methods

[redacted] CD (SD) rats (N=8/sex/group; 7-8 wks old; 274-360 g M, 183-228 g F) received No Treatment, Vehicle, LD (0.375 mg/kg/day), LMD (1.125 mg/kg/day), HMD (1.5 mg/kg/day), or HD (3.0 mg/kg/day) SDZ ENA 713 (1.5 mL/kg/day) in a 50:50 ethanol:water mixture applied dermally (onto dorsum, between limb girdles, ~10% of body surface clipped free of hair) once daily for 2 weeks. Toxicokinetic (TK) groups included 12/sex/group LD, LMD, HMD, and HD evaluated at 0.25, 0.5, 1, 2, 4, 8, and 24 hrs postdose Days 1 and 14. Parameters measured included application site inspection, clinical signs, body weights, food consumption, hematology, blood chemistry, organ weights, necropsy, and histopathological evaluation (No Treatment, Vehicle, MHD, and HD only; 27 tissues, including test site). A second study was conducted using the TK animals (after a 12-13 day washout) at 6/sex/group at LD (15 mg/kg/day), MD (30 mg/kg/day), and HD (50 mg/kg/day) SDZ ENA 713 via dermal application at 1.5 mL/kg/day in 50% ethanol for 7 days; evaluations included clinical signs, body weight, food intake, TK (at 0, 4, 8, and 24 hrs postdose Days 1 and 7), and gross pathology. b(4)

Results

In the main study, no treatment-related differences were observed in clinical signs, body weight, food intake, hematology, blood chemistry, gross pathology, organ weights, or histopathology. Blood levels of SDZ ENA 713 and ZNS 114-666 were below levels of detection at some time points in all dose groups.

In the additional study at 15 (LD), 30 (MD) and 50 (HD) mg/kg/day, reduced feces were observed in all treated animals from Day 2 onward. Systemic twitching was observed in 5/6 MDF, 2/6 HDM, and 6/6 HDF from Day 3 onward; incidence increased with dose. Reductions in body weight and food intake were observed in all groups on Days 4 and 7 compared to Day 1, though all three dose groups were affected similarly; Day 7 values were greater than Day 4 values, though still below baseline. Systemic exposures to SDZ ENA 713 and ZNS 114-666 on Day 7 were higher than those on Day 1.

NV97262

Table 11-2-1 Plasma concentration of SDZ ENA 713 in male rats administered SDZ ENA 713 BASE percutaneously for 1 week

- The 1st day of administration -

Group	Plasma concentration of SDZ ENA 713 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	0.00 ± 0.00	14.01 ± 9.35	6.57 ± 2.28	1.85 ± 0.41	14.01	4.00	136.54
30 mg/kg	0.00 ± 0.00	23.57 ± 10.92	10.30 ± 8.95	5.19 ± 0.75	23.57	4.00	238.80
50 mg/kg	0.00 ± 0.00	41.24 ± 6.51	45.76 ± 25.00	9.18 ± 2.67	45.76	8.00	696.00

- The 7th day of administration -

Group	Plasma concentration of SDZ ENA 713 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	2.69 ± 1.12	17.20 ± 1.93	11.84 ± 2.06	2.58 ± 1.12	17.20	4.00	213.22
30 mg/kg	2.90 ± 5.03	40.73 ± 12.32	21.37 ± 8.19	6.69 ± 0.40	40.73	4.00	435.94
50 mg/kg	19.25 ± 8.94	103.80 ± 8.84	53.73 ± 8.69	12.05 ± 2.73	103.80	4.00	1087.40

Each value represents Mean ± S.D. (n=3)

NV97262

Table 11-2-2 Plasma concentration of ZNS 114-666 in male rats administered SDZ ENA 713 BASE percutaneously for 1 week

- The 1st day of administration -

Group	Plasma concentration of ZNS 114-666 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	0.00 ± 0.00	2.29 ± 1.09	2.75 ± 1.09	0.41 ± 0.71	2.75	8.00	30.94
30 mg/kg	0.00 ± 0.00	7.89 ± 2.66	10.14 ± 12.14	13.09 ± 10.86	13.09	24.00	237.68
50 mg/kg	0.00 ± 0.00	12.84 ± 2.75	19.65 ± 7.28	5.54 ± 1.46	19.85	8.00	294.18

- The 7th day of administration -

Group	Plasma concentration of ZNS 114-666 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	11.88 ± 10.04	9.01 ± 1.47	7.51 ± 0.50	3.57 ± 3.51	11.88	0.00	163.46
30 mg/kg	11.88 ± 12.83	20.64 ± 5.85	13.68 ± 4.54	6.11 ± 1.46	20.64	4.00	292.00
50 mg/kg	35.09 ± 17.73	59.28 ± 15.80	38.56 ± 2.57	9.48 ± 2.99	59.28	4.00	766.74

Each value represents Mean ± S.D. (n=3)

NV97262

Table 12-2-1 Plasma concentration of SDZ ENA 713 in female rats administered SDZ ENA 713 BASE percutaneously for 1 week

- The 1st day of administration -

Group	Plasma concentration of SDZ ENA 713 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	0.00 ± 0.00	8.35 ± 4.62	9.35 ± 1.68	2.40 ± 0.71	9.35	8.00	146.10
30 mg/kg	0.00 ± 0.00	33.83 ± 10.15	20.23 ± 1.73	5.21 ± 1.07	33.83	4.00	379.30
50 mg/kg	0.00 ± 0.00	56.60 ± 2.88	29.18 ± 2.98	12.42 ± 1.53	56.60	4.00	617.56

- The 7th day of administration -

Group	Plasma concentration of SDZ ENA 713 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	3.31 ± 1.36	22.11 ± 4.57	10.97 ± 0.07	2.54 ± 0.42	22.11	4.00	225.08
30 mg/kg	5.16 ± 1.34	45.65 ± 19.10	19.55 ± 1.38	3.41 ± 2.97	45.65	4.00	415.70
50 mg/kg	11.47 ± 2.47	131.99 ± 23.97	34.61 ± 4.70	10.53 ± 2.78	131.99	4.00	981.24

Each value represents Mean ± S.D. (n=3)

NV97262

Table 12-2-2 Plasma concentration of ZNS 114-666 in female rats administered SDZ ENA 713 BASE percutaneously for 1 week

- The 1st day of administration -

Group	Plasma concentration of ZNS 114-666 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	0.00 ± 0.00	3.94 ± 1.84	6.08 ± 1.79	2.37 ± 0.09	6.06	8.00	95.32
30 mg/kg	0.00 ± 0.00	23.09 ± 2.25	14.80 ± 1.67	5.84 ± 2.00	23.09	4.00	287.08
50 mg/kg	0.00 ± 0.00	36.02 ± 3.43	22.35 ± 1.06	11.38 ± 2.27	36.02	4.00	458.62

- The 7th day of administration -

Group	Plasma concentration of ZNS 114-666 (ng/mL) 0.00 h	4.00 h	8.00 h	24.00 h	Cmax (ng/mL)	Tmax (h)	AUC 0-24 h (ng·h/mL)
15 mg/kg	16.16 ± 7.21	22.68 ± 7.17	13.34 ± 1.21	3.03 ± 0.09	22.68	4.00	280.68
30 mg/kg	23.24 ± 5.54	50.32 ± 18.45	26.08 ± 2.78	6.02 ± 0.93	50.32	4.00	556.72
50 mg/kg	46.24 ± 6.96	116.73 ± 25.93	36.84 ± 5.18	10.41 ± 2.28	116.73	4.00	1011.08

Each value represents Mean ± S.D. (n=3)

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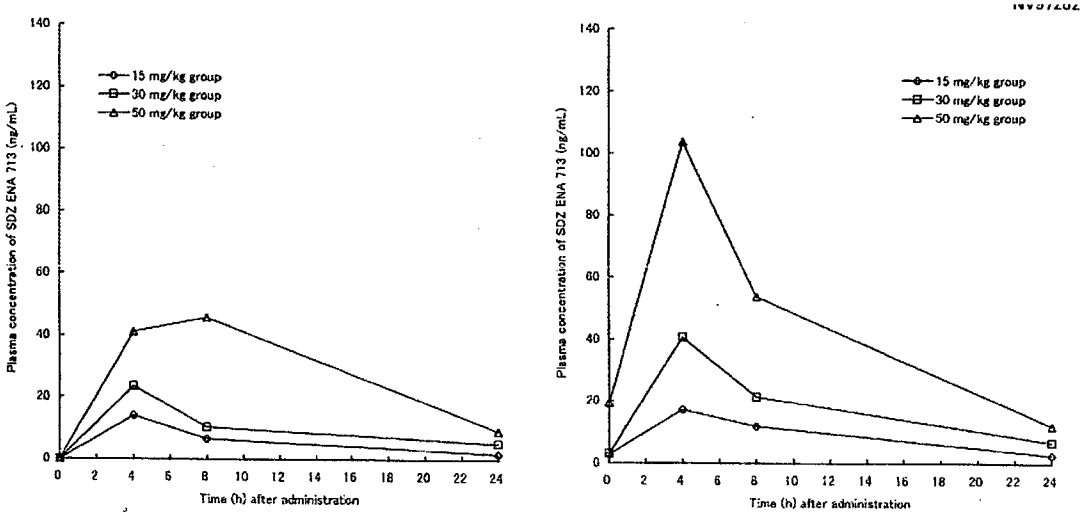

Fig. 5-2-1 Mean plasma concentration of SDZ ENA 713 in male rats administered SDZ ENA 713 BASE percutaneously for 1 week

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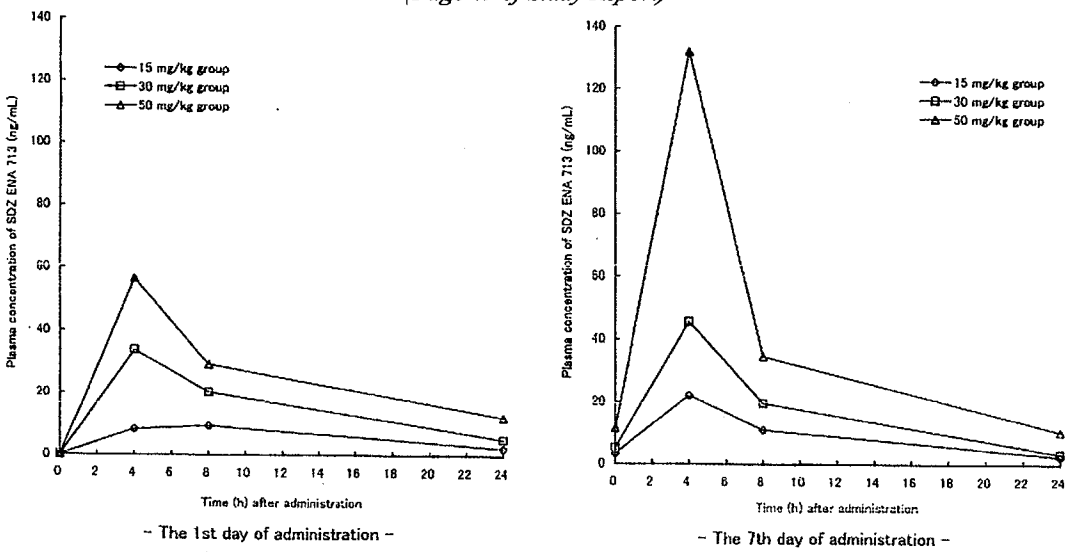

Fig. 6-2-1 Mean plasma concentration of SDZ ENA 713 in female rats administered SDZ ENA 713 BASE percutaneously for 1 week

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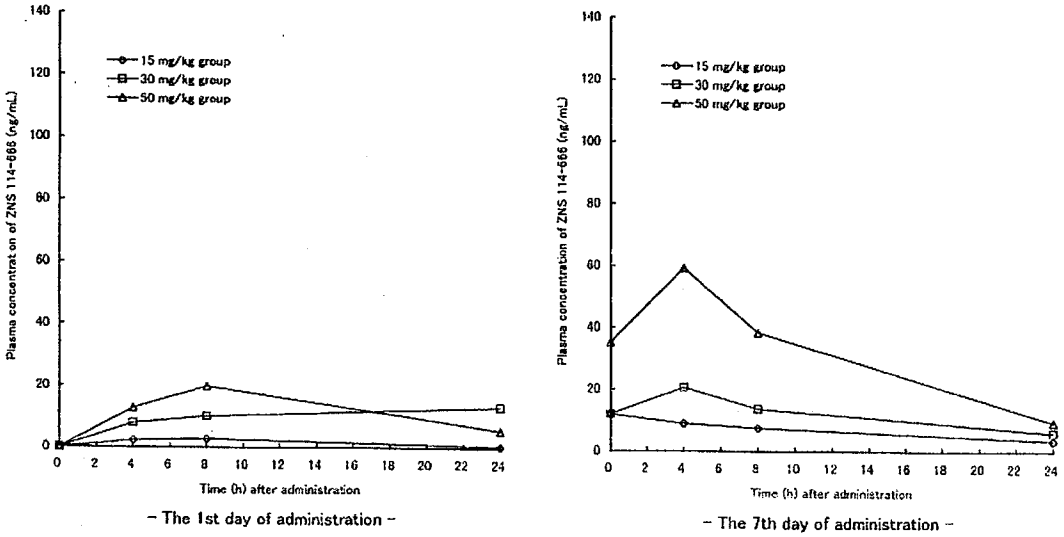

Fig. 5-2-2 Mean plasma concentration of ZNS 114-666 in male rats administered SDZ ENA 713 BASE percutaneously for 1 week

(Page 50 of Study Report)

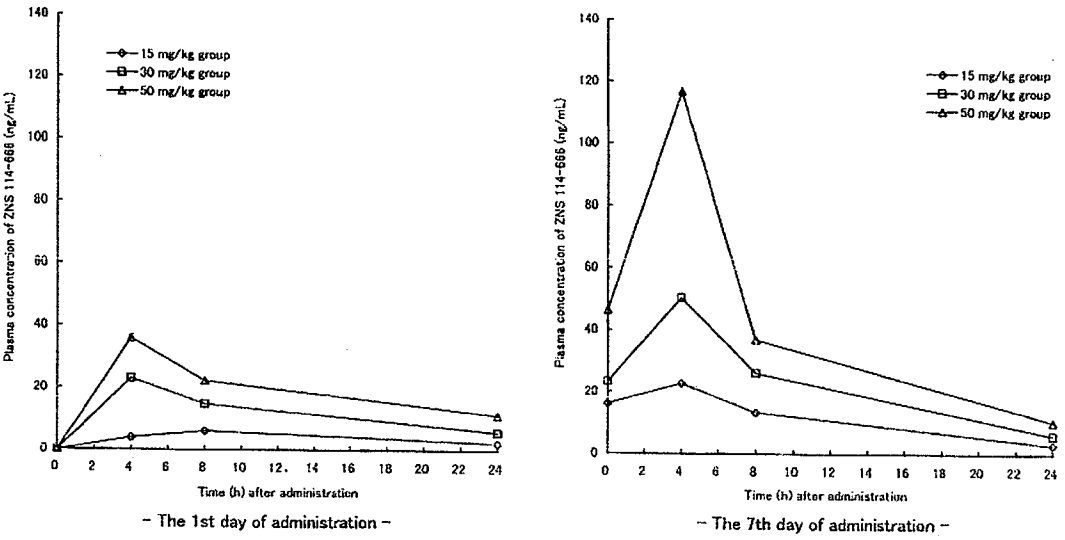

Fig. 6-2-2 Mean plasma concentration of ZNS 114-666 in female rats administered SDZ ENA 713 BASE percutaneously for 1 week

(Page 54 of Study Report)

A 4-week percutaneous dose toxicity study of SDZ ENA 713 base in rats with a 2-week recovery period

(Study Report NV98084; [redacted] Dosing commenced 07-08 JUL 1998; GLP; QA; SDZ ENA 713 Lot #96912, Purity by HPLC 99.9%, by titration 100.6%) b(4)

Key Points

- Dermal application of 5, 15, or 50 mg/kg/day in 50:50 ethanol:water resulted in twitching (1/16 MDM, 11/16 HDM, and 16/16 HDF); salivation (6/16 HDF); tremors (4/16 HDF); and lacrimation (2/16 HDF).
- Body weight was slightly decreased in HDM on Day 4, and food intake was slightly decreased in HDF on Day 4.
- Increased salivary gland weights in HDF (29%) correlated with microscopic findings in the submaxillary gland (hypertrophy of acinar cells), also seen in some MDM and HDM rats. Increased incidence and severity of vacuolation in acinar cells (MDM, HDM, HDF) and eosinophilic granules in the striated portion of the submaxillary gland (HDM) were also noted. These effects were attributed to increased cholinergic stimulation, and were reversible over the 2 week recovery period.
- Day 28 mean Cmax values associated with doses of 5, 15, and 50 mg/kg/day were 8, 55, and 226 ng/mL, respectively, for M; and 16, 95, and 287 ng/mL, respectively, for F. Mean Cmax values for the metabolite NAP 226-90 were lower than the parent (0.23-0.36X for M; 0.35-0.44X for F).
- Day 28 mean $AUC_{0\text{-}24\ hr}$ values associated with doses of 5, 15, and 50 mg/kg/day were 78, 332, and 1380 ng*hr/mL, respectively, for M; and 100, 410, and 1380 ng*hr/mL, respectively, for F. Mean $AUC_{0\text{-}24\ hr}$ values for the metabolite NAP 226-90 were lower than the parent (0.33-0.53X for M; 0.65-0.83X for F).

Methods

[redacted] CD (SD) rats (N=10/sex/group + 6/sex/group for 2-wk recovery [all groups except LD]; 7-8 wks old; 256-326 g M, 167-220 g F) received No Treatment, Vehicle, LD (5 mg/kg/day), MD (15 mg/kg/day), or HD (50 mg/kg/day) SDZ ENA 713 (1.5 mL/kg/day) in a 50:50 ethanol:water mixture applied dermally (onto dorsum, between limb girdles, ~10% of body surface clipped free of hair) once daily for 4 weeks. Toxicokinetic (TK) groups included 12/sex/group LD, LMD, HMD, and HD evaluated at 1, 4, 8, and 24 hrs postdose Days 1 and 28. Parameters measured included clinical signs, body weights, food intake, ophthalmology, urinalysis, hematology, blood chemistry, organ weights, necropsy, and histopathological evaluation (Untreated, Vehicle, and HD only; 49 tissues, including test site; submaxillary glands and gross lesions were examined in all groups). b(4)

Results

All animals survived to the end of the treatment period. Clinical signs observed included twitching (1/16 MDF Days 1-2; 11/16 HDM sporadically; 16/16 HDF **"nearly throughout the administration period"); salivation (6/16** HDF); tremors (4/16 HDF); and lacrimation (2/16 HDF). The salivation, tremors, and lacrimation were generally observed within 2 hrs of dosing, had abated by 24 hrs after dosing, and only occurred on one or two days during the treatment period.

Body weight and body weight gain were slightly reduced in HDM early in the treatment period (Day 4) compared to vehicle controls. Body weight and food intake in M and F Untreated controls was higher than all other groups throughout the treatment period. Food intake was transiently decreased in HDF early in the dosing period (Days 1-4).

Sodium, potassium, and/or chloride urinary excretion rates were significantly decreased in MDF, HDM, and HDF compared to Vehicle controls during Wk 4, correlating with slightly decreased urine volume in HDM and HDF that reached significance in HDF.

Platelet count was increased in HDF (14.6%), total cholesterol was increased in HDM 19.2%), and albumin was decreased in HDF (6.2%) compared to Vehicle controls.

Salivary gland weights were increased in HDF (29% absolute, 29% relative).

Treatment-related microscopic changes included vacuolation in acinar cells of the submaxillary gland (1/10 M Untreated, very slight; 3/10 M Vehicle, very slight to slight; 2/10 LDF, very slight to slight; 5/10 MDM, very slight to slight; 7/10 HDM, very slight to moderate; 2/10 HDF, very slight to moderate); hypertrophy of acinar cells in the submaxillary gland (3/10 MDF, slight; 2/10 HDM, slight to moderate; 6/10 HDF, slight to moderate); and slightly increased eosinophilic granules in the striated portion of the submaxillary gland (1/10 Vehicle M; 1/10 LDM; and 4/10 HDM). Recovery groups showed vacuolation in acinar cells of the submaxillary gland (1/6 Vehicle M, very slight; 1/6 Vehicle F, very slight; and 1/6 HDM, slight).

No treatment-related changes were observed in ophthalmology or gross pathology.

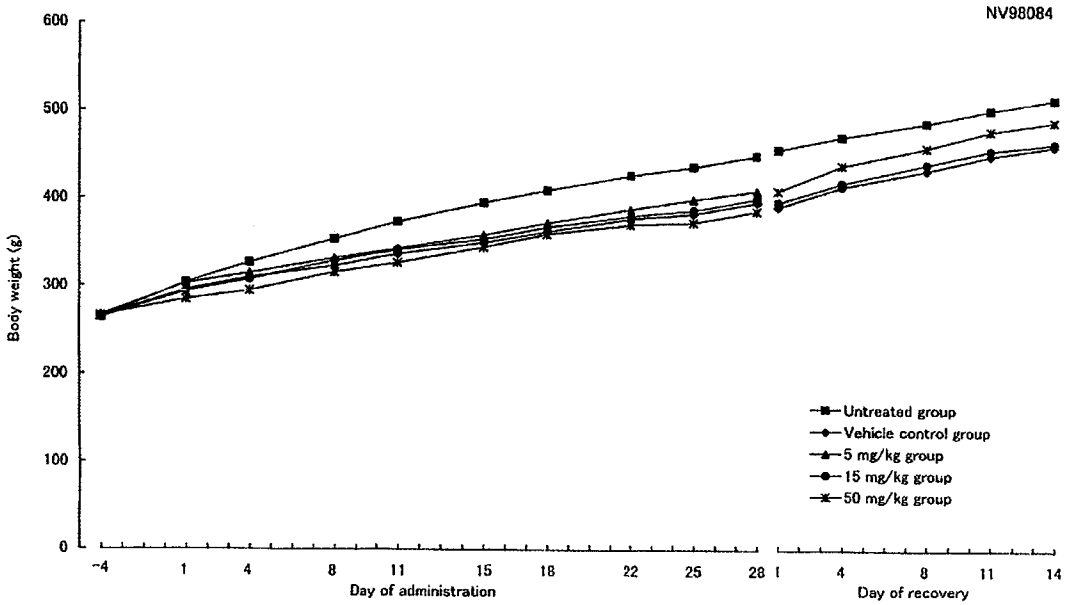

Fig. 1 Body weight in male rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

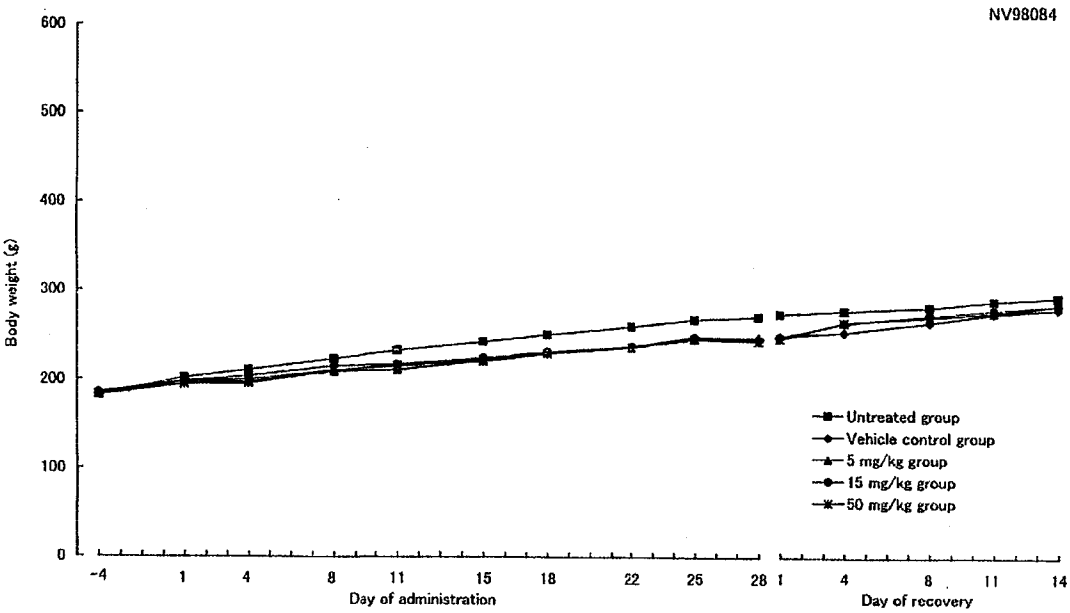

Fig. 2 Body weight in female rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

(Pages 40-41 of Study Report)

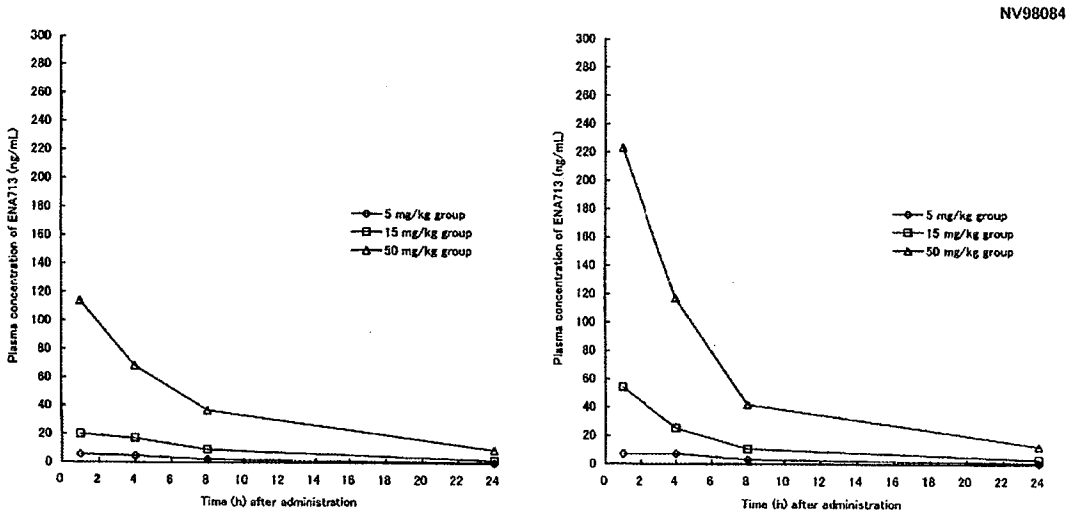

Fig. 5-1 Mean plasma concentration of ENA713 in male rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

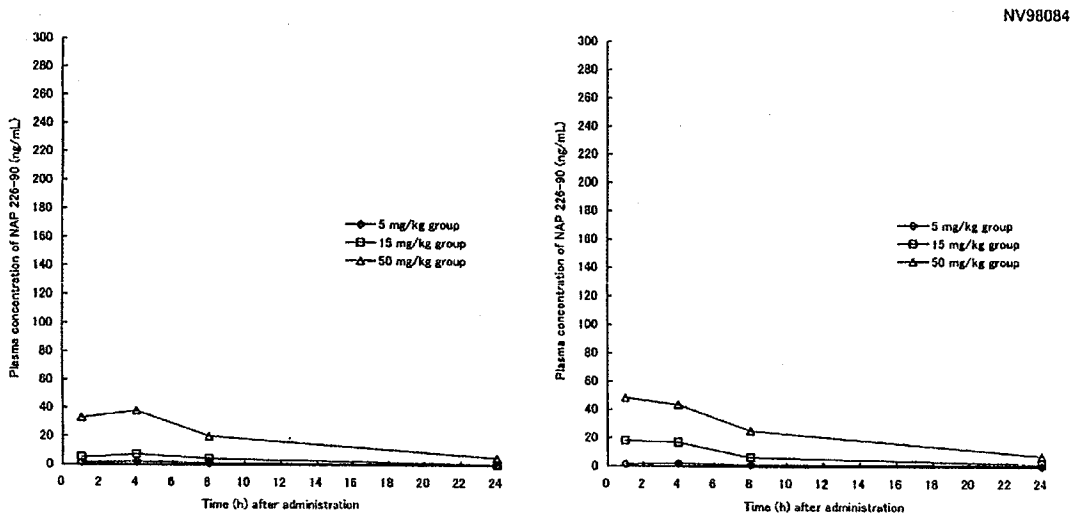

Fig. 5-2 Mean plasma concentration of NAP 226-90 in male rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

(Pages 44-45 of Study Report)

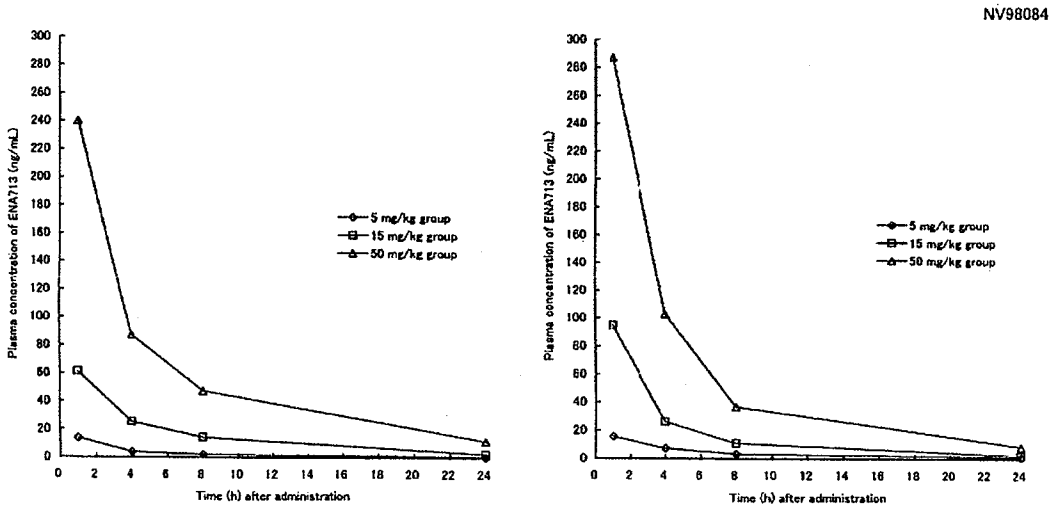

Fig. 6-1 Mean plasma concentration of ENA713 in female rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

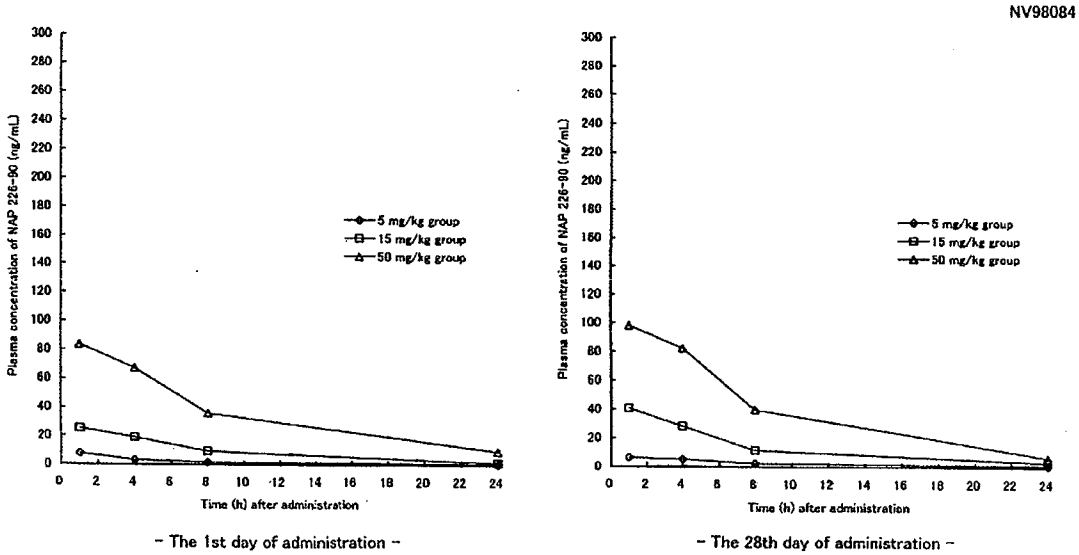

Fig. 6-2 Mean plasma concentration of NAP 226-90 in female rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

(Pages 46-47 of Study Report)

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Table 15-1 Plasma concentration of ENA713 in male rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

- The 1st day of administration -

Group	Plasma concentration of ENA713 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	5.75 ± 3.33	4.65 ± 1.40	2.26 ± 0.983	0.0693 ± 0.139	6.47 ± 2.18	2.5	51.0 ± 16.8
15	20.0 ± 13.7	17.0 ± 4.80	9.13 ± 2.66	1.91 ± 0.222	23.0 ± 11.0	2.5	206 ± 66.5
50	114 ± 25.5	68.4 ± 5.55	36.9 ± 6.48	9.22 ± 1.09	114 ± 25.5	1	911 ± 132

- The 28th day of administration -

Group	Plasma concentration of ENA713 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	7.10 ± 2.24	7.27 ± 1.56	3.23 ± 0.441	0.755 ± 0.171	7.63 ± 1.73	1	78.4 ± 14.2
15	54.2 ± 23.6	25.2 ± 7.53	10.8 ± 4.62	3.29 ± 1.02	54.7 ± 23.0	1	332 ± 78.4
50	223 ± 87.0	117 ± 17.8	42.0 ± 8.94	12.6 ± 2.86	226 ± 83.2	1	1380 ± 226

Each value represents Mean ± S.D. (N=4)

Day 1 : The concentration values at time 0 h were taken as 0 in the AUC calculation.

Day 28 : The concentration values at time 24 h were also taken as the concentration values at time 0 h.

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Table 15-2 Plasma concentration of NAP 226-90 in male rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

- The 1st day of administration -

Group	Plasma concentration of NAP 226-90 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	1.64 ± 1.10	2.19 ± 0.852	1.08 ± 0.438	0.192 ± 0.383	2.27 ± 0.849	4	23.3 ± 6.28
15	5.23 ± 3.75	7.49 ± 1.43	4.50 ± 1.10	0.538 ± 0.0712	7.93 ± 1.76	4	86.0 ± 22.0
50	33.3 ± 11.0	38.2 ± 8.20	20.4 ± 6.65	5.26 ± 1.12	38.4 ± 8.27	4	446 ± 123

- The 28th day of administration -

Group	Plasma concentration of NAP 226-90 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	1.61 ± 0.315	2.10 ± 0.340	1.16 ± 0.260	0.449 ± 0.0847	2.10 ± 0.340	4	25.9 ± 4.21
15	18.2 ± 6.54	16.9 ± 2.32	6.30 ± 2.85	1.94 ± 0.385	20.4 ± 4.21	1	175 ± 25.7
50	48.4 ± 22.1	43.5 ± 6.19	24.9 ± 3.97	7.64 ± 5.59	53.0 ± 17.1	2.5	563 ± 138

Each value represents Mean ± S.D. (N=4)

Day 1 : The concentration values at time 0 h were taken as 0 in the AUC calculation.

Day 28 : The concentration values at time 24 h were also taken as the concentration values at time 0 h.

Table 16-1 Plasma concentration of ENA713 in female rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

- The 1st day of administration -

Group	Plasma concentration of ENA713 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	14.0 ± 6.96	3.99 ± 2.17	2.28 ± 1.55	0.194 ± 0.335 [a]	14.0 ± 6.96	1	64.9 ± 25.6
15	61.1 ± 26.0	25.4 ± 9.12	14.4 ± 5.17	2.49 ± 0.422	61.1 ± 26.0	1	375 ± 127
50	240 ± 61.7	87.2 ± 28.6	47.4 ± 13.4	11.7 ± 2.30	240 ± 61.7	1	1350 ± 266

- The 28th day of administration -

Group	Plasma concentration of ENA713 (ng/mL)				C_{max}	T_{max}	AUC 0-24 h
(mg/kg)	1 h	4 h	8 h	24 h	(ng/mL)	(h)	(ng·h/mL)
5	15.8 ± 8.95	7.38 ± 1.95	3.41 ± 0.812	0.968 ± 0.414	15.8 ± 8.95	1	99.7 ± 32.8
15	94.7 ± 44.5	26.2 ± 5.72	11.0 ± 2.70	2.17 ± 0.687	94.7 ± 44.5	1	410 ± 139
50	287 ± 58.7	103 ± 9.85	36.8 ± 3.33	8.71 ± 1.74	287 ± 58.7	1	1380 ± 155

Each value represents Mean ± S.D. (N=4)

Day 1 : The concentration values at time 0 h were taken as 0 in the AUC calculation.

Day 28 : The concentration values at time 24 h were also taken as the concentration values at time 0 h.

[a] The value based upon 3 animals

NV98084

Table 16-2 Plasma concentration of NAP 226-90 in female rats administered SDZ ENA 713 BASE percutaneously for 4 weeks

- The 1st day of administration -

Group (mg/kg)	Plasma concentration of NAP 226-90 (ng/mL) 1 h	4 h	8 h	24 h	C_{max} (ng/mL)	T_{max} (h)	AUC 0-24 h (ng·h/mL)
5	7.87 ± 3.14	3.23 ± 1.22	1.74 ± 0.833	0.466 ± 0.106 [a]	8.00 ± 2.92	1	45.9 ± 9.90
15	25.4 ± 10.1	19.0 ± 7.58	9.51 ± 2.70	1.43 ± 0.433	26.5 ± 10.0	1	224 ± 62.3
50	83.6 ± 31.8	67.3 ± 20.5	36.0 ± 9.70	9.62 ± 2.54	83.8 ± 31.7	1	838 ± 229

- The 28th day of administration -

Group (mg/kg)	Plasma concentration of NAP 226-90 (ng/mL) 1 h	4 h	8 h	24 h	C_{max} (ng/mL)	T_{max} (h)	AUC 0-24 h (ng·h/mL)
5	6.38 ± 0.812	5.25 ± 1.20	2.47 ± 0.885	1.02 ± 0.240	6.38 ± 0.812	1	64.5 ± 15.7
15	40.8 ± 13.9	28.3 ± 3.51	11.7 ± 2.46	3.16 ± 2.47	41.7 ± 12.5	1	325 ± 50.3
50	98.1 ± 28.3	82.5 ± 19.7	40.0 ± 10.2	6.46 ± 0.761	101 ± 26.8	1	940 ± 195

Each value represents Mean ± S.D. (N=4)

Day 1 : The concentration values at time 0 h were taken as 0 in the AUC calculation.

Day 28 : The concentration values at time 24 h were also taken as the concentration values at time 0 h.

[a] The value based upon 3 animals

(Pages 75-78 of Study Report)

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5-day repeated dose dermal toxicity study with SDZ ENA 713 TDS in rabbits (range finding)
(Study report 282058; [redacted] b(4)
Dosing commenced 15 OCT 1990; GLP; QA; SDZ ENA 713 TDS Batch #W 0720890 and #W 0730890)

Key Points
- Dermal administration of SDZ ENA 713 TDS patches at 1, 2, 4, or 8 cm^2 per rabbit per day (~0.13, 0.26, 0.53, or 1.06 mg/kg/day) induced local signs of very slight to well-defined erythema (focal and/or general) and very slight to slight scaling starting 3-5 days into the 5-day treatment period, and extending 1-5 days into the recovery period. Neither the area of the patch nor the number of patches per animal appeared to correlate with the extent of the local toxicity observed.
- Placebo patches did not induce local toxicity.
- Plasma cholinesterase activity was dose-dependently reduced down to 13% of baseline activity in HDM and 49% in HDF at 24 hrs into the treatment period.
- No treatment-related differences were observed in clinical signs, body weights, food intake, ophthalmology, or necropsy.

Methods
New Zealand White rabbits (N=1/sex/group; 15-16 wks old; 2.59-2.86 kg M, 2.66-3.01 kg F) received Placebo Patch (2x4 cm^2; M#1, F#6), LD (1x1 cm^2; M#2, F#7), LMD (2x1 cm^2; M#3, F#8), HMD (1x4 cm^2; M#4, F#9), or HD (2x4 cm^2; M#5, F#10) SDZ ENA 713 TDS (patch) applied onto shaved skin of back once daily for 24 hrs/day for 5 days, followed by 5 days untreated. The amount of drug applied was 0, 0.37, 0.73, 1.46, and 2.93 mg/rabbit/day, respectively, or 0, ~0.13, 0.26, 0.53, and 1.06 mg/kg/day based on a mean mass of ~2.8 kg per rabbit. Patches were covered by a semi-occlusive dressing and wrapped with an elastic adhesive bandage. Parameters measured included clinical signs, local signs, body weights, food intake, ophthalmology, plasma cholinesterase, and necropsy.

Results
No local findings were observed in the placebo-treated rabbits, but test article-treated rabbits showed very slight to well-defined focal and/or general erythema and very slight to slight scaling varying in intensity and duration throughout the observation period, without clear differences between the 4 groups (see local findings tables on the next page).

Plasma cholinesterase activity decreased dose-dependently, reaching maximum decrease from baseline at 24 hrs (HDM-13%; HMDM-58%; LMDM-80%, LDM-83%, Placebo M-105%; HDF-49%; HMDF-68%; LMDF-88%; LDF-85%; Placebo F-88%).

No treatment-related differences were observed in clinical signs, body weights, food intake, ophthalmology, or necropsy.

CHANGE (MAX.GRADE) (LOCATION)	WEEKS:	ACCLIMATIZATION 1.....	TREATMENT 1....	RECOVERY 1.....
ANIMAL 2				
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	...11	11....
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	....1.
ANIMAL 3				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	1	11111.
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	...1..
ANIMAL 4				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	..112	2.....
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	.....	.2111.
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	...11.
ANIMAL 5				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	..111	1.....
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	.....	.11111
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	..111.

CHANGE (MAX.GRADE) (LOCATION)	WEEKS:	ACCLIMATIZATION 1.....	TREATMENT 1....	RECOVERY 1.....
ANIMAL 7				
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	...11	11111.
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	...11.
ANIMAL 8				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	..111	221111
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	..111.
ANIMAL 9				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	..112	2.....
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	.....	.1111.
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	..112.
ANIMAL 10				
GENERAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	..112	2.....
FOCAL ERYTHEMA (4) (THORACO-DORSAL REGION)	G:	.....	.....	.2111.
SCALING (3) (THORACO-DORSAL REGION)	G:	.....	.....	..111.

(Pages 51-59 of Study Report)

Subacute 28-day repeat-dose dermal toxicity study with SDZ ENA 713 TDS in rabbits

(Study report 282857: [redacted], Dosing commenced 21 NOV 1990; GLP; QA; SDZ ENA 713 TDS Batch #W 0720890 and #W 0730890) b(4)

Key Points

- Dermal administration of SDZ ENA 713 TDS patches at 2 or 4 cm^2 per rabbit per day (~0.3 and 0.6 mg/kg/day, respectively) induced dose-dependent local signs of very slight to well-defined erythema (focal and/or general) and very slight to well-defined edema.
- Placebo patches did not induce local toxicity.
- Plasma cholinesterase activity was inhibited in LD and HD rabbits, while erythrocyte cholinesterase was inhibited only in the HD group.
- No treatment-related changes were observed in systemic signs, body weights, food intake, ophthalmoscopy, hematology, blood chemistry, necropsy, organ weights, or histopathology.

Methods

New Zealand White rabbits (N=5/sex/group; 13 wks old; 2.3-2.5 kg M, 2.3-2.5 kg F) received Placebo Patch (1x4 cm^2), LD (2x1 cm^2), or HD (1x4 cm^2) SDZ ENA 713 TDS (patch) applied onto shaved skin of back once daily for 24 hrs/day for 29 days. The amount of drug applied was 0, 0.73, and 1.46 mg/rabbit/day, respectively, or 0, ~0.30, and 0.61 mg/kg/day based on a mean mass of ~2.4 kg per rabbit. Patches were covered by a semi-occlusive dressing and wrapped with an elastic adhesive bandage. Parameters measured included clinical signs, local signs, body weights, food intake, ophthalmology, hematology, coagulation, blood chemistry, plasma and erythrocyte cholinesterase activity, necropsy, organ weights, and histopathology (skin, adrenals, heart, kidneys, liver, and gross lesions from all animals).

Results

All animals survived to scheduled sacrifice. No erythema or edema was observed at the application site in the placebo group. The test article induced dose-dependent increases in the incidence and severity of very slight to well-defined erythema (focal and/or general) and very slight to well-defined edema at the application site (see tables on next page).

Plasma cholinesterase was inhibited by 41-65% in LD M & F and by 48-52% in HD M & F on Day 28, 24 hrs after the patch was applied. Erythrocyte cholinesterase was inhibited 29-38% in HDM and HDF on Day 28, 24 hrs after the patch was applied.

No treatment-related changes were observed in systemic signs, body weights, food intake, ophthalmoscopy, hematology, blood chemistry, necropsy, organ weights, or histopathology.

Local Toxicity Tables

LDM:

CHANGE (MAX.GRADE) LOCATION		PRETEST WEEKS: 1.....	TREATMENT 1.....2.....3.....4.....5..
GENERAL ERYTHEMA (4)	G:	.....	..1111111111111211111111112111
(THORACO-DORSAL REGION)	%:	.....	..888A888888666266666888888AAA
FOCAL ERYTHEMA (4)	G:	.....	..........1111111111..........
(THORACO-DORSAL REGION)	%:	.....	..........2222222222..........
GENERAL EDEMA (4)	G:	.....	........111111111.........1...
(THORACO-DORSAL REGION)	%:	.....	........222222422.........2...

HDM:

CHANGE (MAX.GRADE) LOCATION		PRETEST WEEKS: 1.....	TREATMENT 1.....2.....3.....4.....5..
GENERAL ERYTHEMA (4)	G:	.....	.1122122222111111111122222222
(THORACO-DORSAL REGION)	%:	.....	.4668AAAAAAAAAAAAAAAAAAAAAAAA
GENERAL EDEMA (4)	G:	.....	1111111111111111112222111
(THORACO-DORSAL REGION)	%:	.....	.....2488888888888666888888AAA

LDF:

CHANGE (MAX.GRADE) LOCATION		PRETEST WEEKS: 1.....	TREATMENT 1.....2.....3.....4.....5..
GENERAL ERYTHEMA (4)	G:	.....	..1111111111111111112211111111
(THORACO-DORSAL REGION)	%:	.....	..88AA886666444244444666666666
FOCAL ERYTHEMA (4)	G:	.....	1111111111111111111
(THORACO-DORSAL REGION)	%:	.....	2222224444444444444
GENERAL EDEMA (4)	G:	.....	................1..11111111111
(THORACO-DORSAL REGION)	%:	.....	................2..22222224444

HDF:

CHANGE (MAX.GRADE) LOCATION		PRETEST WEEKS: 1.....	TREATMENT 1.....2.....3.....4.....5..
GENERAL ERYTHEMA (4)	G:	.....	.1122211111112211111111111122222
(THORACO-DORSAL REGION)	%:	.....	.AAAAAAAAAAAA88AAAAAAAAAAAAAAAA
GENERAL EDEMA (4)	G:	.....	.........111111111111......1111
(THORACO-DORSAL REGION)	%:	.....	.........444444444222......2222
GENERAL EDEMA (4)	G:	.....	.1111111111111111111..........
(SHOULDER LEFT)	%:	.....	.22222222222222222222..........

G: Median value of the highest individual daily grades
%: Percent of affected animals (0 = less than 5%, 1 = between 5% and 15%,..., A = more than 95%)

(Pages 52-56 of Study Report)

Subacute 28-day repeat-dose dermal toxicity in rabbits
(Study report 643285; [redacted] b(4)
Dosing commenced 02 DEC 1996; GLP; QA; SDZ ENA 713 TDS Batch #X 087 0496)

Key Points

- Dermal administration of SDZ ENA 713 TDS patches at 9 mg/5 cm^2 per rabbit per day (~3 mg/kg/day) for 28 days resulted in slight reductions in body weight gain and food consumption in females.
- Local toxicities observed at the application site were similar in frequency and severity in placebo patch and drug-containing patch groups, and were therefore attributed to mechanical injury sustained during removal of the adhesive patches rather than to the presence of the test article itself. These local toxicities included erythema; edema; localized reddish to reddish-blue skin discoloration (bruising, sometimes followed by scab formation); scaling of the skin; mononuclear and inflammatory cell infiltration; dermal hyperplasia; akanthosis; fibroplasia; and necrosis.
- No treatment-related changes were observed in systemic signs.

Methods

New Zealand White rabbits (N=4/sex/group; 14-15 wks old; 2.4-3.3 kg) were left Untreated, received Placebo Patch (½ of a 29 x 37.5 mm, 10 cm^2 patch), or SDZ ENA 713 TDS Patch (½ of a 18 mg/10 cm^2 patch, or 9 mg/day) applied onto shaved skin of back once daily for 24 hrs/day for 28 days. The patch was rotated among 14 sites per animal, with a 15^{th} site left untreated for comparison. Patches were overlaminated with ½ of a 40 x 48 mm rectangular adhesive patch, covered with a gauze patch and a semi-occlusive adhesive dressing wrapped around the abdomen and secured with tape. Parameters measured included clinical signs, local signs, body weights, food consumption, necropsy, and histopathology (treated and untreated skin, and gross lesions).

Results

One out of 4 Untreated control M showed minor reddening and scaling of the shaved skin, and 3/4 Untreated F showed marked irritation, with occasional open wounds and scaling/scabbing. These findings were attributed to the adhesive semi-occlusive bandage, so the treatment procedure was changed on Day 3, by shortening the bandage for extra flexibility and adding a thin layer of gauze to prevent the adhesive of the bandage from contacting the shaved skin. All signs of generalized local irritation in the Untreated group (and the Placebo Patch group) disappeared within a few days of these changes.

Erythema and edema were not seen after Day 3 in the Untreated groups, and were observed at the application sites in Placebo Patch and SDZ ENA 713 TDS Patch groups with generally similar frequency, severity, and duration. Erythema was present at all test sites after the first and second applications, whereas edema was present at very slight severity or (occasionally) absent after the first application, but present at almost all sites at increased severity after the second application.

Occasional observations of localized reddish to reddish-blue skin discoloration (bruising, sometimes followed by scab formation) were noted in 3/4 M and 3/4 F in the Placebo Patch group, especially during the first week of the study, and were attributed to injuries sustained during the removal of the adhesive patch and overlaminate, perhaps exacerbated by removal of the adhesive of the bandage before the change in procedure. Frequent observations of scaling of the skin at various individual application sites was observed in all Placebo Patch animals throughout the treatment period, and was also attributed to the removal of the adhesive patches/overlaminates.

The SDZ ENA 713 TDS Patch group showed local toxicity similar to the Placebo Patch group, with marginally higher incidence of bruising and/or scab formation at individual application sites, again attributed to the removal of the adhesive patches/overlaminates.

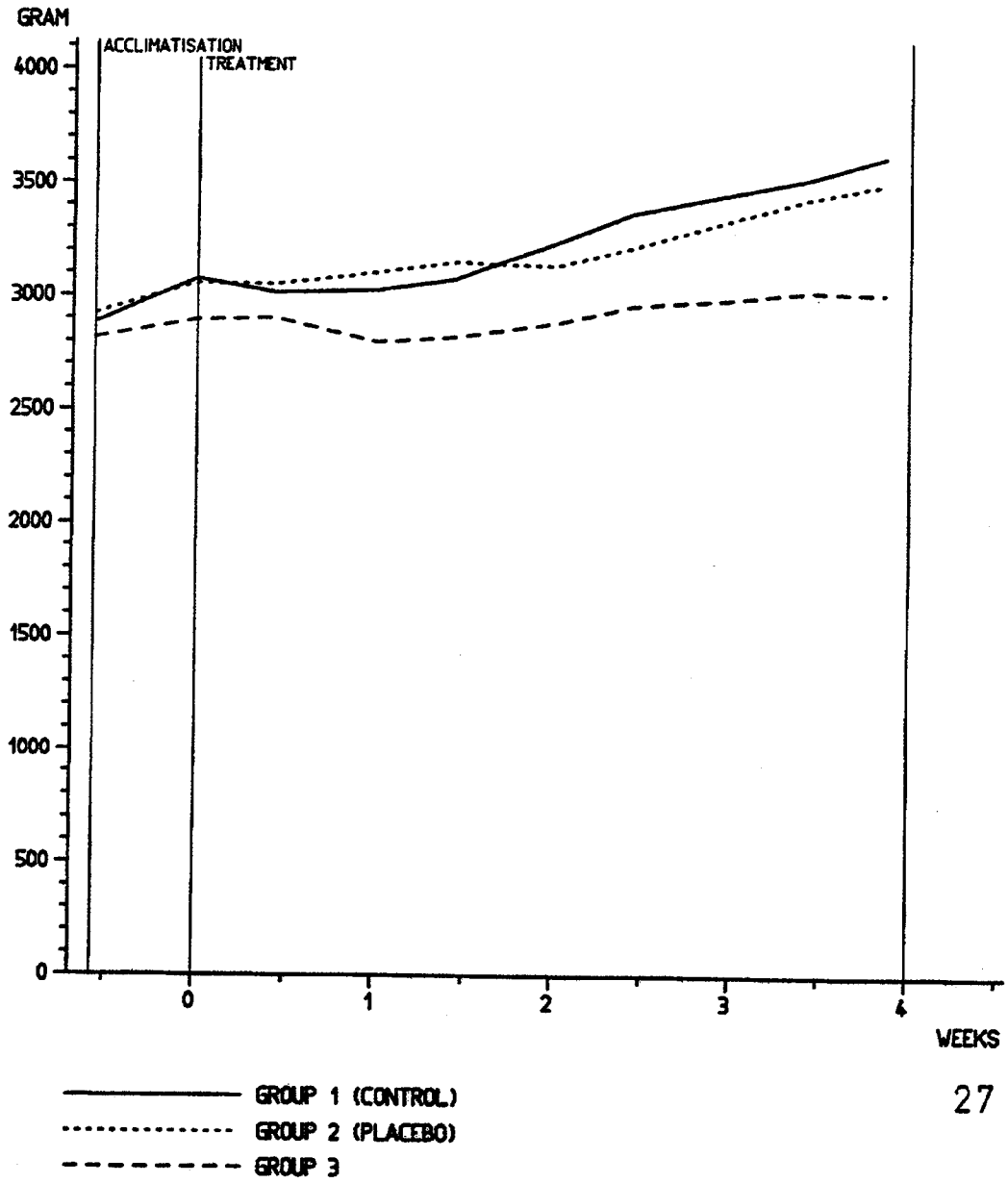
No treatment-related changes were observed in systemic clinical signs.

Food consumption was reduced in all 3 groups during the first week, attributed to the constrictive semi-occlusive bandage. A slight reduction in food consumption in SDZ ENA 713 TDS Patch females compared to both control groups throughout the treatment period was considered treatment-related, and correlated with a slight reduction in body weight gain in F of this group (see graph on the following page).

Reddish or dark red discoloration of the most recently treated skin site was note upon necropsy in 1/4 M and 3/4 F Placebo Patch animals, and in 3/4 M and 4/4 SDZ ENA 713 TDS Patch rabbits, and was considered to be caused by the physical stress during removal of the patches rather than by the presence of the test article.

Microscopic findings were similar in Placebo and SDZ ENA 713 TDS groups, and were indicative of minor irritant effects; they included: mononuclear and inflammatory cell infiltration, dermal hyperplasia, akanthosis, fibroplasia, and necrosis. Once again, these **changes "were considered to be the result** of mechanical injury incurred during the removal of the adhesive placebo or test article patches, rather than a test article-specific **effect."** *(Page 32 of Study Report)* This conclusion seems reasonable to this reviewer.

BODY WEIGHTS
FEMALES



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4-week oral (gavage) toxicity study in minipigs
(Study report 645985; [redacted] Dosing commenced 18 FEB 1997; GLP Switzerland 1986; QA; SDZ ENA 713 (HTA salt) Batch #96603, Purity 99.7%) b(4)

Key Points

- Treatment of minipigs by oral gavage with 6 mg/kg/day SDZ ENA 713 for 4 weeks resulted in frequent, transient, slight to moderate tremors in 4/5 M and 5/5 F; occasional slightly decreased activity in 1/5 HDM and 4/5 HDF; occasional slight salivation in 1/5 HDM and 5/5 HDF; and slight lateral recumbency in 1/5 HDF.
- No treatment-related changes were observed in body weights, food consumption, ophthalmoscopy, ECG, hematology, blood chemistry (except decreases in cholinesterase activity of 21-38% in HDF on Day 1 and in HDM and HDF on Day 28), urinalysis, organ weights, macroscopic findings, or microscopic findings.
- The NOAEL of 2 mg/kg/day was associated with Day 28 Cmax = 1.9 ng/mL (M) and 2.8 ng/mL (F), and Day 28 AUC 0-24 hr = 2.8 ng*hr/mL (M) and 4.5 ng*hr/mL (F).

Methods

Göttingen minipigs (N=3/sex/group + 2/sex/group Veh and HD for 2-wk recovery; 3.5-4.5 months old; 6.8-9.1 kg) received 0, 0.6, 2, or 6 mg/kg/day SDZ ENA 713 via oral gavage in 1% carboxymethylcellulose and 0.2% Polysorbate 80 at 5 mL/kg. Parameters measured included clinical signs, body weights, food consumption, ophthalmoscopy, ECG (prior to dosing, at pretest, 4 wks, and after 2-wk recovery period), hematology, coagulation, blood chemistry, urinalysis, toxicokinetics (Day 1 and Day 28), necropsy, organ weights, and histopathology (50 tissues and all gross lesions from all animals).

Results

All animals survived to terminal sacrifice. Treatment-related clinical signs were limited to the HD group, and included tremor, salivation, decreased activity, and recumbency. No clinical signs were observed in 1/5 HDM during the treatment period, or in 2/2 HDM or 2/2 HDF during the recovery period.

Clinical signs in HDM:

SIGN (MAX.GRADE) (LOCATION)	WEEKS:	PRETEST 1.....2.....	TREATMENT 1.....2.....3.....4.....5
ANIMAL 12			
SPASMS			
TREMOR (3)	G:	.....	.....1..11..1.....
ANIMAL 13			
SPASMS			
TREMOR (3)	G:	.....	.111111111222112212121111111111
ANIMAL 14			
SPASMS			
TREMOR (3)	G:	.....	.1111111111111211....11111111
GAIT / MOTILITY			
DECREASED ACTIVITY (3)	G:	.....	.....1....11
SECRETION / EXCRETION			
SALIVATION (3)	G:	.....	.....1.
ANIMAL 15			
SPASMS			
TREMOR (3)	G:	.....	.1111111111111111..11111...2
ANIMAL 16			
NO CLINICAL SIGNS NOTED			

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Clinical signs in HDF:

SIGN (MAX.GRADE) (LOCATION)	WEEKS:	PRETEST 1.....2.....	TREATMENT 1.....2.....3.....4.....5
ANIMAL 28			
SPASMS			
TREMOR (3)	G:	.....	1111112222222111111111111.1111
GAIT / MOTILITY			
DECREASED ACTIVITY (3)	G:	.....	.....111.
SECRETION / EXCRETION			
SALIVATION (3)	G:	.....	.....1111111111111111111.
ANIMAL 29			
SPASMS			
TREMOR (3)	G:	.....	..1....11...1.....11
SECRETION / EXCRETION			
SALIVATION (3)	G:	.....	.....1.
ANIMAL 30			
SPASMS			
TREMOR (3)	G:	.....	1111111211111.....111...2.
GAIT / MOTILITY			
DECREASED ACTIVITY (3)	G:	.....	.....1.
SECRETION / EXCRETION			
VOMITING OF FEED (1)	G:	.....	1.....
SALIVATION (3)	G:	.....	.....11.1.
ANIMAL 31			
SPASMS			
TREMOR (3)	G:	.....	1111112222222222122212 1.1.121
GAIT / MOTILITY			
DECREASED ACTIVITY (3)	G:	.....	.....1111.1111.11111...11
SECRETION / EXCRETION			
SALIVATION (3)	G:	.....	.....111111111111111.21111
ANIMAL 32			
SPASMS			
TREMOR (3)	G:	.....	1111111111212221212221122112 21
POSTURE			
LATERAL RECUMBENCY (1)	G:	.....	.....111..11..11.
GAIT / MOTILITY			
DECREASED ACTIVITY (3)	G:	.....	.....11111...1111111111
SECRETION / EXCRETION			
SALIVATION (3)	G:	.....	.....1.1

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No treatment-related changes were observed in body weights, food consumption, ophthalmoscopy, ECG, hematology, blood chemistry (except decreases in cholinesterase activity of 21-38% in HDF on Day 1 and in HDM and HDF on Day 28), urinalysis, organ weights, macroscopic findings, or microscopic findings.

The toxicokinetic data shown in the tables below revealed large increases in Cmax and AUC of SDZ ENA 713 from Day 1 to Day 28, especially in the HD groups (2-5 fold). Also, exposures to the metabolite ZNS 114-666 were much greater than exposures to the parent drug.

Toxicokinetic parameters of SDZ ENA 713

Dose [mg/kg]	Sex	Day 1 AUC(0-24h) [(ng/mL)h]	Day 1 C_{max} [ng/mL]	Day 1 t_{max} [h]	Day 28 AUC(0-24h) [(ng/mL)h]	Day 28 C_{max} [ng/mL]	Day 28 t_{max} [h]
0.6	M	**	0.4 ± 0.01*	0.5 - 1*	**	0.6 ± 0.03	0.5 - 1
	F	**	0.5 ± 0.03*	0.5 - 1*	**	0.5 ± 0.2	0.5 - 1
2	M	2.1 ± 0.2*	1.5 ± 0.4	0.5 - 0.5	2.8 ± 1.7	1.9 ± 0.8	0.5 - 0.5
	F	2.8 ± 0.7	1.6 ± 0.1	0.5 - 1	4.5 ± 3.0	2.8 ± 1.8	0.5 - 1
6	M	13.6 ± 9.9	4.2 ± 2.6	1 - 1	69.2 ± 49.9	27.8 ± 20.0	0.5 - 2
	F	12.1 ± 4.9	5.5 ± 3.9	0.5 - 1	24.4 ± 13.9	14.4 ± 11.0	0.5 - 1

* N=2 ** not enough values to calculate AUCs

Toxicokinetic parameters of ZNS 114-666

Dose [mg/kg]	Sex	Day 1 AUC(0-24h) [(ng/mL)h]	Day 1 C_{max} [ng/mL]	Day 1 t_{max} [h]	Day 28 AUC(0-24h) [(ng/mL)h]	Day 28 C_{max} [ng/mL]	Day 28 t_{max} [h]
0.6	M	82 ± 6	22 ± 3	0.5 - 0.5	83 ± 20	32 ± 11	0.5 - 0.5
	F	111 ± 16	26 ± 5	0.5 - 1	110 ± 28	34 ± 3	0.5 - 1
2	M	392 ± 9*	160 ± 49	0.5 - 0.5	369 ± 43	114 ± 21	0.5 - 1
	F	454 ± 122	165 ± 34	0.5 - 1	413 ± 172	134 ± 98	0.5 - 2
6	M	1184 ± 123	249 ± 62	1 - 2	1682 ± 243	392 ± 88	1 - 2
	F	1234 ± 301	400 ± 158	0.5 - 0.5	1483 ± 213	457 ± 63	0.5 - 1

* n=2

(Pages 275-276 of Study Report)

Appears This Way
On Original

A dermal dose-escalating study in minipigs
(Study report 60DEMP; Novartis Pharma AG, Basle, Switzerland; Report released 18 AUG 1997; GLP Switzerland 1986; not QA; SDZ ENA 713 TDS Batch #U 001 0195)

Key Points

- Treatment of 1 M and 1 F minipig by dermal application of 2, 4, 6, 8, 10, and 12 SDZ ENA 713 TDS patches per animal in six successive days (total dose of 36, 72, 108, 144, 180, and 216 mg/animal/day, respectively) resulted in no changes in clinical signs, body weight, or food consumption.
- Analysis of residual drug in used patches revealed delivery of 34-52% of SDZ ENA 713 over the 23 hr application period.
- Only minimal to slight erythema was observed after repeated application to the same site for six consecutive days.
- Plasma butyryl cholinesterase was inhibited by 19-23% after the final dose of 216 mg/animal/day.

Methods

Göttingen minipigs (N=1/sex) were treated with 2, 4, 6, 8, 10, and 12 SDQ ENA 713 patches (each 18 mg/10 cm^2) on six successive days, to achieve total doses of 36, 72, 108, 144, 180, and 216 mg/animal. Patches were applied to the same skin area and covered with [redacted] adhesive tape, and replaced with the new patches one hour after the 23-hr application period had ended. Parameters measured included clinical signs, body weights, food consumption, plasma cholinesterase activity, and residual drug in used patches. No necropsy was performed. b(4)

Results

No treatment-related changes were observed in clinical signs, body weight, or food consumption. Analysis of residual drug in used patches revealed delivery of 34-52% of SDZ ENA 713 over the 23 hr application period. Repeated application to the same skin site resulted in only minimal to slight skin erythema in both the M and the F minipig. Skin color measured with a reflectometer correlated well with the findings of erythema. The sponsor believes that the slight erythema might be caused by removal of the adhesive patch rather than by the presence of the drug substance. Plasma butyryl cholinesterase was inhibited by 19-23% after the final dose of 216 mg/animal/day.

Reviewer's Note:
No data tables or study report were provided to support this brief summary.

A 2-week dermal dose-range-finding study in minipig
(Study report 208DFP; ——————————, Dosing commenced JUN 1995; GLP Switzerland 1986; not QA; SDZ ENA 713 TDS Batch #U 002 0195) b(4)

Key Points

- Treatment of minipigs by dermal application of 1, 3, or 6 SDZ ENA 713 TDS 36 mg/20 cm^2 patches per animal per day for 2 weeks (total dose of 36, 108, and 216 mg/animal/day, respectively, or ~3.4, 10.2, or 20.4 mg/kg/day at ~10.6 kg per animal) resulted in no changes in clinical signs, body weight, food consumption, rectal body temperature, hematology, blood chemistry, ECG, or organ weights.
- Severe erythema occurred by Day 9 of repeated application to the same skin sites, necessitating changing the application site in one placebo, one MD, and 1 HD animal; the new sites showed moderate to severe erythema after only 2 applications. The other animals showed slight to moderate erythema, increasing in severity with repeated dosing, except for the Placebo F, which showed no erythema.
- Erythema was correlated with minimal to moderate superficial perivascular dermatitis in all dose groups.
- Analysis of residual drug in used patches revealed delivery of 46-68% of SDZ ENA 713 over the 23 hr application period on Day 1 and 34-63% on Day 14.
- Plasma butyrylcholinesterase activity was inhibited 14-37% on Day 14 in all 3 SDZ ENA 713 TDS Patch groups, without relation to dose.

Methods

Göttingen minipigs (N=1/sex/group; 15-18 wks old; 9.5-11.7 kg) were treated with six 20 cm^2 placebo patches or 1, 3, or 6 SDQ ENA 713 patches (each 36 mg/20 cm^2) per day for 2 weeks, to achieve total doses of 36, 108, or 216 mg/animal/day (~3.4, 10.2, or 20.4 mg/kg/day at ~10.6 kg per animal). Patches were applied to the same skin area and covered with ——— adhesive tape, and replaced with the new patches one hour after the 23-hr application period had ended. Parameters measured included clinical signs, body weights, food consumption, rectal body temperature, ECG, hematology, clinical chemistry, plasma butyrylcholinesterase activity, toxicokinetics (Days 1 and 14 at 2, 6, and 24 hrs post-application), residual drug in used patches, necropsy, organ weights, and microscopic evaluation (41 tissues + gross lesions). b(4)

Results

All animals survived to scheduled sacrifice. No treatment-related changes were observed in clinical signs, body weight, food consumption, rectal body temperature, hematology, blood chemistry, ECG, or organ weights. Local reactions varied in the severity of erythema observed, from no erythema in the F Placebo to severe erythema appearing from Day 9 onward in the Placebo M, a MD, and a HD animal; the severity of the skin reaction necessitated changing the application site on Day 12 in these three animals. Moderate to severe erythema appeared at the new site after only 2 applications. Previously treated sites recovered after 1-4 days. The other animals tolerated repeated applications with slight to moderate erythema. Since one placebo control animal showed this severe reaction, the patch itself rather than the drug substance must be responsible.

The sites covered by the ——— ape also showed slight skin erythema in several animals. b(4)

Plasma butyrylcholinesterase activity was inhibited 14-37% on Day 14 in all 3 SDZ ENA 713 TDS Patch groups, without relation to dose.

Toxicokinetic measurements on Days 1 and 14 revealed great inter-animal variability and lack of clear dose-dependence in exposure between the MD and HD groups.

Day 14 Toxicokinetic Parameters:

	SDZ ENA 713		ZNS 114-666	
	Male	Female	Male	Female
C_{max} (ng/ml)				
36 mg/day	11	14	14	17
108 mg/day	121	10	103	16
216 mg/day	120	53	102	60
AUC_{0-24h} (h.ng/ml)				
36 mg/day	185	199	247	242
108 mg/day	1810	168	1637	239
216 mg/day	1113	735	1003	942

(Page 11 of Study Report)

Macroscopic changes at the application site were limited to slight to moderate reddened and/or thickened skin, correlating with microscopic observations of minimal to moderate superficial perivascular dermatitis. These changes were seen in all dose groups, including the placebo control. No other treatment-related changes were observed.

A 4-week dermal toxicity study in minipigs

(Study report 442P: [redacted], Dosing commenced 11 SEP 1995; GLP Switzerland 1986; QA; SDZ ENA 713 TDS Batch #X 135 0595, Purity 100.5%) b(4)

Key Points

- Treatment of minipigs by dermal application of 2, 6, or 12 SDZ ENA 713 TDS 18 mg/10 cm^2 patches per animal per day for 4 weeks (total dose of 36, 108, and 216 mg/animal/day, respectively, or ~3.4, 10.2, or 20.4 mg/kg/day at ~10.6 kg per animal) resulted in no changes in systemic clinical signs, body weights, food consumption, ophthalmoscopy, ECG, hematology, clinical chemistry, or organ weights.
- Severe erythema occurred by Day 9 of repeated application to the same skin sites every other day, necessitating unscheduled sacrifice in 2/10 placebo, 1/6 MD, and 2/10 HD animals on Days 12-19. Most animals showed no, minimal or slight erythema.
- Minimal to marked dermatitis was observed at the application site in all dose groups, but was moderate to marked in the 5 animals sacrificed early with moderate to marked erythema.
- Analysis of used patches revealed mean percent of total drug delivered over 24 hours did not change from Day 2 (44-59%) to Day 26 (47-63%), and was independent of dose and sex.
- Plasma butyrylcholinesterase was inhibited 20-31% in LDF, 18-40% in MD M/F and 38-44% in HD M/F on Day 25, but was not changed consistently on Day 1.

Methods

Göttingen minipigs (N=5/sex/group for Placebo & HD, 3/sex/group for LD & MD; 15-21 wks old; 8.6-10.6 kg) were treated with twelve 10 cm^2 placebo patches or 2, 6, or 12 SDQ ENA 713 patches (each 18 mg/10 cm^2) per day for 4 weeks, to achieve total doses of 36, 108, or 216 mg/animal/day (~3.4, 10.2, or 20.4 mg/kg/day at ~10.6 kg per animal). Each patch was applied alternately to each of two application sites on the clipped dorsal trunk, so that the same site was used every other day. MDM #B9M had to have a third site added on Days 10 and 11 due to severe skin reaction. Patches were covered with [redacted] adhesive tape, and removed after ~24 hrs. Parameters measured included clinical signs, test site appearance, body weights, food consumption, ECG (Pretest, Day 4, Day 23; ~8 AM), ophthalmoscopy, hematology, clinical chemistry, plasma butyrylcholinesterase activity, endocrinology (ACTH, cortisol, insulin, progesterone), toxicokinetics (Days 1 and 25 at 0, 2, 6, and 24 hrs post-application), residual drug in used patches (Days 2 and 26), necropsy, organ weights, and microscopic evaluation (43 tissues + gross lesions). b(4)

Results

No treatment-related changes were observed in systemic clinical signs, body weights, food consumption, ophthalmoscopy, ECG, hematology, clinical chemistry, or organ weights.

Due to severe skin reactions (ecchymotic skin with crusts), unscheduled sacrifice was performed on 2 Placebo F (Days 12 and 19), 1 MDM (Day 12), 1 HDM (Day 12), and 1 HDF (Day 16).

Analysis of used patches revealed mean percent of total drug delivered over 24 hours did not change from Day 2 (44-59%) to Day 26 (47-63%), and was independent of dose and sex.

Moderate to severe skin erythemas (Grade 3-4) were observed in individual Placebo and drug-treated groups from Day 9 onward, but were limited to the application sites and did not show edema. The erythemas persisted until 24 hrs after patch removal, and increased in severity with increasing number of patches and number of applications on the same site. Since placebo controls showed similar skin reactions, they were attributed to the patch formulation rather than to the drug substance. Most animals showed no erythemas or minimal to slight erythemas (Grade 0-2). The 2-patch dosing pattern in the LD group induced only mild erythema.

Skin Reactions (recorded according to OECD Guideline No. 404)

Incidence of highest severity grades of skin erythema:

Group (mg/animal/day)	0	36	108	216
Σ Animals	5M/5F	3M/3F	3M/3F	5M/5F
Grade 0	4/1	0/0	0/1	0/0
Grade 1	1/2	3/2	2/1	2/2
Grade 2	0/0	0/1	0/1	2/2
Grade 3	0/1 (12)	0/0	0/0	0/1 (16)
Grade 4	0/1 (19)	0/0	1* (12)/0	1 (12)/0

M = males
F = females
0 = day of unscheduled necropsy
* = changing to a new application site (naive site) on day 10; erythema, severity grade 2 occured after a single 24h application.

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Plasma butyrylcholinesterase was inhibited 20-31% in LDF, 18-40% in MD M/F and 38-44% in HD M/F on Day 25, but was not changed consistently on Day 1.

No systemic toxicity was revealed in macroscopic or microscopic examinations. All groups showed minimal to severe dermatitis at the application site. In the few animals that had moderate to marked erythema and dermatitis at the test site, less severe dermatitis was observed at the ——— covered sites and the naïve skin areas. The dermatitis was characterized by varying degrees of epidermal parakeratosis, acanthosis, spongiosis, occasional epidermal neutrophils, transepidermal inflammatory infiltrates and superficial dermal perivascular infiltrates, edema, and hemorrhage. b(4)

Plasma concentrations of SDZ ENA 713 and the major metabolite ZNS 114-666 were very similar, and were increased ~5-10-fold from Day 1 to Day 25. No correlation was observed between the amount of drug released from the patch and the blood concentration of the parent drug or its major metabolite.

	Dose mg/animal/day	DAY	MALES		FEMALES		M + F	
			Mean	SD	Mean	SD	Mean	SD
Cmax (ng/ml)	36	1	1.3	1.4	2.8	0.3	2.1	1.2
		25	11	3	12	5	12	4
	108	1	10	5	4	2	7	5
		25	55	9	43	14	48	12
	216	1	18	7	15	13	17	10
		25	117	40	137	49	127	43
AUC(0-24h) (h*ng/ml)	36	1	22	28	40	15	31	23
		25	202	43	233	75	218	58
	108	1	194	91	71	32	133	91
		25	932	114	762	226	830	194
	216	1	328	147	272	255	300	199
		25	2033	589	2244	709	2138	614

Summary of the toxicokinetic characteristics of ZNS 114-666

	Dose mg/animal/day	DAY	MALES		FEMALES		M + F	
			Mean	SD	Mean	SD	Mean	SD
Cmax (ng/ml)	36	1	2.5	1.1	3.4	0.8	2.9	1.0
		25	12	2	9	5	10	4
	108	1	10	3	5	2	7	4
		25	48	5	41	4	44	5
	216	1	18	8	15	9	17	8
		25	169	76	155	61	162	64
AUC(0-24h) (h*ng/ml)	36	1	32	27	42	17	37	21
		25	225	11	179	87	202	61
	108	1	170	72	64	38	117	78
		25	801	37	705	44	744	64
	216	1	335	170	243	189	289	176
		25	2826	1356	2471	909	2649	1085

(Pages 101-104 of Study Report)

A 4-week dermal tolerability study in minipigs

(Study report 445P· ——————————— Dosing commenced 20 NOV 1995; GLP Switzerland 1986; QA; SDZ ENA 713 TDS Batch #X 135 0595, Purity 100.5%) b(4)

Key Points

- Treatment of minipigs by dermal application of 1, 2, or 4 SDZ ENA 713 TDS 18 mg/10 cm^2 patches per animal per day for 4 weeks (total dose of 18, 36, and 72 mg/animal/day, respectively, or ~1.9, 3.8, or 7.6 mg/kg/day at ~9.5 kg per animal) resulted in no changes in systemic clinical signs, body weights, food consumption, hematology, or clinical chemistry.
- Minimal to moderate erythema was observed at the application site of all animals except for 2/6 Placebo controls. Severity slightly increased with dose and with the frequency of re-use of application sites (severity was greater in the HD group rotating patches among two distinct sites compared to the HD group rotating patches among 6 distinct sites).
- Minimal to slight dermatitis was observed at the application site in all dose groups, with severity slightly increased in the HD-2 site group vs. other groups.
- Analysis of used patches revealed mean percent of total drug delivered over 24 hours did not change from Day 2 (32.5-73.3%) to Day 26 (34.4-75.6%), and was independent of dose and sex.
- Plasma butyrylcholinesterase activity was reduced dose-dependently up to 39% in HDM, but not in F.

Methods

Göttingen minipigs (N=3/sex/group for Placebo & HD; 4.5-5 months old; 8.1-10.8 kg) were treated with 4 10 cm^2 placebo patches or 1, 2, or 4 SDQ ENA 713 patches (each 18 mg/10 cm^2) per day for 4 weeks, to achieve total doses of 18, 36, or 72 mg/animal/day (~1.9, 3.8, or 7.6 mg/kg/day at ~9.5 kg per animal). Each patch was applied alternately to each of six application sites on the clipped dorsal trunk, so that the same site was used every sixth day; except in a second HD group (Group D) in which the triple patch was alternated between only two application sites, so that each site was used every other day. Patches were covered with ——— adhesive tape, and removed after ~24 hrs. b(4)
Parameters measured included clinical signs, test site appearance, body weights, food consumption, hematology, clinical chemistry, plasma butyrylcholinesterase activity, toxicokinetics (Days 1 and 25 at 0, 2, 6, and 24 hrs post-application), residual drug in used patches (Days 2 and 26), necropsy, organ weights, and microscopic evaluation (42 tissues + gross lesions).

Results

No treatment-related changes were observed in systemic clinical signs, body weights, food consumption, hematology, or clinical chemistry.

Analysis of used patches revealed mean percent of total drug delivered over 24 hours did not change from Day 2 (32.5-73.3%) to Day 26 (34.4-75.6%), and was independent of dose and sex.

Minimal to slight erythema was observed at the application sites in all animals of all dose groups rotating patches among 6 sites, lasting up to 24 hrs after removal of the patch. One MD animal showed moderate erythema from Day 15 onward, and some animals occasionally showed erythema lasting for up to 72 hrs after patch removal. Group D HD animals (rotating patches among only 2 distinct sites) showed minimal to moderate erythema persisting for 24 hrs post-removal; moderate erythema was noted in 1/3 M from Day 11 and 1/3 F from Day 17 in this group. Edema at the test site was not observed in any animal of any group. Severity of skin erythema increased with repeated dosing in individual animals, and with more frequent re-use of applications sites (i.e., Group 5 vs. Group 4 in the table below). The sponsor argues that, since placebo patches induced similar skin irritation, the changes must be due to the patch rather than to the drug substance. However, in this case it appears that the incidence and severity of erythema was slightly increased in the drug-treated groups (Groups 2, 3, and 4 in the table below) compared to the Placebo group (Group 1 in the table below).

Incidence of highest severity grades of skin erythema:

Group (mg/animal/day)	1(0)	2(18)	3(36)	4(72 I)	5(72 II)
Σ Animals	3M/3F	3M/3F	3M/3F	3M/3F	3M/3F
Grade 0	1/1				
Grade 1	2/1	3/3	2/2	3/0	1/
Grade 2	0/1		1*/0	0/3*	1/2
Grade 3			0/1*		1/1
Grade 4					

M = males
F = females
* = erythema persisted 2-3 days
I = 6 application regions
II = 2 application regions

(Page 49 of Study Report)

Plasma butyrylcholinesterase activity was reduced dose-dependently up to 39% in HDM, but not in F.

Minimal to slight dermatitis was observed at the application sites in Placebo and drug-treated groups, with slightly increased severity in Group D vs. other groups. Although clinically observed slight to moderate skin erythema disappeared with 24 to 72 hrs after patch removal, histopathologically observed dermatitis was still apparent at the test site up to 6 days after patch removal. In animals showing moderate erythema at the application site, dermatitis was also observed histopathologically at naïve skin sites, though it was less severe. No other treatment-related findings were observed in the histopathological evaluation.

Summary of the toxicokinetic characteristics of SDZ ENA 713

	Dose mg/animal/day	DAY	MALES Mean	MALES SD	FEMALES Mean	FEMALES SD	M+F Mean	M+F SD
Cmax (ng/ml)	18	1	2.1	0.7	2.1	0.7	2.1	0.6
		25	3.4	0.8	3.7	0.2	3.5	0.5
	36	1	6.4	3.2	4.0	0.9	5.2	2.5
		25	9.6	1.2	8.7	2.5	9.1	1.8
	72(C)	1	7.4	2.5	6.5	1.5	7.0	1.9
		25	16.4	1.7	23.7	5.9	20.0	5.5
	72(D)	1	17.8	9.4	4.6	1.0	11.2	9.4
		25	41.3	16.9	39.1	18.7	40.2	16.0
AUC(0-24h) (h*ng/ml)	18	1	36	13	32	16	34	13
		25	69	16	71	7	70	11
	36	1	104	44	70	23	87	36
		25	177	34	168	40	173	34
	72(C)	1	109	52	107	41	108	42
		25	322	15	430	42	376	66
	72(D)	1	269	126	82	25	176	131
		25	624	115	646	255	633	152

Summary of the toxicokinetic characteristics of ZNS 114-666

	Dose mg/animal/day	DAY	MALES Mean	MALES SD	FEMALES Mean	FEMALES SD	M+F Mean	M+F SD
Cmax (ng/ml)	18	1	1.8	0.9	1.8	0.1	1.8	0.6
		25	3.4	1.5	3.5	0.8	3.5	1.1
	36	1	6.7	3.2	3.6	0.4	5.1	2.6
		25	8.9	4.0	6.9	0.7	7.9	2.8
	72(C)	1	7.3	2.7	6.3	3.9	6.8	3.0
		25	17.3	1.7	19.6	7.6	18.4	5.1
	72(D)	1	17.5	9.1	5.5	0.9	11.5	8.7
		25	43.1	8.6	38.6	14.4	40.8	10.9
AUC(0-24h) (h*ng/ml)	18	1	31	23	23	11	27	17
		25	64	19	63	7	63	13
	36	1	118	50	54	6	86	47
		25	171	70	142	10	156	48
	72(C)	1	115	69	91	63	103	61
		25	363	25	391	106	377	71
	72(D)	1	277	116	90	4	183	126
		25	686	68	624	189	661	111

(Pages 132-135 of Study Report)

SDZ-ENA 713 - A 26-week dermal toxicity study in minipigs

Key study findings:

- Very slight to well-defined erythema was observed at the application site from Week 3 onward in drug-treated groups (18 or 36 mg/day), but not in the placebo patch group. Erythema lasted up to 3 days after patch removal, and frequency and severity increased with the number of patches applied per day (2 > 1) and with the frequency of reapplication to previously used sites (every 6th day > every 12th day).
- No edema was observed, and no correlating histopathological changes were observed at the application site.
- No treatment-related changes were observed in mortality, clinical signs (except erythema), body weight, food consumption, ophthalmoscopy, EKG, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, or histopathology.
- Toxicokinetic analysis showed mean steady state Cmax of 2.4-5.4 ng/mL and $AUC_{0\text{-}24\ hr}$ of 40-90 ng*hr/mL at the HD of two 18 mg 10 cm^2 patches per day, resulting from a mean delivery of 15-23 mg per day (based on assessment of residual drug in the used patches). Metabolite ZNS 114-666 ranged from 0-6.6 ng/mL.

Study no.: ————Lab No. 17727
Volume #, and page #: e-NDA 22-083/5 NonClinical Pharmacology and Toxicology/Toxicology Studies/Repeat-Dose Toxicity/Study Report 17727: A 26-week dermal toxicity study in minipigs/Pages 1-402
Conducting laboratory and location: ———— b(4)
Date of study initiation: Dosing started on 15 MAY 1996
GLP compliance: Yes, GLP statement signed by Peter Brinck, DVM, the Study Director, 06 NOV 1997
QA report: yes (X) no ( ), QA statement signed by Vita Boeck, Head of QA, 06 DEC 1997
Drug, lot #, and % purity: SDZ ENA 713 Batch # X008 0196 and #X087 0496; Placebo Batch #X009 0196 and #X088 0496

Methods

Doses:

Group	Dose (mg SDZ ENA 713/animal/ day	No. of patches applied per day	Dose* (mg SDZ ENA 713/kg b.wt./day)	No. of application sites	Approx. size of each application area (cm²)	Main study animals		4-week post-treatment animals	
						♂	♀	♂	♀
1	0	2	0	12	20	1 - 4	5 - 8	33 - 34	35 - 36
2	18	1	2*	12	10	9 - 12	13 - 16		
3	36	2	4*	12	20	17 - 20	21 - 24		
4	36	2	4*	6	20	25 - 28	29 - 32	37 - 38	39 - 40

* The dose given here is based on a theoretical body weight of 9 kg.

(from Study Report, page 14)

Species/strain: Göttingen SPF minipigs

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: transdermal patch, one or two 10 cm^2 patches per day, rotated among 6 or 12 applications sites on the back; each patch contained 18 mg rivastigmine, and delivered ~50% of it over 23 hrs.

Satellite groups used for toxicokinetics or recovery: 2/sex for 4-wk recovery groups receiving placebo (Group 1) and 2-patch/day/6 sites (Group 4)

Age: 3-4 months old

Weight: 5.6-7.4 kg

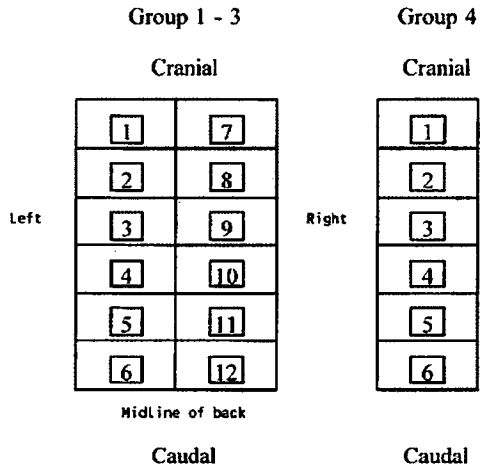
Sampling times: 26 weeks or 30 weeks

Unique study design or methodology: Amount of drug remaining in patch was measured in one patch from each of 4 animals/sex/group on Days 5, 86, and 180.

Administration of patches

Shortly before commencement of application of the patches the hairs were clipped from the application area on the back of the minipigs using an electrical shaver. Repeated clipping took place as required.

In group 1 - 3 one longitudinal row of 6 application sites was marked on the left and right side of the back of the animals. In group 4 one longitudinal row of 6 application sites was marked on the right side of the back of the animal. The distance between application sites was about 2 cm. The application sites were identified by numbers as follows:



The patches were applied on application site No. 1 on the first day of dosing, on site No. 2 on the second day of dosing, etc. Therefore the patches were applied on the same application site every 12 days (group 1 - 3) or every 6 days (group 4).

The test article or placebo patches were held in contact with the skin with tape [redacted] In addition the patch and tape were retained by a net-like body-stocking [redacted] or [redacted] attached to a neck collar.

b(4)

At the end of the 23 hour treatment period the treated area was cleaned gently with soap [redacted] and water and dried.

The animals were treated with the test article or placebo patches for 23 hours (between 22 and 24 hours) each day.

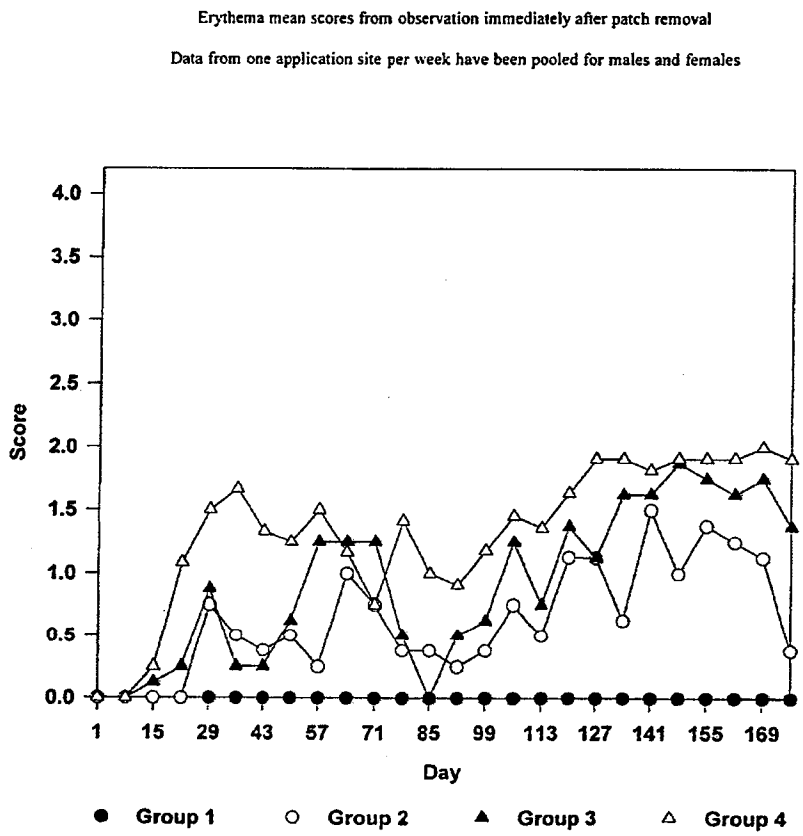
(from Study Report, pages 14-15)

Results

Mortality: (observed daily)
Male #26 (Group 4) was found dead on Day 88, with no prior clinical signs observed. Findings included: swollen, discolored kidneys; discolored urine; hemorrhage in urinary bladder, lungs, heart, and jejunum; infarcts in these and other organs. The cause of death was unknown, but was not considered to be associated with treatment.

Clinical signs: (observed daily)
Intermittent loose stools and/or distension of the abdomen were observed for several weeks in one Group 3 male (Male #18) until Day 125. GI function of this M was stabilized by addition of 100 mL yogurt-like milk product twice daily to the diet and increasing the ration of regular food from Day 111 to Day 135.
Male #34 (Group 1) showed many small red, slightly elevated areas in the skin on Days 74-80.

Skin Reactions: (observed daily)
Very slight (Score 1) to well-defined (Score 2) erythema was observed in all treated groups from Wk 3-26 (highest in Gr 4) but not with placebo.



(from Study Report, page 30)

The erythema was observed for up to 3 days after removal of the patch. No edema was observed.

Body weights: (assessed weekly)
No treatment-related effects were observed.

Food consumption: (estimated daily by weighing remaining food)
No treatment-related effects were observed.

Ophthalmoscopy: (assessed predose, Wk 13, and Wk 26)
No treatment-related effects were observed.

EKG: (assessed predose, Wk 6, Wk 13, and Wk 26)
No treatment-related effects were observed.

Hematology: (assessed predose, Wk 6, Wk 13, Wk 26, and Wk 20; parameters included hemoglobin, red blood cells, hematocrit, mean cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, white blood cells, differential leukocyte count (neutrophils, lymphocytes, eosinophils, basophils, and monocytes; absolute and %), platelet count, reticulocyte count, activated partial thromboplastin time, thrombin time, prothrombin time, and fibrinogen)
No treatment-related effects were observed.

Clinical chemistry: (assessed predose, Wk 6, Wk 13, Wk 26, and Wk 20; parameters included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, gamma-glutamyl transferase, cholesterol, triglycerides, carbamide [urea], creatinine, glucose, Na, K, Ca, Mg, P, Cl, total protein, albumin, alpha 1 and 2 globulins, beta globulin, gamma globulin, and A/G ratio)
No treatment-related effects were observed.

Urinalysis: (assessed predose, Wk 13, Wk 26, and wk 30; parameters included Volume, Specific gravity, creatinine, pH, N-acetyl-β-D-glucosaminase, Color, Protein, Leukocytes, Nitrite, Blood, Glucose, Ketones, Bilirubin, Urobilinogen, and microscopic examination of spun sediment)
No treatment-related effects were observed.

Gross pathology: (upon early death, moribund sacrifice, or scheduled sacrifice at Wk 26 or Wk 30, by exsanguination after i.p. Mebumal® anesthesia; parameters included examination of the tissues *in situ* after opening the cranial, thoracic and abdominal cavities; tissues collected for fixation in phosphate buffered neutral 4% formaldehyde [except testes fixed in Bouin and the eyes **in Davidson's fluid] are listed in the** Histopathology Inventory below)
No treatment-related effects were observed.

Organ weights: (see Histopathology Inventory below for list of organs weighed)
No treatment-related effects were observed.

Histopathology: (Adequate Battery: yes (X); Peer review: yes (X), on 2/sex/group plus selected slides from other animals; see Histopathology Inventory below for list of tissues evaluated; paraffin-embedded tissues were cut at ~4-5 um, and stained with haematoxylin and eosin)

No treatment-related effects were observed.

Toxicokinetics: (Day 1, Wk 13, and Wk 26; 3 mol Li-Heparin stabilized blood, from bijugular trunk predose, and 2, 6, and 24 hrs (1 hr after patch removal at 23 hrs post-application)

Day 1 plasma level of SDZ ENA 713 was below the limit of quantification (0.26 ng/mL) in 3/4 M and 3/4 F in Group 2 (one patch/day); in 1/4 M and 3/4 F in Group 3 (2 patches/day/12 sites; but was detectable in all animals in Group 4 (2 patches/day/6 sites). By Wk 13, parent drug was detectable in plasma of all but 1/4 F in Group 2 and 1/4 M in Group 3. By Wk 26, all surviving animals had detectable levels of parent drug. Mean plasma levels are presented in the table and graphs below:

Rounded Mean Blood Concentrations of SDZ ENA 713 (ng/ml)
After Dermal Administration of SDZ ENA 713

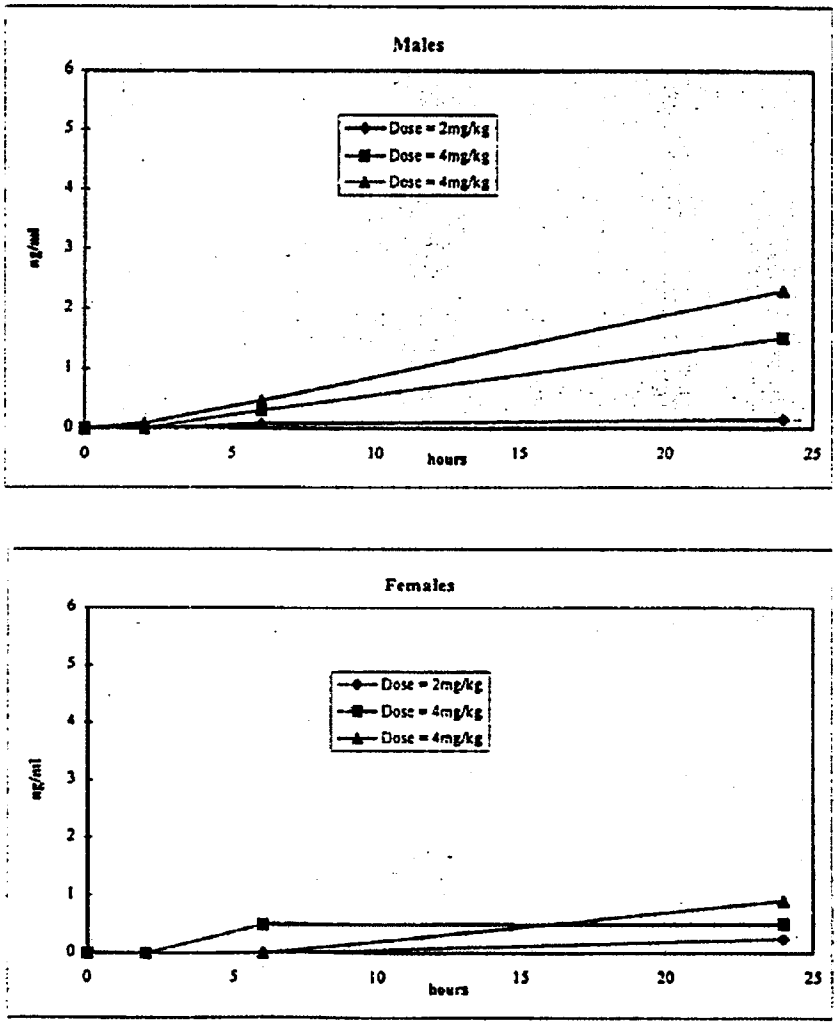
Dose	Time	Day1		Week 13		Week 26	
mg/kg/day	h	Males	Females	Males	Females	Males	Females
	0	0.0	0.0	0.7	0.4	1.4	0.8
2*	2	0.0	0.0	0.7	0.2	0.8	0.5
	6	0.1	0.0	2.2	0.9	0.7	0.6
	24	0.1	0.3	1.2	0.8	1.0	0.7
	0	0.0	0.0	1.4	0.5	1.5	2.5
4*	2	0.0	0.0	0.6	0.4	0.9	1.7
	6	0.3	0.5	0.4	0.5	1.8	1.3
	24	1.5	0.5	0.8	0.8	2.7	2.4
	0	0.0	0.0	1.6	2.8	2.3	2.6
4**	2	0.1	0.0	1.0	1.2	1.5	1.9
	6	0.5	0.0	4.4	5.1	1.7	2.8
	24	2.3	0.9	2.1	3.0	1.6	2.4
	0	0.0	0.0				
4***	2	0.1	0.0				
	6	0.4	0.3				
	24	1.9	0.7				

* 12 sites of application ** 6 sites of application
*** Common results for 4* and 4** on day 1 (one site)

(from Study Report, page 382)

Mean Blood Concentrations of SDZ ENA 713 in Minipigs
After One Dermal Administration of SDZ ENA 713

(Day 1)



(from Study Report, page 384)

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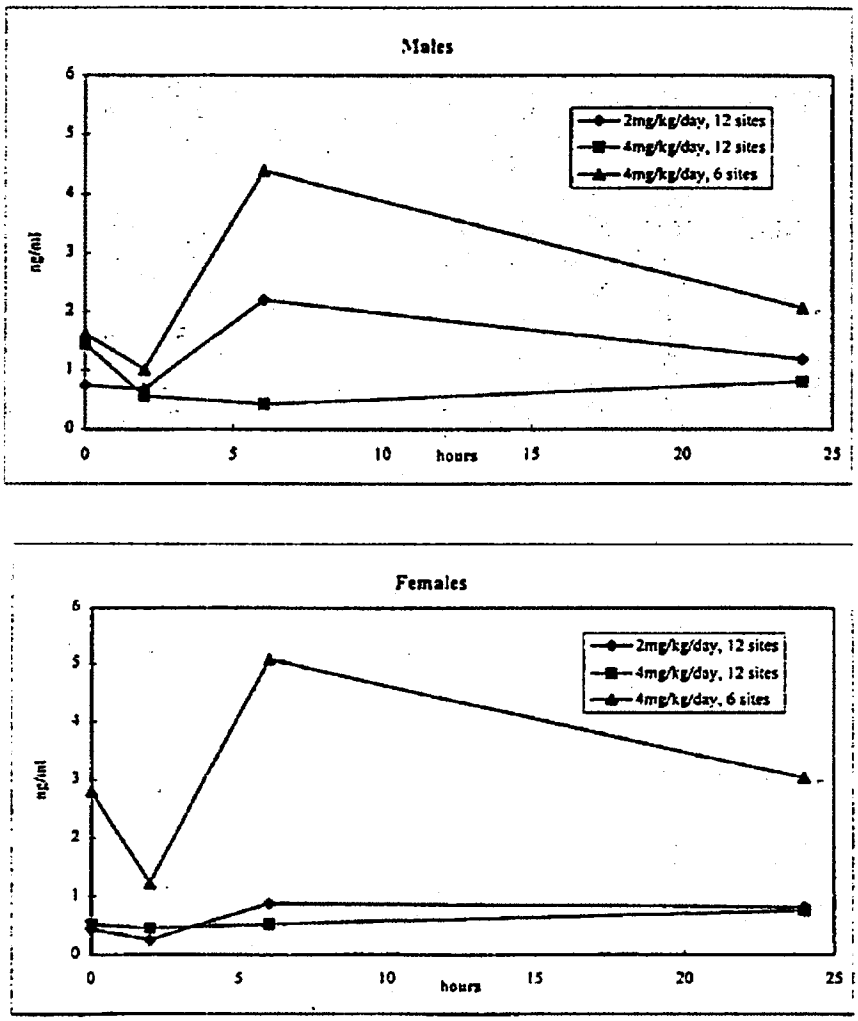
Mean Blood Concentrations of SDZ ENA 713 in Minipigs After Repeated Dermal Administration of SDZ ENA 713

Week 13



(from Study Report, page 385)

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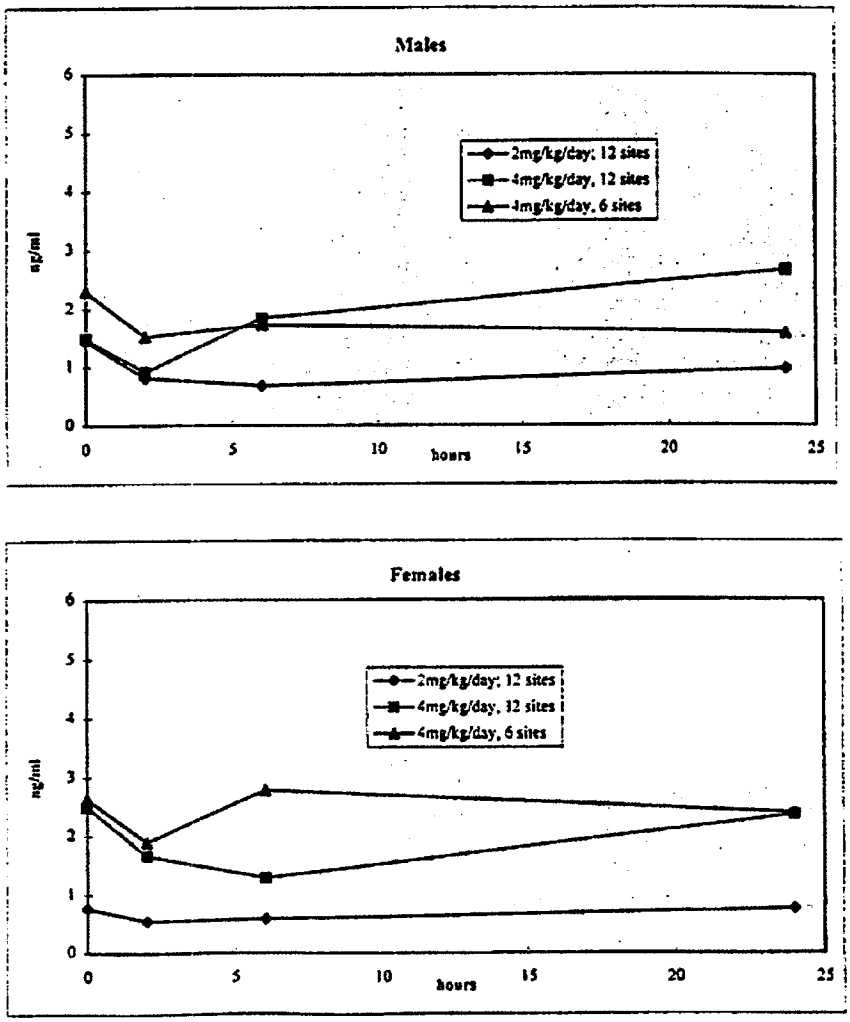
Mean Blood Concentrations of SDZ ENA 713 in Minipigs After Repeated Dermal Administration of SDZ ENA 713

Week 26



(from Study Report, page 386)

The table below shows the mean toxicokinetic parameters (Note: because of the small number of timepoints used per animal, the accuracy of the derived TK parameters is limited). After Day 1, on which absorption of drug was generally lower in F than in M, no consistent gender effects were observed. Parent drug exposure appeared to be maximal by Week 13 in Groups 2 and 4, but not until Week 26 in Group 3.

Rounded Toxicokinetic Parameters (Means and Standard Deviations) of SDZ ENA 713 in Minipigs After Dermal Application of SDZ ENA 713

Parameters	Dose	Day 1				Week 13				Week 26			
		males		females		males		females		males		females	
	mg/kg/day	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
	2	0.1	0.3	0.3	0.5	2.3	3.3	1.0	0.9	1.5	0.2	1.2	0.6
Cmax	4	1.5	1.0	0.5	1.0	1.5	1.1	1.1	0.8	2.9	0.6	2.9	1.0
(ng/ml)	4*	2.3	0.9	0.9	0.4	4.6	6.0	5.4	1.3	2.4	1.3	3.4	2.2
Cmax/dose	2	0.1	0.1	0.1	0.3	1.2	1.7	0.5	0.5	0.7	0.1	0.6	0.3
(ng/ml for a dose	4	0.4	0.3	0.1	0.3	0.4	0.3	0.3	0.2	0.7	0.2	0.7	0.3
of 1 mg/kg/day)	4*	0.6	0.2	0.2	0.1	1.2	1.5	1.3	0.3	0.6	0.3	0.9	0.6
	2	2	4	2	5	37	52	18	16	20	4	15	13
AUC(0-24h)	4	17	12	10	20	15	14	14	13	48	18	43	14
(h·ng/ml)	4*	26	8	8	4	72	92	90	24	40	34	60	44
AUC(0-24h)/dose	2	1	2	1	2	19	26	9	8	10	2	8	6
(h·ng/ml for a dose	4	4	3	3	5	4	3	4	3	12	4	11	4
of 1 mg/kg/day)	4*	6	2	2	1	18	23	22	6	10	9	15	11

*: 6 application sites instead of 12

(from Study Report, page 383)

Blood concentrations of the major metabolite, ZNS 114-666, are presented in the table below; these data were not analyzed further since the levels did not consistently rise above the limit of quantification (2 ng/mL).

Rounded Blood Concentrations [ng/ml] of ZNS 114-666 in Minipigs after Dermal Administration of SDZ ENA 713

Dose mg/kg/day	Week	Minipig	Sex	Time (h)	concentration
2	13	10	M	6	2.3
				24	2.3
		16	F	24	2.3
	26	9	M	0	2.1
4	26	20	M	24	2.3
		23	F	0	3.3
				2	2.1
4*	1	25	M	24	2.8
	13	26	M	6	6.6
				24	2.1
		27	M	24	2.2
		29	F	6	3.1
		30	F	24	2.3
		31	F	0	3.0
		32	F	0	3.1
				2	2.1
				6	3.1
				24	3.9
	26	25	M	24	2.5
		28	M	0	3.5
				2	3.7
				6	3.1
		30	F	0	2.3
		31	F	6	2.6
				24	2.5

*: 6 application sites instead of 12

The concentrations below LOQ (2 ng/ml) are not reported

(from Study Report, page 396)

Other: The amount of drug delivered over 23 hrs was generally ~41-65% of the 18 or 36 mg contained in the patch(es), though individual values varied from 29% to 95%.

Conclusion

Dermal exposure of minipigs to one or two patches (each containing 18 mg rivastigmine) for 23 hrs per day for 26 weeks resulted in no treatment-related toxicity other than very slight to well-defined erythema at the site of application. The erythema began to appear during Weeks 3-4 and lasted up to three days after removal of the patch. The frequency and severity of the erythema appeared to depend on the dose (two patches induced a stronger reaction than one) and on the frequency of re-application to previously used skin sites (every six days induced a stronger reaction than every twelve days). The results in the second HD group indicated that re-use of skin sites every 6 days was tolerable, since the mean erythema score never rose above Grade 2 (well-defined), and no individual animals required changes in frequency of patch re-application.

Evaluation of the residual drug in the used patches revealed that ~41-65% (mean 53%) of the 18 mg in each patch was generally delivered over the 23 hr application period (i.e., the dose delivered was ~9.5 mg per patch per day, the same as the delivery rate reported in humans). Therefore, the high dose groups treated with 2 patches per day received ~19 mg/day, or (since the mean weight increased over 26 wks from ~7 kg to 14 kg per animal) ~2.7-1.4 mg/kg/day, equivalent to a human dose of 79-52 mg/kg/day on a mg/m^2 basis, which is much higher than the recommended human dose of one 10 cm^2 Exelon® Patch 9.5 mg/24 hours per day. However, the mean Week 26 Cmax and AUC rivastigmine exposures in the HD groups (Cmax = 2.9 ng/mL; $AUC_{0\text{-}24\ hr}$ = 50 ng*hr/mL), however, were 0.37 and 0.39 times ***lower***, respectively, than those observed in humans at steady state at the recommended clinical dose of one Exelon® Patch 9.5 mg/24 hours per day (Cmax = 7.9 ng/mL; $AUC_{0\text{-}24\ hr}$ = 127 ng*hr/mL). The mean Week 26 Cmax for the primary metabolite, ZNS 114-666, was 2.8 ng/mL in HD minipigs, compared to 4.0 ng/mL in humans at steady state at the recommended clinical dose of one Exelon® Patch 9.5 mg/24 hours per day. These data indicate that despite identical rates of delivery from the patch to the skin, the systemic absorption of rivastigmine was much more efficient in humans than in minipigs.

The lack of toxicity observed in the high dose groups in this study (other than slight to well-defined erythema) suggests that two 10 cm^2 Exelon® Patch 9.5 mg/24 hours per day does not represent a maximum tolerated dose (MTD) for minipigs. The 4-week minipig studies demonstrated that doses of twelve 10 cm^2 Exelon® Patch 9.5 mg/24 hours per day were limited by occasional severe local reactions at sites that were re-used every day or every other day rather than by systemic toxicity.

Therefore, a maximum feasible dose can be calculated by estimating the approximate skin area available on the minipig for patch application, allowing six sites per 10 cm^2 patch (60 cm^2 total per patch) as the demonstrated highest tolerable frequency of re-use. Minipigs in the weight range of 25-64 kg have a mean total body surface area of approximately 1.14 m^2, or 11,400 cm^2 *(c.f., Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Health Volunteers, CDER, July 2005, page 22, Appendix B, Table 3)*. Assuming, conservatively, that only one tenth of the total body surface area are available for patch application, then 19 patches per day is the maximum feasible dose, since 1,140 cm^2 divided by 60 cm^2 =19. The high dose used in this study, two 10 cm^2 Exelon® Patch 9.5 mg/24 hours per day, is quite a bit lower than the approximate maximum feasible dose of 19 patches per day.

The minipigs used in this study were much younger and smaller (7-14 kg) than the adults (25-64 kg) used for the calculation of the estimated maximum feasible dose. Therefore, the maximum feasible dose for young minipig studies may be lower than 19 patches per animal per day (depending upon the rough estimate that only ~10% of the total body surface area is available for patch application). One 4-week minipig study showed that application of four 10 cm^2 patches per animal per day, rotating among six sites per patch, was feasible.

In conclusion, the repeated once daily application of one or two 10 cm^2 Exelon® Patch 9.5 mg/24 hours per day to minipigs for 26 weeks caused mild skin irritation, but no systemic toxicity. Exposures to rivastigmine at the highest doses tested (2 patches/day) were only ~40% of those in humans at steady state at the recommended clinical dose of one Exelon® Patch 9.5 mg/24 hours per day. The high dose of 2 patches per animal per day was neither a maximum tolerated dose nor a maximum feasible dose.

Histopathology inventory (optional)

Study	17727			
Species	minipig			
Adrenals	*X			
Aorta	X			
Bone Marrow smear				
Bone (femur)	X			
Brain	*X			
Cecum	X			
Cervix	X			
Colon	X			
Duodenum	X			
Epididymis	X			
Esophagus	X			
Eye	X			
Fallopian tube				
Gall bladder	X			
Gross lesions	X			

Harderian gland				
Heart	*X			
Ileum	X			
Injection site				
Jejunum	X			
Kidneys	*X			
Lachrymal gland				
Larynx				
Liver	*X			
Lungs	X			
Lymph nodes, cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	*X			
Pancreas	X			
Parathyroid	X			
Peripheral nerve				
Pharynx				
Pituitary	*X			
Prostate	*X			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	*X			
Sternum	X			
Stomach	X			
Testes	*X			
Thymus	*X			
Thyroid	*X			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	*X			
Vagina	X			
Zymbal gland				

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

No new studies were submitted.

2.6.6.5 Carcinogenicity

Carcinogenicity Study by Dermal Administration in Mice

Key study findings: No treatment-related changes were observed, except for reduced body weight gain (16-17%) in HDF.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The study was considered adequate, and the model was considered appropriate.

Evaluation of tumor findings: No significant differences were observed.

Study no.: 15151 TCS
Volume #, and page #: eNDA Section 5: Nonclinical Pharmacology and Toxicology/Toxicology Studies/Carcinogenicity/Study Report 15151, pages 1-1997
Conducting laboratory and location: [redacted] b(4)
Date of study initiation: 18 FEB 1997
Date of study completion: 28 MAR 2001
GLP compliance: Yes, statement signed by J. Richard, Study Director, 28 MAR 2001
QA report: yes (X) no ( ), statement signed by L. Valette-Talbi, 28 MAR 2001
Drug, lot #, and % purity: SDZ ENA 713 base, Batch # 96912, Purity 99.9% HPLC
CAC concurrence: Neither sought nor given.

Methods

Doses: Untreated Control, Vehicle Control, 0.25, 0.50, and 0.75 mg/kg/day
Basis of dose selection (MTD, MFD, AUC etc.): MTD; dermal application of 1.6 (M) and 2.0 (F) mg/kg/day rivastigmine (50 uL of 1.0 mg/mL in 50% EtOH) induced severe signs (tremors [5/16 F], underactivity [16/16 M, 15/16 F], piloerection [16/16 M, 9/16 F], prostrate posture [13/16 M, 11/16 F], and involuntary back spasms in the region of the test site [2/16 F]) after the first dose, necessitating humane sacrifice of 1/22 M and 2/22 F mice in a 13-week repeat-dose dermal toxicity study in CD-1 mice (Study Report 96/SPM106/0739). Survivors tolerated resumption of dosing at a reduced level (1.2 mg/kg/day in M; 1.5 mg/kg/day in F) after a 3-day dose holiday, but still showed underactivity (15/16 M, 16/16 F), piloerection (16/16 M, 16/16 F), prostrate posture (12/16 M, 9/16 F), hunched posture (3/16 M, 7/16 F), mostly during the first few days, but no tremors. The mid-dose group (0.8 mg/kg/day M, 1.0 mg/kg/day F) showed underactivity (7/16 M, 15/16 F), piloerection (5/16 M, 14/16 F), prostrate posture (2/16 M, 12/16 F), and hunched posture (0/16 M, 3/16 F), mostly during Days 2-3 for M and Days 1-4 for F.
Species/strain: [redacted].CD-1® (ICR) BR mice, COBS-VAF®, from [redacted] b(4)
Number/sex/group (main study): 50/sex/group

Route, formulation, volume: Dermal application in ethyl alcohol of 1.25 mL/kg from Day 1 to 23 (24 for F); or 1.50 mL/kg from Day 24 (25 for F) to end. Hair was clipped from an area of approximately 2 X 3 cm (~6 cm^2) comprising the dorsal retro-scapular region, on both sides of the spinal column, the back and the two flanks. Clipping was repeated as needed, generally once a week, at least two hours prior to treatment. Test article or vehicle control solution was administered to the skin using an adjustable volume pipette fitted with a plastic tip, which was used to spread the solution around the application site. The site was not rinsed or dressed. Negative controls were clipped at the same frequency, but no solution was applied. Volume applied was adjusted on an individual basis according to the most recently recorded body weight. Application volumes were generally lower than 50 uL.

Frequency of dosing: Once daily for 98-99 weeks (682 to 690 days)

Satellite groups used for toxicokinetics or special groups: 24/sex/group TK

Age: Approximately eight weeks

Body weight at initiation: 25.6-33.7 g (M), 19.4-29.0 g (F)

Animal housing: Individually, in polycarbonate cages, w/autoclaved sawdust on floor

Food and water: Free access to food pellets and filtered tap water

Drug stability/homogeneity: Formulations made at 0.167, 0.200, 0.333, and 0.400 mg/mL solutions for up to nine days of treatment, stored at 4°C, protected from light, until delivery to animal room on dry ice for use. Concentration was assayed for samples of each solution in Weeks 1, 4, 13, 26, 39, 52, 64, 78, and 89. All samples were frozen at -20 °C pending dispatch on dry ice to the sponsor for analysis.

Dual controls employed: Yes. Negative (no treatment) and Vehicle (ethyl alcohol).

Interim sacrifices: None

Deviations from original study protocol: *(from page 37 of study report)*

2.11.1 Deviations to treatment/dose-level

. on day 17, the male Q12003 (given 0 mg/kg/day) was treated with an erroneous volume of application (55 µl instead of 47 µl),
. on day 400, males (Q11964 to Q11973) from group 2 (given 0 mg/kg/day) were treated with the formulation of group 3 (i.e. given 0.25 mg/kg/day),
. on days 408 and 409, the female Q12449 from group 3 (given 0.25 mg/kg/day) was treated with an erroneous volume of application (26 µl instead of 35 µl),
. on day 470, the male Q12101 (given 0.5 mg/kg/day) was treated with an erroneous volume of application (46 µl instead of 41 µl),
. on day 457, the female Q12449 (given 0.25 mg/kg/day) was treated with an erroneous volume of application (26 µl instead of 34 µl),
. on day 397, no trace of dosing was available for animal Q12622 (female of the 0.75 mg/kg/day principal group),

2.11.2 Other deviations

. on day 603, the males from groups 2 and 3 were weighed after the treatment application for that day,
. during the study, some cutaneous lesions due to the clipper were observed; these results were noted in the study raw data but are not presented in the study report,
. some animals received a volume of application higher than 50 µl since their body weight exceeded 40 grams,
. the concentration was checked for samples of each solution (including the control) prepared for use in weeks 1, 4, 13, 26, 39, 52, 64, 78 and 89 instead of 1, 4, 13, 26, 39, 52, 65, 78 and 90. Thus throughout analytical report presented in Appendix 2 "week 65" should be replaced by "week 64" and "week 90" should be replaced by "week 89",
. the food consumption was recorded in week 78 instead of week 77 and until week 97 for all surviving animals (first time of final necropsy),
. the racks were not moved rack by rack in a clockwise direction around the room in weeks 39, 83 and 93,
. the apparatus/method used for the MCV determination was [redacted] instead of [redacted] b(4)
. in week 4, the test substance formulations were used for up to nine days after preparation.

2.11.3 Conclusion

These deviations were not considered to have compromised the validity or integrity of the study.

This reviewer concurs with the sponsor's conclusion above.

Observation times

Mortality: At least twice daily.

Clinical signs: At least once daily. Animals were palpated for possible masses every 2 weeks from week 27 on.

Body weights: Weekly

Food consumption: Weekly first 13 weeks, once every 4 weeks until Week 78, then weekly. Food intake per animal was calculated based on the difference between food given and food left in the food-hopper.

Hematology: Weeks 52 and 78 (Differential White Cell count), and at termination (Wk 98-99, complete hematological investigation). The following parameters were evaluated: Erythrocytes (RBC), Haemoglobin (HB), Mean Cell Volume (MCV), Packed Cell Volume (PCV), Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), Thrombocytes (PLAT), Leucocytes (WBC), Differential White Cell count with cell morphology [Neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes (M)], and Reticulocytes (RETIC). *Bone marrow differential cell count (from femoral bone marrow smear) was not determined since no abnormalities were observed in the hematological investigations.*

Toxicokinetics: Blood samples were taken during Weeks 13, 26, and 52 from the TK groups, and during Week 97 (F) or 98 (M) from the main study groups, at 1, 2, 4, 7, and 24 hrs postdose. Plasma levels of parent drug (SDZ ENA 713 base) and the major metabolite (ZNS 114-666) were measured. 3 M and 3 F were sampled per timepoint. Approximately 0.5 mL blood was taken from the orbital sinus under light anesthesia and place in a tube containing lithium heparinate and 10 uL 0.01 M eserine salicylate to avoid possible enzymatic degradation of SDZ ENA 713 base. TK animals were killed after the last sampling in week 52, by carbon dioxide asphyxiation and discarded without necropsy.

Gross Pathology: At termination (Week 98, F, Week 99, M), or upon early death or sacrifice. Scheduled sacrifice was performed by carbon dioxide asphyxiation and exsanguination after at least 14 hours of fasting. Complete macroscopic examinations were performed on all animals.

Histopathology: Peer review: yes (X), no ( )
Macroscopic lesions and tissues specified in the Tissue Procedures Table (see below), were preserved in 10% buffered formalin (except for eyes with Harderian glands, which were fixed in Davidson's fixative; and testes and epididymides, which were preserved in Bouin's fluid), embedded in paraffin was, sectioned at ~4 microns thickness, and stained with hematoxylin-eosin. Embedding, sectioning, and staining was performed by [redacted] Microscopic examination was performed on all sampled tissues (except tongue and carcass) from all animals from all control and treated groups by the Principal Investigator, Dr. K. Yoshitomi, and the Peer Reviewer, Dr. V. Pace, both employed by the sponsor. b(4)

TISSUE PROCEDURES TABLE

Organs	Preservation of tissue	Microscopic examination
Macroscopic lesions	X	X
Adrenals	L+R	L+R
Aorta	X	X
Brain (including medulla/pons cerebrum and cerebellum)	X	X
Caecum	X	X
Carcass	X	
Colon	X	X
Duodenum	X	X
Epididymides	L+R	L+R
Eyes with Harderian glands	L+R	L+R
Femoral bone with articulation	X	X
Gall bladder	X	X
Heart	X	X
Ileum	X	X
Jejunum	X	X
Kidneys	L+R	L+R
Lacrymal glands	L+R	L+R
Liver	X	X
Lungs with bronchi	X	X
Lymph nodes axillary	X	X
Lymph nodes mandibular	X	X
Lymph nodes mesenteric	X	X
Lymph nodes regional to masses	X	X
Lymph nodes tracheobronchial	X	X
Mammary glands	X	X
Nasal turbinates	X	X
Oesophagus	X	X
Ovaries	L+R	L+R
Pancreas	X	X
Pituitary gland	X	X
Prostate	X	X
Rectum	X	X
Salivary glands (sublingual and submaxillary)	X	X
Sciatic nerve	X	X
Seminal vesicles	L+R	L+R
Skeletal muscle	X	X
Skin (one control site + one treated site)	X	X
Spinal cord (cervical, thoracic and lumbar)	X	X
Spleen	X	X
Sternum with bone marrow	X	X
Stomach with forestomach	X	X
Testes	L+R	L+R
Thymus	X	X
Thyroids with parathyroids	L+R	L+R
Tongue	X	
Trachea	X	X
Urinary bladder	X	X
Uterus (horns and cervix)	X	X
Vagina	X	X

X: action was undertaken L: left R: right

(from page 33 of study report)

The list above omits the following tissues listed in the standard Histopathology Inventory: cervix, Fallopian tubes, larynx, cervical lymph nodes, optic nerve, pharynx, and Zymbal gland.

Results

Formulations analysis: Samples were within ± 12% of targeted concentration, and test compound was not detected in placebo formulations.

Mortality: No treatment-related differences in mortality or morbidity were observed. The most common cause of death/moribundity for control and treated mice in both sexes was systemic amyloidosis (primarily the small and large intestine, stomach, salivary glands, heart, kidney, spleen, and/or liver), which is a spontaneous, age-related finding common in CD-1 mice.

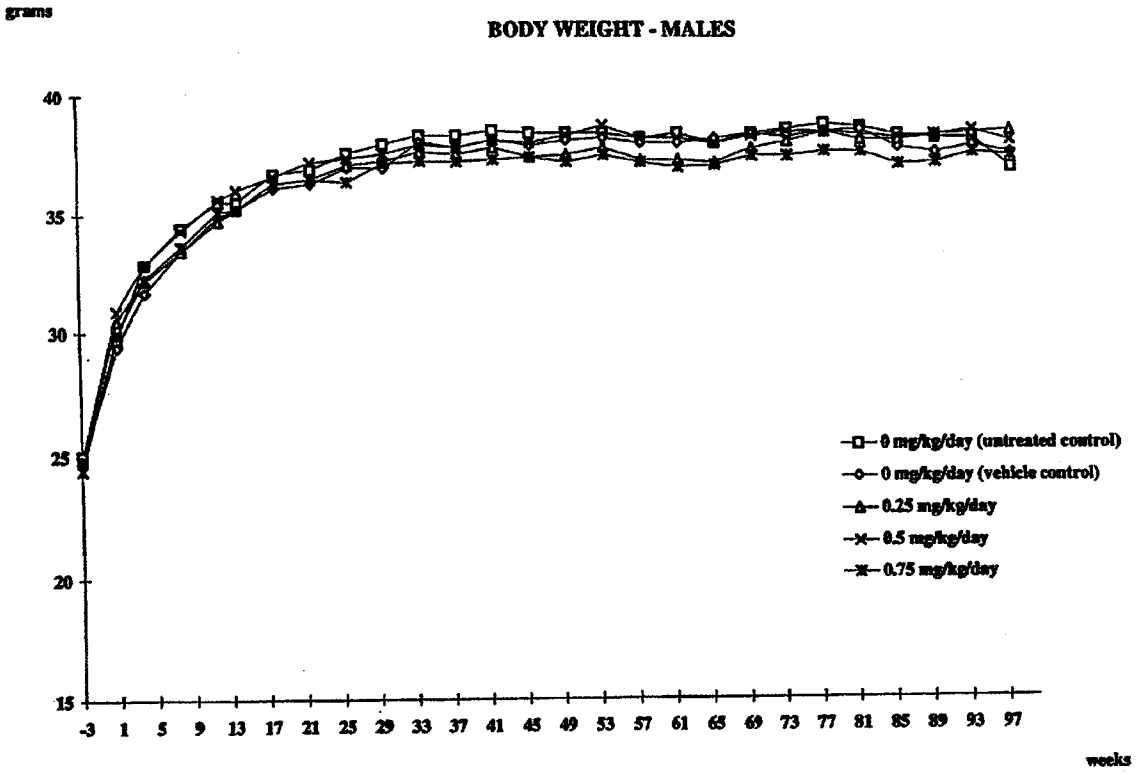
Survival rates (expressed in %), fate (number of animals) and mean duration of treatment (weeks)

Dose-levels (mg/kg/day)	untreated	0	0.25	0.50	0.75
Males					
SR after 52 weeks	96	98	90	96	96
SR after 78 weeks	86	90	84	88	78
SR at study termination (week 99)	48	58	66	50	52
Fate:					
- found dead (FD)	17	12	9	15	16
- killed prematurely (KP)	9	9	8	10	8
Ratio FD/KP	1.9	1.3	1.1	1.5	2.0
Mean duration of treatment	91	91	90	91	88
Females					
SR after 52 weeks	92	96	92	96	96
SR after 78 weeks	78	82	84	80	82
SR at study termination (week 99)	50	70	54	58	68
Fate:					
- found dead (FD)	16	9	14	13	11
- killed prematurely (KP)	9	6	9	8	5
Ratio FD/KP	1.8	1.5	1.6	1.6	2.2
Mean duration of treatment	86	91	89	90	90

(from page 38 of study report)

Clinical signs: No treatment-related differences were observed between treated and control groups, or between untreated and vehicle control groups; no systemic or local reactions were noted.

Body weights: The mean body weight gain of untreated and vehicle control groups was similar throughout the treatment period for both males and females. No noteworthy differences in the mean body weight gain of any treated male group or of the 0.25 and 0.50 mg/kg/day female groups compared to the control groups. The high dose female group (0.75 mg/kg/day), however, showed a treatment-related reduction in body weight gain reaching 16% (compared to untreated controls) or 17% (compared to vehicle controls) from Week 1 to 97. This group showed a slight decrease in body weight gain during the first 26 weeks of treatment, and then slight body weight loss during the last 17 weeks of treatment. The mean final body weight at Week 98 was reduced 3.9% in HDF compared to vehicle control F, but this was not considered significantly different at the $P<0.05$ level. The reduction in body weight gain observed in the HDF group did not correlate with lower mean food consumption.



(from page 52 of study report)

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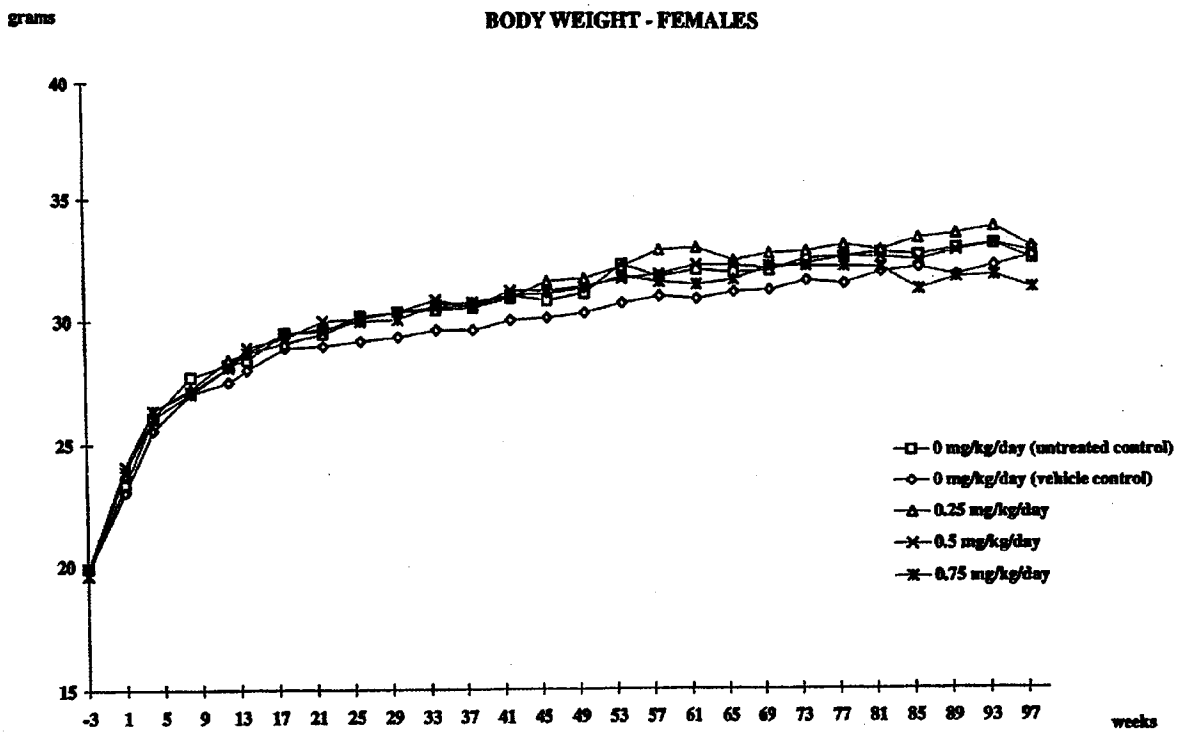

(from page 53 of study report)

Food consumption: No test article-related differences in mean food consumption were observed in this study. In females, the untreated control group showed slightly higher (10%) mean food consumption compared to the vehicle control group (perhaps due to reduced handling of the untreated controls), and the LDF and MDF groups showed slightly higher (3-8%) mean food consumption compared to the vehicle control group, but these changes were not dose-related.

Hematology: No toxicologically important treatment-related differences were observed. HDM mice showed a higher mean neutrophil percentage (+29%) and a lower mean lymphocyte percentage (-22%) compared to vehicle controls, but these differences were not considered to be of toxicological importance since the were not found at later time points (Wks 78 and 98/99), and since all individual values were within or close to the normal range of historical background values. This reviewer concurs.

Gross pathology: No treatment-related differences in the onset, incidence, location, or morphological type of palpable masses were observed in this study. Likewise, no indication of treatment or dose-relationship was noted in the size, number, or distribution of the masses and nodules found in some organs and tissues upon necropsy.

Palpable masses (number of animals)

Dose-levels (mg/kg/day)	untreated	0	0.25	0.50	0.75
Males					
. Number of animals in study	50	50	50	50	50
. Number of palpable masses*	1	1	0	1	0
. Number of animals bearing palpable masses	1	1	0	1	0
. Mean per animal	1	1	0	1	0
. Week of appearance of the first mass	65	77	-	98	-
Females					
. Number of animals in study	50	50	50	50	50
. Number of palpable masses*	2	0	3	1	4
. Number of animals bearing palpable masses	2	0	3	1	2
. Mean per animal	1	0	1	1	2
. Week of appearance of the first mass	25	-	43	87	57

*: confirmed as neoplastic lesions at microscopic examination

(from page 40 of study report)

Histopathology:

Non-neoplastic: No treatment-related non-neoplastic findings were observed. All findings observed occurred in both control and treated groups approximately equally and/or occurred with an incidence and severity commonly observed in aging CD-1 mice. No differences were seen in the skin from the site of application in treated groups compared to control groups, or between the untreated and vehicle control groups.

Neoplastic: No treatment-related neoplastic findings were observed. The incidence and types of tumors observed occurred in both control and treated groups approximately equally and/or occurred with an incidence and severity commonly observed in aging CD-1 mice.

The Statistical Reviewer concurred with the sponsor that no treatment-related neoplastic findings were observed: "**Tests showed no** statistically significant dose response in the incidence of any tested tumor types (individual tumor types and combinations of tumor types suggested by the reviewing pharmacologist) with respect to the untreated control or the vehicle control.

In pairwise comparisons none of the tested tumor types showed statistically significant increased incidence in the high dose group compared to the untreated control or the vehicle **control in the tested tumor types.**" *(page 7 of the Statistical Review and Evaluation of the mouse dermal carcinogenicity study [Study 15151 TCS] in NDA 22-083)*

Note: The summary tables below report the number of animals with neoplastic lesions by organ/group/sex; the study report did not include a summary of the number of tumors.

PATHOLOGY REPORT — PAGE : 11
SUMMARY TABLES — STUDY NO. : 15151 TCS

TEST ARTICLE : ENA 713 BASE — *PATHOL. NO.*: 15151 KAT
TEST SYSTEM : MOUSE, CARCINOGENICITY, DERMAL — *DATE* : 24-OCT-00
SPONSOR : NOVARTIS PHARMA AG, BASEL — *PathData® System V5.1b*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	1	2	3	4	5	
NO.ANIMALS:	50	50	50	50	50	
FORESTOMACH :	49	48	49	50	49	
- Squamous Carcinoma :	-	-	-	1	-	
FEMORAL BONE(+ART.) :	50	50	50	50	50	
- Hemangiosarcoma :	-	-	-	1	-	
GALL BLADDER :	46	46	43	48	46	
- Adenoma :	-	4	2	-	1	
LIVER :	50	50	50	50	50	
- Hemangioma :	-	1	-	-	-	
- Hemangioma,Multiple :	-	-	-	-	1	
- Hemangiosarcoma :	-	-	-	1	-	
- Hptcl Adenoma :	4	5	5	5	3	
- Hptcl Adenoma,Mtpl :	3	1	-	1	-	
- Hptcl Carcinoma :	1	2	2	2	2	
- Hptcl Carcinoma,Mtpl:	-	1	-	1	-	
PITUITARY GLAND :	50	49	50	48	50	
- Adenoma,Intermedia :	-	1	-	-	-	
STERNUM (+ B.M.) :	50	50	50	50	50	
- Hemangiosarcoma :	-	-	-	1	-	
THYROID GLANDS :	50	50	50	50	50	
- Adenoma,F.Cell :	1	-	-	-	-	
ADRENAL GLANDS :	50	50	50	50	50	
- Adenoma,Subcap.Cell :	1	-	-	-	1	
- Adenoma,Cortical :	-	-	-	-	1	
PANCREAS :	50	50	50	50	50	
- Adenoma,Islet Cell :	-	-	1	-	-	

(from page 134 of study report)

PATHOLOGY REPORT PAGE : 12
SUMMARY TABLES STUDY NO. : 15151 TCS

TEST ARTICLE : ENA 713 BASE *PATHOL. NO.*: 15151 KAT
TEST SYSTEM : MOUSE, CARCINOGENICITY, DERMAL *DATE* : 24-OCT-00
SPONSOR : NOVARTIS PHARMA AG, BASEL *PathData® System V5.1b*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						MALE
DOSE GROUP:	1	2	3	4	5	
NO.ANIMALS:	50	50	50	50	50	
SPLEEN :	50	50	50	50	50	
- Hemangiosarcoma :	1	1	-	1	-	
THYMUS :	46	49	47	45	48	
- Carcinoma,Thyroid :	-	-	1	-	-	
TRACHEOBRONCHIC L.N. :	28	25	28	25	29	
- Metastasis,Carcinoma:	1	-	-	1	2	
LUNGS WITH BRONCHI :	50	50	50	50	50	
- Metastasis,Osteosarc:	-	1	-	-	-	
- A/B Adenoma :	8	7	6	8	9	
- A/B Adenoma,Multiple:	2	1	1	1	2	
- A/B Carcinoma :	8	5	4	5	7	
- A/B Carcinoma,Mtpl :	1	1	1	1	-	
TESTES :	50	50	50	50	50	
- Hemangioma :	-	-	-	-	1	
- Adenoma,Interst.Cell:	3	2	5	-	2	
HARDERIAN GLANDS :	50	50	50	50	50	
- Adenoma :	3	4	2	3	1	
HEMOLYMPHORET. SYS. :	3	1	4	5	3	
- Lymphoma,Malignant :	3	1	4	3	2	
- Histiocytic Sarcoma :	-	-	-	2	1	
SKIN :	8	5	1	9	5	
- Hemangioma :	-	-	-	1	-	
- Osteosarcoma :	-	1	-	-	-	

(from page 135 of study report)

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SUMMARY TABLES — STUDY NO. : 15151 TCS

TEST ARTICLE : ENA 713 BASE — *PATHOL. NO.*: 15151 KAT
TEST SYSTEM : MOUSE, CARCINOGENICITY, DERMAL — *DATE* : 24-OCT-00
SPONSOR : NOVARTIS PHARMA AG, BASEL — *PathData® System V5.1b*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	1	2	3	4	5	
NO.ANIMALS:	50	50	50	50	50	
HEART :	50	50	50	50	50	
- Metastasis,A/B Carc.:	-	-	-	1	-	
MAMMARY GLAND(S) :	50	49	50	50	49	
- Adenocarcinoma :	2	-	-	-	-	
- Adenocarcinoma,Mtpl :	-	-	-	-	1	
BRAIN :	50	49	50	50	50	
- Meningioma :	1	-	-	-	-	
FORESTOMACH :	50	49	48	50	50	
- Squamous Papilloma :	-	-	-	1	-	
GALL BLADDER :	39	44	48	50	45	
- Adenoma :	-	-	-	-	1	
LIVER :	50	50	50	50	50	
- Metastasis,M.F.H. :	-	-	-	1	-	
- Hemangioma,Multiple :	-	-	1	-	-	
- Hemangiosarcoma,Mtpl:	-	-	-	-	1	
- Hptcl Adenoma :	-	2	1	1	-	
PITUITARY GLAND :	49	48	50	50	50	
- Adenoma,Intermedia :	-	-	-	-	1	
- Adenoma :	4	1	1	-	-	
- Adenoma,Multiple :	-	-	-	1	-	
ADRENAL GLANDS :	50	49	50	50	50	
- Pheochromocytoma,Bgn:	-	-	-	1	-	
OVARIES :	50	49	50	50	50	
- Luteoma :	-	1	2	-	-	
- Granulosa Cell Tumor:	-	-	1	-	-	
- Adenoma,Tubulostrom.:	-	-	-	1	-	

(from page 136 of study report)

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SUMMARY TABLES — STUDY NO. : 15151 TCS

TEST ARTICLE : ENA 713 BASE — *PATHOL. NO.*: 15151 KAT
TEST SYSTEM : MOUSE, CARCINOGENICITY, DERMAL — *DATE* : 24-OCT-00
SPONSOR : NOVARTIS PHARMA AG, BASEL — *PathData® System V5.1b*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	1	2	3	4	5	
NO.ANIMALS:	50	50	50	50	50	
UTERUS :	50	50	50	50	50	
- Leiomyoma :	2	1	-	-	-	
- Leiomyosarcoma :	1	-	-	-	1	
- Hemangiosarcoma :	-	1	-	3	-	
- Stromal Polyp :	4	6	5	5	2	
- Stromal Polyp,Mtpl :	2	1	-	-	1	
- Stromal Sarcoma :	-	1	2	2	1	
- Adenocarcinoma :	-	-	1	-	-	
CERVIX :	48	50	50	48	50	
- Metastasis,Carcinoma:	-	-	1	-	-	
- Leiomyosarcoma :	-	-	-	1	-	
- Leiomyoma :	1	1	-	2	1	
- Stromal Sarcoma :	-	1	1	1	1	
- Stromal Polyp :	1	3	1	1	2	
SPLEEN :	50	49	50	50	50	
- Hemangiosarcoma :	-	-	-	1	-	
LUNGS WITH BRONCHI :	50	50	50	50	50	
- A/B Adenoma :	4	6	5	2	4	
- A/B Adenoma,Multiple:	1	1	2	1	-	
- A/B Carcinoma :	2	5	4	4	2	
- A/B Carcinoma,Mtpl :	2	-	-	-	-	
KIDNEYS :	50	50	50	50	50	
- Metastasis,Carcinoma:	-	-	1	-	-	
HARDERIAN GLANDS :	50	49	50	50	50	
- Adenoma :	4	3	3	3	3	
NASAL TURBINATES :	50	49	50	50	50	
- Metasta.,Meningioma :	1	-	-	-	-	

(from page 137 of study report)

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SUMMARY TABLES STUDY NO. : 15151 TCS

TEST ARTICLE : ENA 713 BASE *PATHOL. NO.*: 15151 KAT
TEST SYSTEM : MOUSE, CARCINOGENICITY, DERMAL *DATE* : 24-OCT-00
SPONSOR : NOVARTIS PHARMA AG, BASEL *PathData® System V5.1b*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

SEX :						FEMALE
DOSE GROUP:	1	2	3	4	5	
NO.ANIMALS:	50	50	50	50	50	
ORAL CAVITY :	3	3	1	2	4	
- Squamous Carcinoma :	-	-	-	-	1	
RIB(S) :	-	-	-	1	-	
- Metastasis,A/B Carc.:	-	-	-	1	-	
HEMOLYMPHORET. SYS. :	5	9	8	7	6	
- Lymphoma,Malignant :	3	7	5	5	6	
- Histiocytic Sarcoma :	2	2	3	2	-	
ILIAC LYMPH NODES :	2	5	6	3	5	
- Metastasis,Carcinoma:	-	-	1	-	-	
SKIN :	9	5	9	8	5	
- Fib.Histiocytoma,Mal:	-	1	1	1	-	
- Osteosarcoma :	1	-	1	-	-	
- Sarcoma,NOS :	-	-	1	-	-	
- Squamous Papilloma :	-	-	-	1	-	
- Squamous Carcinoma :	-	1	-	-	-	

(from page 138 of study report)

Appears This Way
On Original

Toxicokinetics: The mean exposures to the test compound SDZ ENA 713 (rivastigmine) and its major metabolite, ZNS 114-666, increased roughly in proportion to dose. The mean $AUC_{0\text{-}24\ hr}$ values for rivastigmine in HDM (56.15 ng*hr/mL) and HDF (42.62 ng*hr/mL) were only 44% and 28%, respectively, those observed in AD patients receiving Exelon® Patch 9.5 mg/24 hour (rivastigmine $AUC_{0\text{-}24\ hr}$ = 127 during application of the 18 mg 10 cm^2 24-hr patch). Similarly, the mean $AUC_{0\text{-}24\ hr}$ values for ZNS 114-666 in HDM (36.34 ng*hr/mL) and HDF (53.16 ng*hr/mL) were only 48% and 70%, respectively, of those observed in AD patients receiving Exelon® Patch 9.5 mg/24 hour (ZNS 114-666 $AUC_{0\text{-}24\ hr}$ = 75.5 during application of the 18 mg 10 cm^2 24-hr patch). *(see pages 57-58 of Study ENA713D 2331)*

Toxicokinetic parameters of SDZ ENA 713 base

Dose-levels (mg/kg/day)	0.25		0.50		0.75	
Sex	M	F	M	F	M	F
Week 13						
$AUC_{(0\text{-}24h)}$	5.10	6.34	25.95	19.55	43.14	39.21
C_{max}	1.50	1.34	2.98	3.96	7.04	8.58
Week 26						
$AUC_{(0\text{-}24h)}$	11.32	4.37	24.66	17.63	43.82	35.17
C_{max}	1.44	1.89	4.18	4.42	7.03	6.81
Week 52						
$AUC_{(0\text{-}24h)}$	11.16	5.47	29.20	28.07	47.48	38.63
C_{max}	1.17	1.36	5.39	5.75	6.92	5.31
Week 97-98						
$AUC_{(0\text{-}24h)}$	15.29	10.60	36.47	25.60	56.15	42.62
C_{max}	2.03	1.17	6.18	3.52	5.97	5.16

****: week 97 (female mice) or week 98 (male mice).**
$AUC_{(0\text{-}24h)}$ in ng.h/mL C_{max} in ng/mL

Toxicokinetic parameters of ZNS 114-666

Dose-levels (mg/kg/day)	0.25		0.50		0.75	
Sex	M	F	M	F	M	F
Week 13						
$AUC_{(0\text{-}24h)}$	9.46	12.47	23.11	24.71	28.75	42.74
C_{max}	1.59	2.50	2.82	4.56	4.91	6.91
Week 26						
$AUC_{(0\text{-}24h)}$	5.11	5.25	17.91	24.46	33.65	44.12
C_{max}	1.47	2.33	2.45	3.44	5.20	6.68
Week 52						
$AUC_{(0\text{-}24h)}$	*	*	21.38	33.89	29.00	45.36
C_{max}	*	*	3.16	6.58	5.03	7.98
Week 97-98						
$AUC_{(0\text{-}24h)}$	8.69	13.96	24.15	35.19	36.34	53.16
C_{max}	1.46	1.47	3.20	5.38	3.92	6.31

***: ZNS 114-666 was not detected, most likely due to a technical problem during sample preparation.**
****: week 97 (female mice) or week 98 (male mice).**
$AUC_{(0\text{-}24h)}$ in ng.h/mL C_{max} in ng/mL

(reproduced from page 16 of Study Report 15151)

Conclusions:

The sponsor concluded that the daily administration of SDZ ENA 713 (rivastigmine) base for 98-99 weeks by dermal application to mice at 0.25, 0.50, or 0.75 mg/kg/day resulted in slightly reduced body weight gain and (during the final 17 weeks) slight body weight loss in females at 0.75 mg/kg/day, but did not show a carcinogenic potential or any effect on the incidence or severity of any microscopic or macroscopic findings, including **tumors. The sponsor's No Effect Level for carcinogenic effects in mice was 0.75** mg/kg/day, which was associated with a mean Cmax of 5.16-8.58 ng/mL and a mean $AUC_{0\text{-}24\ hr}$ of 35.17-56.15 ng*hr/mL.

The Statistical Reviewer concurred with the sponsor that no treatment-related neoplastic findings were observed in this study. This reviewer concurs with the sponsor and the Statistical Reviewer. The study appears to have been adequately performed.

One point of concern is that the systemic exposures achieved at the high dose in mice were quite low compared to those observed at steady state in AD patients given Exelon® Patch 9.5 mg/24 hour (18 mg/10 cm^2 24-hr patch): $AUC_{0\text{-}24\ hr}$ = 28-44% of human for parent drug, and 20-29% for the major metabolite, ZNS 114-666. The reduction in body weight gain of 16-17% in HDF compared to controls argues that this dose is close to a maximum tolerated dose (MTD), despite the apparent lack of toxicity. Stronger evidence that 0.75 mg/kg/day is reasonably close to an MTD comes from the results of the 13-week mouse study in which even a single dose of rivastigmine applied dermally at 1.6 (M) or 2.0 mg/kg was not tolerated; severe signs included underactivity, piloerection, prostrate posture, tremors (F only), and back spasms (F only), and 1/22 M and 2/22 F had to be sacrificed moribund. The 13-week study also showed that a reduced HD of 1.2 (M) and 1.5 (F) mg/kg/day dermal rivastigmine was well tolerated for the duration of the treatment period, after initially inducing signs of underactivity, piloerection, prostrate posture, hunched posture during the first week.

In conclusion, though the MTD for dermally applied rivastigmine in mice is probably closer to 1.2 mg/kg/day in M and 1.5 mg/kg/day in F, the high dose of 0.75 mg/kg/day used in the present 98-99 week study is a reasonable fraction of the lethal dose (47% in M, 38% in F).

Recommendations

This study should be considered adequate. Rivastigmine was negative for carcinogenicity in mice up to 0.75 mg/kg/day applied dermally for 98-99 weeks. However, the labeling should clearly state that the highest dose tested yielded mean plasma exposures (AUC) of rivastigmine only 0.28-0.44 times as high as those expected in patients administered the highest recommended clinical dose (Exelon® Patch 9.5 mg/24 hour).