Table 23 Effects of SDZ ENA 713 on postnatal development in rat pups (F_2) of offspring (F_1) of the treated dams (F_0)

Dose (mg/kg/day)	0	0.4	1.3	4
No. of dams examined (F_1)	10	10	8	11
Separation of ear auricle (on day 4)	78 / 78[a)]	80 / 80	57 / 57	86 / 86
Appearance of dorsal hair (on day 4)	78 / 78	80 / 80	57 / 57	86 / 86
Eruption of upper incisors (on day 14)	78 / 78	80 / 80	52 / 52	86 / 86
Separation of eyelids (on day 17)	78 / 78	80 / 80	52 / 52	86 / 86

a) : No. of developing pups / No. of pups examined

SEGMENT II REPRODUCTION IN RABBITS:

A) Dosage

18 F at 0, 0.6, 1.8, and 3.6 mg/kg/day, by gavage

(Doses given as hydrogen tartrate salt. Doses as free base = 0.4, 1.1, and 2.2 mg/kg).

Dosing was from days 7-19 of gestation. (Day of successful mating = day 0 of gestation). Does sacrificed on day 30 of gestation. Fetuses were examined externally, viscerally (by dissection "unless a particular finding suggested that more useful data would be obtained by fixing the specimen in Bouin's fluid for later dissection"; dead fetuses "as a rule" were fixed in Bouin's and "examined by cross - sectioning and dissecting with a razor blade") and skeletally (Alizarin Red - S). (Attached page shows numbers of pregnant does, and numbers of fetuses examined, in each group.)

Strain: New Zealand White

Drug lot #: 88902

B) Results

1) Observed signs in does

Narrative results only. It was stated that the following were seen in most HD and in "a few" MD: ataxia, tactile hypersensitivity, aggressiveness, tremors, increased/decreased locomotor activity, kicking/thumping, elevated abdomen, licking, chomping/chewing, lacrimation, loose stool. No drug-related signs were said to have occurred at LD.

2) Doe mortality

No drug effect. (One control and 1 LD died, associated with abortion. Two LD and 1 HD were sacrificed following abortion. One MD "appeared to have immobile or paralyzed hind limbs" on day 8 of gestation and was sacrificed; thought to be due to accidental spinal fracture.) (In a range finding study, 4 of 5 pregnant rabbits died at 12 mg/kg/day; no deaths at the next lowest dose of 6 mg/kg/day).

3) Doe bodyweight

Slight decrease at HD. (During treatment period, HD lost 3.8% of weight compared to a loss of 0.5% in controls and gains of 0.9% and 1.7% in LD and MD, resp.). Weight gain slightly increased at HD during days 19-30 of gestation. No drug effect on gravid uterus weight (day 30 of gestation).

4) Doe food consumption

Not measured.

5) Reproductive parameters

Results shown in attached table 5. The text notes a slight increase in resorptions and post-implantation loss at HD. However, note that the increase were relatively slight in magnitude (e.g. total resorptions and post-implantation loss less than 1.5x control), were not statistically significant, and values were within the historical control range (with the implied [in the text] exception of the incidence of litters with at least 1 or more resorptions, which was 75% at HD compared to a historical mean of 49%, although the historical range was not given). Also note that no increase in resorptions was seen in the range finding study at higher doses (although in that study does were sacrificed earlier, i.e. day 20 of gestation). Although the text concludes that the slight increase in resorptions "was probably related to ... maternal toxicity" this conclusion may be questioned on the grounds of a lack of such an increase in the range finding study despite the use of higher doses causing more pronounced maternal toxicity.

There were no drug effects on numbers of live or dead fetuses, or on fetal weight. (Table 5).

6) Fetal exams

No drug effects. (# of fetuses examined shown in attached table cited earlier).

IV. METHODS

A. EXPERIMENTAL DESIGN

The purpose of this investigation was to detect possible effects on developing rabbit fetuses after oral administration of various dose levels of ENA 713 to pregnant rabbits during the period of Days 7 through 19 of gestation.

The following table summarizes groups, doses, female animal identification, and numbers of animals (and fetuses) evaluated:

Group	Control	Low	Mid	High
Dose (mg/kg/day)[a]	0	0.6	1.8	3.6
Triturated Dose (mg/kg/day)[b]	32.4	6	18	36
Animal No. 89- (female-odd numbers)	3237-3271	3273-3307	3309-3343	3345-3379
# Animals Mated	18	18	18	18
# Animals Pregnant	14	15	18	17
# Pregnant Animals Examined at Day 30	13	12	17	16[d]
Fetal Exam (Day 30 Necropsy)				
Total # external exam[c]	95	116	130	113[d]
Total # skeletal exam - Alizarin Red-S	94	111	117	106
Total # visceral exam	94	111	117	107
# alcohol fixed	94	111	117	106
# Bouin's fixed	0	0	0	1
Total # fetuses not suitable for skeletal or visceral exam (e.g. late resorptions)	1	5	13	6[d]

[a]These salt dose levels represent equivalent base dose levels of 0.36, 1.09 and 2.18 mg/kg/day for the Low, Mid and High dose groups, respectively, (using salt factor of 1.65).

[b]The test article ENA 713 was triturated with lactose in a ratio of the 1:10 to facilitate mixing. The Control group animals received lactose in vehicle (1% CMC/0.2% Tween 80) at a concentration equivalent to the High dose group.

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[c]Includes late resorptions.

[d]Includes High dose group animal 89-3379 which delivered early on Day 30 prior to scheduled necropsy.

TABLE 5

INVESTIGATION OF TERATOGENIC POTENTIAL OF ENA 713 (212-713)
IN THE RABBIT - SEGMENT II
SANDOZ PROJECT T-2620

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

	DOSAGE	0 MG/KG /DAY	0.6 MG/ KG/DAY	1.8 MG/ KG/DAY	3.6 MG/ KG/DAY
Viable Fetuses	N	94	111*	117	101
	%	86	96	87	80
	MEAN	7.2	9.3	6.9	6.7
	S.D.	3.3	1.9	2.7	3.3
Viable Male Fetuses	N	38	53	55	55
	%	40	48	47	54
Live Fetal Body Weight (g)	MEAN	46.3	43.7	49.3	46.6
	S.D.	10.6	7.3	5.2	8.2
Male Fetuses	MEAN	46.2	44.9	50.3	47.6
	S.D.	10.8	8.1	6.6	9.3
Female Fetuses	MEAN	46.3	42.7	48.2	44.5
	S.D.	10.7	7.2	4.3	6.3

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

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TABLE 5

INVESTIGATION OF TERATOGENIC POTENTIAL OF BMA 713 (212-713)
IN THE RABBIT - SEGMENT II
SANDOZ PROJECT T-2620

SUMMARY OF MATERNAL AND FETAL DATA AT CESAREAN SECTION

DOSAGE		0 MG/KG /DAY	0.6 MG/ KG/DAY	1.8 MG/ KG/DAY	3.6 MG/ KG/DAY
Females Mated	N	18	18	18	18
Pregnant	N	14	15	18	17
	%	78	83	100	94
Aborted	N	1	2	0	1
Premature Births	N	0	0	0	1
Dams with Viable Fetuses	N	12	12	17	14
Dams with all Resorptions	N	1	0	0	1
Female Mortality	N	1	1	1.	0
Pregnant at C-section	N	13	12	17	15
Corpora Lutea	N	131	134	168	154
	MEAN	10.1	11.2	9.9	10.3
	S.D.	3.3	2.2	1.4	1.9
Implantation Sites	N	109	116	135	126
	MEAN	8.4	9.7	7.9	8.4
	S.D.	2.8	2.0	2.5	2.4
Preimplantation Loss	%	16.8	13.4	19.6	18.2
Postimplantation Loss	%	13.8	4.3	13.3	19.8
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	15	5*	18	25
	%	14	4.3	13	20
	MEAN	1.2	0.4	1.1	1.7
	S.D.	2.1	0.8	2.2	2.1
Early Resorptions	N	14	0**	5*	20
	%	13	0.0	3.7	16
	MEAN	1.1	0.0	0.3	1.3
	S.D.	2.1	0.0	0.7	2.2
Late Resorptions	N	1	5	13**	5
	%	0.9	4.3	9.6	4.0
	MEAN	0.1	0.4	0.8	0.3
	S.D.	0.3	0.8	2.0	0.6

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

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SEGMENT III REPRODUCTION IN RATS (1st of 2 STUDIES):

A) **Dosage**

20 F at 0, 0.4, 1.2, and 2.4 mg/kg/day by gavage.

(Actual # of pregnant F = 19,19,17, and 14 in control, LD, MD, and HD, resp.)

(Above doses expressed as hydrogen tartrate salt. Doses as free base = 0.24, 0.7, and 1.5 mg/kg).

Dosing was from day 15 of gestation (day of evidence of mating = day 0 of gestation) through day 21 PP.

Strain: [redacted]

Drug lot#: 88902

B) **Results**

1) **Observed signs in dams**

Narrative results only. The following occurred in "most" HD: ataxia, whole body twitches, flutters and tremors, decreased locomotor activity, salivation, licking, chomping/chewing, lacrimation, piloerection, diarrhea, and flattened body position. It was stated that, except for the latter sign, "similar observations" were seen, at lower incidence, at MD. It was stated that no drug - related signs were seen at LD.

2) **Dam mortality**

No drug effects

Two accidental deaths occurred due to watering device malfunction (1 MD and 1 HD, days 3 and 5 PP, resp.)

3) **Dam bodyweight**

Weight gain was marginally decreased at HD during days 15-20 of gestation. (Mean weight on day 15 was 103% of control; on day 20, 97%). (Weight gain was statistically significantly different that control; weights were not). An even more equivocal effect was seen at MD (Mean weight on day 15 was 99% of control; on day 20, 96%).

No drug effects on bodyweight gain during post-partum period.

4) **Dam food consumption**

Decreased during days 15-20 of gestation at MD and HD (Means 86% and 73% of control, resp.)

No effect during post-partum period.

5) **Reproductive data**

Results shown in table 11. Duration of gestation was slightly increased at HD. No drug effects on live or stillborn pups, or post-implantation loss (5.8%, 3.1%, 5.6%, and 6.3% in control, LD, MD, and HD, resp.; not calculated in table).

Although there does not appear to be any increase in pup mortality during the post-partum period, the text states that the 93% overall pup survival at HD was statistically significantly different from the 98% in controls. (Note that in table 11, the total number of pups surviving at HD was flagged as significantly different from controls; this might be expected due to the smaller number of pregnant dams in this group; however, in contrast to what is stated in the text, the percent of pups surviving was not so flagged). (This marginally excess mortality was due to 9 pups dying during the first 4 days PP, vs 3 in controls. Examination of individual animal data showed that this occurred among 4 litters at HD, and 3 in controls).

Pup weights were marginally lower than controls, primarily at MD and HD, throughout the post-partum period. (Table 11). (Lowest values ~ 10% below controls. None were statistically significant).

There were no drug effects on developmental milestones (pinna unfolding, surface righting reflex, eye opening).

It was stated that necropsy of stillborn and dead pups, and of pups sacrificed at weaning, showed no drug-related effects.

TABLE 11

CONFIDENTIAL-TRADE SECRET

INVESTIGATION OF THE EFFECTS OF PERINATAL AND POSTNATAL ADMINISTRATION OF ENA 713 (212-713) IN THE RAT - SEGMENT III
SANDOZ PROJECT T-2670

NATURAL DELIVERY AND LITTER DATA -- SUMMARY

	DOSAGE	0 MG/KG /DAY	0.4 MG/ KG/DAY	1.2 MG/ KG/DAY	2.4 MG/ KG/DAY
Litters Delivered (total)	N	19	19	17	17
Pups Delivered (total)	N	244	283	237	192
	MEAN	12.8	14.9	13.9	13.7
	S.D.	3.3	2.8	3.4	3.2
Liveborn	N	243	278	224	192
Stillborn	N	1	5	12**	0
	%	0.4	1.8	5.1	0.0
Uncertain	N	0	0	1	0
Culled (total)	N	0	0	14	12
Cannibalized	N	0	0	0	0
Missing	N	1	6	1	6
Liveborn, not culled prior to day 21	N	243	278	210	180
Pups Dying, Missing, and/or Cannibalized					
day 0	N	0	1	0	3
	%	0.0	0.4	0.0	1.6
days 1-4	N	3	5	5	6
	%	1.2	1.8	2.2	3.1
days 5-7	N	0	4	3	1
	%	0.0	1.4	1.3	0.5
days 8-14	N	2	2	0	2
	%	0.8	0.7	0.0	1.0
days 15-21	N	1	0	0	0
	%	0.4	0.0	0.0	0.0
Pups Surviving 21 days	N	237	266	202	168*
	%	98	96	96	93
Implantation Sites per Litter	N	259	292	251	205
	MEAN	13.6	15.4	14.8	14.6
	S.D.	3.5	2.8	3.6	2.8

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05; ** = P<0.01.

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SEGMENT III REPRODUCTION IN RATS (2nd of 2 STUDIES):

A) **Dosage**

25 F at 0, 0.3, 1, or 3 mg/kg/day, by gavage.

(Doses expressed as hydrogen tartrate salt)

(Numbers of pregnant dams available for evaluation: 21, 24, 25, and 25 in controls, LD, MD, HD, resp.)

Dosing was from day 17 of gestation (day of evidence of copulation = day 0) through day 21 PP. Pup evaluations included postweaning behavioral and reproductive performance.

Strain: Crj: C

Drug batch #: 90904

B) **Results**

1) **Observed signs in Fo dams**

a) LD - miosis

b) MD - miosis, + low incidence/frequency of tremors and salivation

c) HD - miosis, tremors, salivation, decreased spontaneous movement, lacrimation. The text states that transient "quadriplegia accompanied with generalized asthenia" was seen "occassionally", although this was not listed in the summary table. (The table indicated that "decrease of body posture" occurred in a single animal on the first 2 days of dosing)

2) **Fo bodyweight**

Decreased gain at HD; mean weight decreased to 94% of control by day 20 of gestation and remained at 92-96% of control throughout the post-partum period. (The text states that weights were also lower at MD; although weights at MD were similar to those at HD, they were lower than controls prior to treatment such that weight gain at MD was similar to that of controls).

3) **Fo food consumption**

Decreased at HD throughout treatment (mean ~ 70-75% of control during gestation period, ~ 90-95% of control during postpartum period). Decreased at MD during gestation period only (mean ~ 80-90% of control; pretreatment mean ~95% of control).

4) **Reproductive data**

Tables 4 and 5 indicate a very slight increase in length of gestation at HD, and decreased pup weights at MD and HD, D-R. (Mean pup weights at HD decreased from birth onward; means ~ 95% of control at birth [not statistically significant] to ~ 90% during the postpartum period. [Weights of male pups at MD and HD selected for post-weaning evaluations (not shown in table) remained below controls (approx. 5% and 5-10%, resp.) through the last time point measured (11 weeks of age); this was not seen for female pups])

There were no drug effects on numbers of live or stillborn pups, or on pup survival. The text states that at MD and HD "occasional dams failed to exhibit maternal nursing behavior while displaying signs of reaction to treatment." The text also states that necropsy of pups which died, were culled, or were sacrificed at weaning showed no drug effects.

There were no drug effects on post-weaning open field behavior, rotarod performance, learning ability (T maze performance; note that the large variation noted across animals likely limited the ability to detect drug effects in this test), or reproductive performance (fertility, litter size, and F_2 pup weight, survival, and pre-weaning developmental milestones).

Table 4 Effects of SDZ ENA 713 on gestation, litter size and viability of pups(F_1)

Dose(mg/kg/day)			0	0.3	1	3
No. of dams examined(F_0)			21	24	25	25
No. of dams with live pups			21	24	25	25
Gestation index (%)[a]			100.0	100.0	100.0	100.0
Gestation period (day)	Mean ± S.D.		21.9 ± 0.3	21.9 ± 0.4	21.9 ± 0.3	22.3 ± 0.56*
At birth						
No. of implantations	Total		352	378	414	417
	Mean ± S.D.		16.8 ± 2.7	15.8 ± 2.9	16.6 ± 2.8	16.7 ± 2.3
No. of newborns	Total		324	345	384	373
	Mean ± S.D.		15.4 ± 2.8	14.4 ± 3.3	15.4 ± 2.7	14.9 ± 2.0
Delivery index[b]	Mean ± S.D.		92.0 ± 7.7	91.0 ± 11.6	93.0 ± 7.7	89.9 ± 8.6
No. of live pups	Total (Male/Female)		315 (157 / 158[c])	342 (176 / 166[c])	381 (193 / 188[c])	369 (178[c] / 191[c])
	Mean ± S.D.		15.0 ± 2.8	14.3 ± 3.3	15.2 ± 2.8	14.8 ± 2.3
Birth index[d]	Mean ± S.D.		89.4 ± 9.1	90.1 ± 12.1	92.3 ± 8.6	88.7 ± 9.9
No. of stillbirths	Total (Male/Female)		9 (3 / 6)	3 (1 / 2)	3 (0 / 3)	4[e] (2 / 1)
No. of external defects (%)			0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
Lactation period						
No. of perinatal deaths[f]	Total (Male/Female)		13 (4 / 9)	10 (5 / 5)	10 (2 / 8)	13[e] (7 / 5)
Mortality index (%)[g]	Mean ± S.D.	Total	4.1 ± 4.8	2.9 ± 5.1	2.5 ± 3.7	3.6 ± 6.7
		Male	2.1 ± 4.4	2.6 ± 6.2	0.8 ± 2.8	4.0 ± 7.6
		Female	5.7 ± 8.3	3.1 ± 6.2	4.2 ± 6.6	1.8 ± 7.0
Viability index on day 4 (%)[h]	Mean ± S.D.	Total	98.8 ± 2.6	98.1 ± 4.3	98.3 ± 2.8	97.7 ± 5.4
		Male	99.6 ± 1.8	97.8 ± 6.0	99.2 ± 2.8	97.3 ± 5.8
		Female	98.1 ± 4.9	98.2 ± 4.9	97.9 ± 4.4	98.3 ± 6.8
Weaning index (%)[i]		Total	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.0 ± 7.8
	Mean ± S.D.	Male	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	99.0 ± 5.0
		Female	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	97.0 ± 15.0

a) : (No. of females with live pups / No. of pregnant females) × 100
b) : (No. of newborns at birth / No. of implantations) × 100
c) : Including pups died on day 0
d) : (No. of live pups at birth / No. of implantations) × 100
e) : Including 1 stillbirth of which sex was unidentified because of cannibalism
f) : No. of stillbirths + No. of dead pups during 4 days after birth
g) : (No. of perinatal deaths / No. of newborns at birth) × 100
h) : (No. of live pups on day 4 after birth / No. of live pups at birth) × 100
i) : (No. of live pups at weaning / No. of live pups after adjusting on day 4 after birth) × 100
* : Significantly different from control group at P<0.05

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Table 5 Effects of SDZ ENA 713 on viability and body weight of pups(F_1)

Dose(mg/kg/day)	0	0.3	1	3
No. of dams examined(F_0)	21	24	25	25
No. of live pups				
At birth Total (Male/Female)	315 (157 / 158[a])	342 (176 / 166[a])	381 (193 / 188[a])	369[a] (178[a] / 191[a])
On day 4 after birth				
before adjusting Total(M/F)	311 (156 / 155)	335 (172 / 163)	374 (191 / 183)	360 (173 / 187)
after adjusting Total(M/F)	167 (83 / 84)	189 (92 / 97)	208 (103 / 97)	200 (98 / 102)
On day 7 after birth Total(M/F)	167 (83 / 84)	189 (92 / 97)	200 (103 / 97)	200 (98 / 102)
On day 10 after birth Total(M/F)	167 (83 / 84)	189 (92 / 97)	200 (103 / 97)	197 (97 / 100)
On day 14 after birth Total(M/F)	167 (83 / 84)	189 (92 / 97)	200 (103 / 97)	196 (97 / 99)
On day 18 after birth Total(M/F)	167 (83 / 84)	189 (92 / 97)	200 (103 / 97)	196 (97 / 99)
On day 22 after birth Total(M/F)	167 (83 / 84)	189 (92 / 97)	200 (103 / 97)	196 (97 / 99)
Mean body weight of pups (g)				
Male				
At birth	6.6 ± 0.7[b]	6.6 ± 0.6	6.5 ± 0.7[c]	6.2 ± 0.7[c]
Before adjusting	10.6 ± 1.7	11.0 ± 1.6	10.4 ± 1.5	10.0 ± 1.4
After adjusting	10.7 ± 1.6	11.0 ± 1.6	10.5 ± 1.5	10.2 ± 1.3
On day 7 after birth	18.1 ± 1.8	18.3 ± 1.6	17.3 ± 2.1	16.7 ± 1.6
On day 10 after birth	26.5 ± 2.4	26.2 ± 1.8	24.9 ± 2.5	24.0 ± 2.2**[c]
On day 14 after birth	38.2 ± 3.6	37.6 ± 2.5	35.7 ± 2.7	34.0 ± 2.9**[c]
On day 18 after birth	49.2 ± 3.6	48.4 ± 2.2	46.5 ± 3.4	44.4 ± 3.6**[c]
On day 22 after birth	60.4 ± 5.1	67.5 ± 3.5	65.4 ± 4.9	64.0 ± 4.8*[d]
Female				
At birth	6.3 ± 0.6	6.3 ± 0.6	6.2 ± 0.7[c]	5.9 ± 0.7[c]
Before adjusting	10.3 ± 1.5	10.5 ± 1.6	10.0 ± 1.6	9.5 ± 1.5
After adjusting	10.5 ± 1.6	10.5 ± 1.5	10.2 ± 1.6	9.7 ± 1.4
On day 7 after birth	17.6 ± 1.7	17.3 ± 1.6	16.5 ± 2.3	15.9 ± 2.0*
On day 10 after birth	25.6 ± 2.0	25.1 ± 1.7	24.0 ± 2.6	22.7 ± 2.6**[c]
On day 14 after birth	37.1 ± 2.8	36.3 ± 1.7	34.4 ± 2.9**	33.0 ± 2.8**[c]
On day 18 after birth	47.5 ± 3.1	46.5 ± 1.9	44.9 ± 3.9	43.1 ± 3.8**[c]
On day 22 after birth	65.5 ± 3.8	63.9 ± 2.6	62.6 ± 5.0	61.3 ± 4.9*[d]

a) : Including pups died on day 0
b) : Mean ± S.D.
c) : Excluding the value of 1 dam because of sex misjudgment
d) : The value of 1 dam was excluded from mean calculation because the pups from the dam could not drink water by accident.
* ** : Significantly different from control group at $P<0.05$, $P<0.01$

Table 6 Effects of SDZ ENA 713 on postnatal development and reflex functions in rat pups(F_1) of the treated dams(F_0)

Dose(mg/kg/day)	0	0.3	1	3
No. of dams examined(F_0)	21	24	25	25
Postnatal physical development				
Separation of ear auricle (on day 4)	167 / 167[a]	189 / 189	200 / 200	200 / 200
Appearance of dorsal hair (on day 4)	167 / 167	189 / 189	200 / 200	200 / 200
Eruption of upper incisors (on day 14)	167 / 167	189 / 189	200 / 200	196 / 196
Separation of eyelids (on day 17)	167 / 167	189 / 189	200 / 200	196 / 196
Descent of testes (day, Mean ± S.D.)	26.1 ± 1.1 (21)[b]	26.7 ± 1.4 (24)	26.0 ± 1.1 (25)	26.3 ± 1.1 (25)
Opening of vagina (day, Mean ± S.D.)	30.5 ± 1.9 (21)	30.8 ± 1.9 (24)	30.3 ± 1.6 (25)	30.8 ± 2.4 (25)
Reflex functions				
Preyer reflex (on day 19)	167 / 167[c]	189 / 189	200 / 200	196 / 196
Pain reflex (on day 19)	167 / 167	189 / 189	200 / 200	196 / 196
Righting reflex (on day 20)	167 / 167	189 / 189	200 / 200	196 / 196
Righting in mid-air (on day 20)	167 / 167	189 / 189	200 / 200	196 / 196
Corneal reflex (on day 21)	167 / 167	189 / 189	200 / 200	196 / 196
Pupillary reflex (on the 3rd week)	42 / 42	48 / 48	50 / 50	50 / 50

a) : No. of developing pups / No. of pups examined
b) : In parentheses, No. of pups examined
c) : No. of pups with normal reflex / No. of pups examined

GENOTOXICITY:

A) Ames Tests

Three separate studies were done, using 3 separate drug batches (88903, 94702, and 91907). For the study of the first batch, Salmonella strains TA 1535, TA 97, TA 98, TA 100 and TA 102 were used, and the highest drug concentrations were 30-50 mg/plate. For the other 2 studies, TA 97a was used instead of TA 97, and the highest drug concentration was 5 mg/plate. Assays were run with and without metabolic activation (Aroclor-induced rat S9), and with negative (DMSO) and positive controls. Drug did not precipitate at any concentration. Higher doses (primarily above 5 mg/plate) were bacteriotoxic as indicated by decreased numbers of revertants. The drug did not increase revertants in any of the studies.

An Ames Test was also run on a compound identified as "226-90", said to be a by product of ENA 713, although elsewhere in the application it is indicated that it is likely to be a major metabolite of the drug. (Specifically, 226-90 is the optically active phenolic metabolite assumed to be formed from the optically active parent drug [as opposed to compound ZNS 114-666, which is the racemic mixture of this metabolite]). Methodology was similar to the above. (Salmonella strain TA 97 was used rather than TA 97a). The highest concentration used was 25 mg/plate, although this was bacteriotoxic in the absence of metabolic activation with 7.5 mg/plate being the highest evaluable concentration. The test compound caused no increases in revertants.

B) HGPRT Test in V79 Chinese Hamster Cells

Drug concentrations based on preliminary toxicity studies. Concentrations used, and results, shown in attached tables (p. 36a-36h). (Note that in presence of S9, since in the first assay [tables 7a and 7b] the mean cloning efficiency was 18.5% at the highest concentration of 1400 ug/ml, the second assay [tables 8a and 8b] used a high concentration of (1500 ug/ml, yet toxicity was less [mean cloning efficiency of 74.5%]. The reason for this is not clear). There was no clear increase in mutations due to test drug. (Note that increases in mutation frequency of $\geq$ 3x the solvent control were seen in isolated samples. According to the sponsor's criteria, these are not considered drug-related since the absolute values were within the historical control range [upper limit of mutation frequency = $2x10^{-5}$], and were not consistently reproducible in replicate assays. [Also note that there are isolated instances where mutation frequencies in the drug groups were 3x less than the concurrent control frequency]).

C) Unscheduled DNA Synthesis (UDS) in Rat Hepatocyte Culture

ENA 713 was negative. (Highest evaluable concentration was 1250 ug/ml, which was said to be slightly cytotoxic; concentrations of ≥ 2500 ug/ml were said to be too cytotoxic for UDS evaluation). Positive controls were active.

D) Chromosomal Aberrations in V79 Chinese Hamster Cells In Vitro

Results of preliminary cytotoxicity assays are shown in tables I, II, and III, attached (p. 36i-36k). Tables I and II, apparently 2 separate studies done without metabolic activation and using 24 hour treatment time, show divergent results, i.e. Table I shows cell counts of ≤20% of controls at concentrations of 1880 ug/ml and above, whereas table II shows cell counts of 78 % of control at the highest concentration used (2000 ug/ml). The sponsor does not discuss this discrepancy. Table III shows cytotoxicity data in the presence of rat liver S9, using a 3 hour treatment period, and shows a relatively flat dose-response curve with about 50% cell survival at about 3000 ug/ml and above.

Treatment and incubation times, and concentrations used, in the chromosomal aberration assay are shown in the following table:

Based on the results from cytotoxicity studies as well as data concerning the depression of the mitotic index the following concentrations of SDZ ENA 713 (batch 88902) were selected to be analyzed in the chromosomal aberration assay:

Without S9:	**3h treatment 15h incubation**	**18h 45min treatment no further incubation**
	3000 µg/ml	1000 µg/ml
	1500 µg/ml	750 µg/ml
	500 µg/ml	500 µg/ml
With 10% S9:	**3h treatment 15h incubation**	**3h treatment 15h 45min incubation**
	5000 µg/ml	5000 µg/ml
	3000 µg/ml	3000 µg/ml
	1000 µg/ml	1000 µg/ml

Table IV (p. 36 l) shows the mitotic index data, presumably taken from the chromosomal aberration assay although this was not explicitly stated. A treatment time of 3 hours followed by a fixation time of 18 hours did not result in a decrease in mitotic index in the absence or presence of S9 at drug concentrations up to 3000 and 5000 ug/ml, respectively. (Left column of table IV). Somewhat anomalously, when the fixation time in the presence of S9 was increased to 18.75 hours, the mitotic index was decreased, to a maximum of 50 % of controls at 4000 ug/ml; also anomalously, at the next highest concentration of 5000 ug/ml, the mitotic index was 90% of controls (lower right corner of table IV). When a longer treatment time of 18.75 hr. (with no further fixation) was used, in the absence of S9, a dose-related decrease in mitotic index was seen, with a value of 2% control at the highest concentration used (3000 ug/ml). (Upper right corner of table IV.)

A summary of the chromosomal aberration data is shown in table V (p. 36m). In the absence of S9, no drug effect was seen up to the highest concentration tested (3000 ug/ml). In the presence of S9, the % of cells with aberrations was increased at 3000 ug/ml. (The smaller increases seen at 1000 and 5000 ug/ml were apparently not considered drug related, but the reasons for this were not discussed). Attached are tables 1-20 (p. 36n-36w) showing the types of aberrations seen.

E) Chromosomal Aberrations in Human Lymphocytes in Vitro

The assay was performed using duplicate cultures from a single male donor. (The original protocol indicated a female donor would also be used; the study was apparently "abandoned" by the sponsor prior to this). Treatment of cultures in the absence of metabolic activation was for 20 hours. Treatment in the presence of metabolic activation was for 3 hours followed by a 17 hour "recovery period" prior to harvest. Concentrations selected for evaluation were based on reduction of mitotic index; these results are shown in the attached section "5.1" (p. 36x-36y) taken from the report. (Note that in the presence of S9, there was no a clear dose-response). Chromosome aberration results are shown in the attached appendix 1, tables 1 and 2 (summary tables), appendix 2 (abbreviations for types of aberrations), and appendix 3, tables 1 and 2 (types of aberrations found) (p.36z-36dd). Aberrations were clearly increased in the presence of S9. As shown in the summary table (appendix 1, table 2), there was a statisically significant increase at all concentrations, although the value at the lowest concentration was within the historical control range (appendix 6 [p. 36gg]). The increase was not D-R (neither was the decrease in mitotic index). There were no statistically significant increases in aberrations in the absence of metabolic activation (appendix 1 , table 1), although it is noted that the oringinal study protocol stated that if the first experiment was negative or equivocal, additional experiments were to have been performed (i.e. using longer treatment times, or shorter treatment times at higher doses); these were not conducted "at the request of the Sponsor after consideration of the results of [the first experiment]."

An increase in polyploidy was also seen, in the presence of S9 only, at all but the lowest dose level tested (Appendix 4, table 2 [p. 36ff]; historical control data in Appendix 6 [p. 36gg]).

F) Micronuclues Test in Mice

CD-1 mice ———) were given 0 or 5 mg/kg ENA 713 by gavage; bone marrow was sampled at 24, 48, and 72 hours post-dose. Positive control was 0.9 mg/kg TEM; sampling at 24 hours only. N=5/sex/time point.

There were no deaths during the study. Nothing was stated regarding toxic signs observed during the study, although it was stated that in a rangefinding study 5 mg/kg caused "strong signs" consisting of salivation, tremor, prone position, and narrowed eye opening, and a dose of 22 mg/kg caused 2/2 deaths.

Results shown in attached table (p. 36hh). There were no effects of ENA 713 on micronuclei or on % of polychomatic erythtocytes.

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDATIONS:

This NDA is approvable. Comments on the proposed labeling are made above. The sponsor should submit, or indicate the location of, data supporting the statements in the proposed labeling that the drug is a "pseudo-irreversible inhibitor" of acetylcholinesterase and that it "interacts with its target enzymes by forming a covalently bound complex that temporarily inactivates the enzyme".

/S/

Barry N. Rosloff, Ph.D.

cc: NDA 20-823, original jacket + division file
Rosloff
Fitzgerald 12/29/98
Nighswander

APPEARS THIS WAY
ON ORIGINAL

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

16 pages
Raw DATA

APPENDIX 2

Abbreviations and classification of observations

abs	=	aberrations
rep	=	replicate
tot	=	total

Gaps (g)

csg	=	chromosome gap
ctg	=	chromatid gap

Chromosome deletions (Chr del)

del	=	chromosome deletion
d min	=	double minute
f	=	isolocus fragment

Chromosome exchanges (Chr exch)

t*	=	interchange between chromosomes (eg reciprocal translocation)
inv*	=	chromosome intrachange (eg pericentric inversion)
dic	=	dicentric
dic+f	=	dicentric with accompanying fragment
acr	=	acentric ring
r+f	=	centric ring with accompanying fragment
r	=	centric ring

Chromatid deletions (Ctd del)

del	=	chromatid deletion
su	=	isochromatid deletion with sister union of broken ends
mud	=	isochromatid deletion with non-union of broken ends distally
mup	=	isochromatid deletion with non-union of broken ends proximally
min	=	single minute

Chromatid exchanges (Ctd exch)

qr	=	interchange between chromatids of different chromosomes (eg quadriradial)
cx	=	obligate complex interchange
e	=	chromatid intrachange
tr/tr+f	=	isochromatid/chromatid interchange (triradial)/with accompanying fragment

Other structural aberrations

pvz	=	pulverised cell
mabs	=	multiple aberrations (greater than seven aberrations per cell or too many aberrations to permit accurate analysis)

Numerical aberrations (num abs)

E	=	endoreduplicated
H**	=	hyperdiploid (47-68 chromosomes)
P	=	polyploid (greater than 68 chromosomes)

* Reciprocal translocations and inversions are generally not detected in unbanded preparations. These were to be noted if observed but they were not included in the analysis of results.

** Frequencies of hyperdiploid cells were to be recorded but these data were not used to critically assess ability to induce aneuploidy.

SUMMARY:

A) Pharmacodynamics

The sponsor's summary was included earlier. Much of this data was previously reviewed (by Dr. DeGeorge; review attached). Selected aspects will be discussed further in the Labeling section of the present review. Basically, the hypothesized mechanism of action of this drug is inhibition of acetylcholinesterase, leading to increased functional availability of acetylcholine in Alzheimer's Disease, a disease whose postulated pathogenesis involves destruction of central cholinergic pathways involved in cognitive function, especially memory.

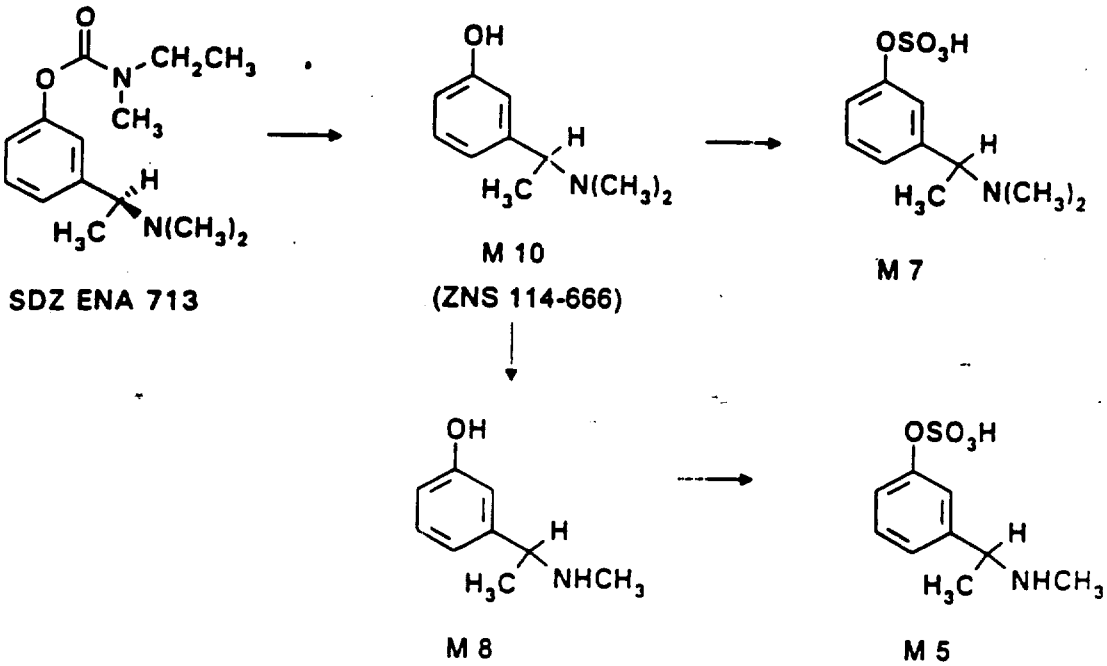
B) ADME/PK

The sponsor's summary was included earlier. Note that for studies using labelled drug, the identity and position of the label was different for animals (tritium in 2-position of phenyl ring) and humans (C^{14} in benzylic carbon). (It was stated that the stability of the tritium label was high [1-6% tritiated water formation] in all species but rat [15 %]). This difference in the position of the label should be kept in mind when comparing ADME across species; however note that both positions are contained in the metabolites which have been identified, and that these metabolites represent a significant fraction of the metabolism of the drug in humans, rats, and dogs (but apparently not in mice and rabbits). (See below).

As indicated in the sponsor's summary, absorption of drug is extensive in all species, except somewhat less so (65%) in mouse. Excretion of label was mainly urinary in all species (again, with the exception of mouse, where urinary excretion was 50 - 65 % of the dose after p.o. dosing, presumably reflecting incomplete absorption since after i.v. dosing urinary excretion of label was 99 % of the dose. (See table C-9 in sponsor's summary).

The drug is extensively metabolized in all species, as indicated by the absence or relatively low levels of parent drug in blood and urine. The identified metabolites and the proposed metabolic pathways are shown in the attached figure taken from the sponsor's summary. (Note that metabolite "M10", which was assayed in plasma in several toxicity studies, is also referred to as "ZNS 114-666" or"226-90".). The metabolites are the products of [redacted]
In human males given single doses of 1 or 2.5 mg of labelled drug orally, the % of plasma label (measured at various time points through 12 hours post-dosing) due to metabolites M5 + M7 + M10 ranged from [redacted] with most of this due to M7. (No M8 or parent drug detected). In 24 hour urines, these 3 metabolites accounted for about 50 % of the administered dose (again, mainly due to M7, with no M8 or parent drug detected). In male rats dosed orally, the sum of M5 + M7 + M8 + M10 in plasma represented about [redacted] of the plasma label (mainly due to M7 and M10, present in approximately equal amounts) and in urine represented [redacted] of the dose (with M10 predominating, i.e. [redacted] the dose). In male dogs dosed orally, the sum of these 4 metabolites in plasma represented 45-56% of the plasma label (mainly due to M10; however note that in a different study M10 represented less than 10 % of the plasma label) and in urine represented 29 %

FIGURE C-4: MAJOR METABOLIC PATHWAYS OF ENA 713 IN MAN



of the dose (with M8 predominating, i.e. 12 % of the dose). In mice and rabbits dosed orally, metabolism to the 4 identified metabolites appeared to be less than in the above species; in male mice only 3-10 % of the dose was excreted in urine as M7 + M8 + M10 (most of this was M10; no M5 or parent drug found); in female rabbits only about 10 % and 3 % of the dose was excreted in urine as M10 and M8, respectively (no M5, M7, or parent drug detected). (Note that part of the reason for the low values in mice is the slightly overall lower total urinary excretion seen in this species).

In summary, regarding inter-species comparisons of drug metabolism, all species tested metabolized the drug extensively. All appeared to use similar types of reactions (.), with some quantitative differences seen in the relative levels of the products of these reactions. In mice and rabbits, however, these pathways appear to represent relatively minor routes of metabolism, with a large amount of drug being metabolized to as yet unidentified products. (Note regarding interspecies comparisons: The studies discussed above used relatively small sample sizes [which were often pooled], used a single gender for each species, usually used only acute dosing, often showed dose-dependent effects, and often used different sampling times, factors which could limit the accuracy and generalizability of the comparisons).

The ability of metabolite M10 to inhibit cholinesterase in vitro and ex vivo in rat brain was studied. (Note that while M10 is found in the circulation, according to the proposed mechanism of action of parent drug, M10 is formed as a byproduct of the inhibition of cholinesterase by parent drug, and is thus presumably available to interact with this enzyme at the sites where it is formed). (Also note that that the report refers to the enzyme as "acetylcholinesterase"; however, the substrate used in the assays [acetylthiocholine] is metabolized by both acetylcholinesterase and butyrylcholinesterase). In vitro, M10 inhibited striatal cholinesterase with an "apparent Ki" of "approximately" 1.8×10^{-5} M. It was stated that "the results suggests [sic] a linear mixed type of inhibition...indicating both competitive and noncompetitive inhibition", with the main component being competitive. It was stated that the inhibition by M10 was "non time dependent" in contrast to that by the parent drug and thus a comparison of the Ki values between these compounds could not be made. It was stated that for M10 the range of IC 50 values was depending on the substrate concentration used, and that "the arbitrary observed IC 50s following preincubation of the enzyme for 15 min. with both compounds and at maximal substrate concentrations" were g. (Note, however, that nothing was stated regarding in vitro assay with parent compound in the "methods" section and no results were shown for parent compound; thus it is not clear if the 2 compounds were compared in the same assay). In the ex vivo study, rats were given M10 at oral doses of 100 or 300 umol/kg (17 and 50 mg/kg, resp.) and were sacrificed after 90 min. (Controls apparently sacrificed at 60 min. post-dose). M10 had no effect on cholinesterase activity in any of the several brain areas examined, whereas parent drug (8 umol/kg; time of sacrifice after dosing not clear) caused about 50 % inhibition. From these studies the report infers (to the best of my ability to understand the somewhat unclear discussion) that a role for M10 in causing central acetylcholinesterase inhibition can be excluded since M10 generated in the periphery poorly penetrates into the brain, and the fact that the

maximum concentration of M10 formed within brain is at most equimolar with that of parent drug. This inference may be questioned on several grounds. The assertion that M10 poorly penetrates into the brain is presumably based on the lack of ex vivo effect as noted above. However, only a single time point after dosing was studied, which may not have coincided with the time when effective brain levels of M10 were present. (Brain levels of M10 were not measured). Also, it is possible that the lack of effect of M10 was due to the fact that it was not well absorbed after oral dosing. Regarding the role of M10 formed within the brain, its contribution to cholinesterase inhibition would depend on its potency relative to that of the parent compound, and as noted above this could nor be accurately determined. Note that a role for compounds other than parent drug in producing pharmacological effects may be suspected based on the fact that in animals, "cholinergic" signs were produced at doses of parent drug after which circulating levels of parent drug were undetectable.

C) Toxicity/carcinogenicity studies

The following studies were performed (doses expressed as mg/kg hydrogen tartrate salt):

1) Rat

   a) Acute toxicity
   b) 4 week gavage (0.6, 2.4, 6.0)
   c) 26 week gavage (0.18, 0.72, 2.4)
   d) 1 year gavage (0.2, 0.6, 1.8, 3.0)
   e) 2 year gavage carcinogenicity (0.2, 0.6, 1.8)

2) Mouse

   a) Acute toxicity
   b) 2 year gavage carcinogenicity (0.4, 1.0, 2.5)

3) Dog

   a) Acute toxicity
   b) 4 week capsule
      1) 0.06, 0.6, 3.6 → 3.0
      2) 0.18, 0.3, 0.42
   c) 26 week capsule (0.18, 0.72, 2.5)
   d) 1 year capsule (0.3, 0.6, 2.5→ 2.1)

(Note that studies of ≤ 26 weeks, and some range-finding studies,

In rats, toxic signs were generally seen at all doses tested, but were relatively minimal at the lower doses. Signs included tremors, twitches, decreased (but sometimes increased) locomotor activity, chomping/chewing/licking/biting/gnawing/excessive grooming, ataxia, salivation, flattened body position, and wet fur; at least some of these were likely due to acetylcholinesterase inhibition. (In a 13 week study using dietary dosing and a rising dose design, no toxic signs were seen up to the highest dose used, i.e. 9.6 mg/kg/day). There were no clearly drug-related deaths in the multiple dose studies; acute oral doses caused deaths at 10 mg/kg and above. Food consumption and bodyweight gain were decreased, generally at 1.8 mg/kg and above. (In the above mentioned 13 week dietary study, there was a slight decrease in food consumption at the highest dose but no effects on bodyweight). Bodyweights near the end of the carcinogenicity study were approximately 90 and 80-85% of control in HD M and HD F, resp. The most consistent effect on lab tests was a decrease in blood triglycerides, which were decreased (up to 50% or more) at the higher doses, although not consistently across sexes (i.e., in M only in the 4 week study, in both sexes in the 26 week study, and primarily in F in the 1 year study). This effect was apparently reversible (as measured after a 2 month recovery period in the 1 year study). There were no drug-related increases in neoplasms. There were a few possibly drug-related histological changes which were not replicated across studies, including reversible bile duct hyperplasia (primarily in HD M) in the one year study, stomach erosion in HD M in the 2 year study, and hypercellularity of sternal bone marrow in MD and HD M in the 2 year study (said to be a response to the hind-limb inflammation and ulceration seen in HD M in this study, although as discussed earlier the bone marrow hypercellularity was often seen in animals without reported hind-limb changes). Toxicokinetic results showed that the blood levels of parent compound were much less than those of the phenolic metabolite ZNS 114-666, and that levels of 1 or both of these compounds tended to increase over the course of the multi-dose studies.

In the mouse carcinogenicity study, toxic signs were seen at MD (tremors, twitches/flutters, decreased motor activity, labored/rapid breathing, rough coat [M only], wet fur [M only], licking/chewing/gnawing) and HD (above + flattened/hunched posture, ataxia, depressed or lost righting reflex, "unusual gait," salivation, piloerection, reduced feces, diarrhea /loose stool [M only], and ptosis [F only]). Mortality was slightly decreased in HD F (survival at termination = 34%, vs 24% and 17% in the two control groups). (Deaths were seen at 10mg/kg in the acute oral toxicity study as well as in an 8 week gavage rangefinding study.) Food consumption and bodyweight gain in the carcinogenicity study were decreased at HD M, HD F, and MD F (weights near end of study approx. 90%, 90% and 90-95% of control, resp.). (Decreased food consumption and weight gain also seen in the gavage rangefinding study at 4 mg/kg and above, and in a 13 week dietary rangefinding study in a group where doses were gradually increased up to 60-120 mg/kg/day.) Routine lab tests were not performed in any of these mouse studies. There were no clearly drug-related effects on neoplasms or non-neoplastic lesions; a borderline result was seen with mammary adenocarcinomas which will be discussed in the "Evaluation" section, below. As in other species, blood levels of the phenolic metabolite ZNS 114-666 were considerably greater than those of the parent compound (which were generally below the limit of quantitation at LD and MD).

In dogs, toxic signs were generally minimal or absent at doses of 0.18 mg/kg and below; higher doses caused increasingly more severe signs (in some cases necessitating a reduction of the HD) including tremors, lacrimation, salivation, licking, erythema, decreased/increased locomotor activity, emesis, diarrhea, bloody stool, increased/labored respiration, ataxia, soiled fur, etc. Tonic-clonic convulsions were occasionally seen at the higher doses. At least some of these signs are probably due to excessive cholinergic stimulation; a brief report of a non-GLP acute study stated that several signs were reversed by atropine. Drug-related deaths occurred at doses of 2.5 mg/kg and above. In a few dogs death or moribund sacrifice was associated with intussusception of the ileum, which was considered to result from an exaggerated pharmacological effect of the drug. Bodyweights and food consumption were transiently decreased at the highest doses. There were no drug effects on heart rate, EKG, or ophthalmoscopic exams. Body temperature was increased (1-2° F) at MD and HD in the 1 year study. There were no consistent drug effects on lab tests. The only clearly drug-related pathological effect was on G.I. tract. In the 1 year study several HD dogs had serosal lesions in the large intestine (nodules, hemorrhage, granulation tissue, etc.; see earlier for more complete description). An apparently similar lesion (involving serosa of both large and small intestine) was also seen in an HD dog in the 26 week study. In addition, an apparently similar lesion was also seen in a 2 week i.v. study at a dose of 1.2 mg/kg/day (which also caused pronounced cholinergic toxic signs including bloody rectal discharge), although in this study there was also degeneration of the adjacent tunica muscularis. Other G.I. effects, again occurring primarily at the highest doses, included intussusception, mucosal and muscularis congestion and/or hemorrhage in large and small intestine, and small intestine muscularis degeneration. These G.I. lesions will be discussed further in the "Evaluation" section, below. As in other species, blood levels of the phenolic metabolite ZNS 114-666 were considerably greater than those of the parent compound.

D) <u>Reproduction Studies</u>

The following studies were performed (doses expressed as mg/kg hydrogen tartrate salt):

1) Segment I rat (0.2, 0.9, 1.8; both M and F dosed)

2) Segment II rat (2 studies)
   a) 0.4, 1.2, 3.6
   b) 0.4, 1.3, 4.0

3) Segment II rabbit (0.6, 1.8, 3.6)

4) Segment III rat (2 studies)
   a) 0.4, 1.2, 2.4
   b) 0.3, 1.0, 3.0

All studies used gavage dosing. Studies 2b and 4b were done in Japan; the others were done in the U.S.

In the segment I study a variety of toxic signs, at least some likely secondary to cholinesterase inhibition, were seen at HD and to a lesser extent at MD. Effects on food consumption and bodyweight were equivocal as discussed earlier; in view of results at similar doses in the other reproduction studies it is likely that there were small decreases at least in MD and HD F during the pre-mating and gestation periods. (During the lactation period there were no effects on food consumption, and bodyweight gain at MD and HD tended to be greater than in controls). There were no drug effects on reproduction parameters among F sacrificed on day 20 (including visceral and skeletal fetal exams). Among F allowed to deliver naturally there was a dose-related slight decrease in pup weights through day 21 PP (and beyond, in male pups) at all doses (mean weight at HD on day 21 was 10% below control); there were no drug effects on F_1 survival, developmental milestones, or behavioral and reproductive performance.

In the 2 segment II rat studies (dosing days 6-15 and 7-17 of gestation, respectively), characteristic toxic signs were seen at MD and HD) (+ miosis at LD in the second study). Dam food consumption and bodyweight gain were decreased at MD and HD in both studies (at HD, food consumption ~75-85% of control; weight at end of treatment period ~90% of control). The second study showed an increase in early resorptions but no effect on number of live fetuses; a trend toward decreased early resorptions was seen in the first study. Fetal weights at c-section were slightly and equivocally decreased at HD in both studies (mean 93-94% of control). Fetal exams showed no drug effects aside from a slight increase in unossified sternebrae and hyoid body at HD in the first study only (although it is not clear if these same areas were examined in the second study). (The text of the second study stated that in a rangefinding study a dose of 8 mg/kg caused retardation of fetal ossification, but no specifics were given). An increase in "lumbar rib", a skeletal variation, was seen at HD in the second study but the incidence was said to be well within the historical control range and was not considered drug-related. Among F allowed to deliver naturally (done in second study only), there were no effects on F_1 external anomalies or necropsy exams, survival, pre-weaning fetal weights or developmental milestones, or post-weaning behavioral and reproductive performance; there was a not clearly dose-related decrease in bodyweights at week 11 in female F_1 pups chosen for mating; this decrease continued through the gestation and lactation periods and was associated with slightly decreased F_2 pup weights at MD and HD. (No bodyweight effects on F_1 pups were seen from birth through week 8 PP).

In the segment II rabbit study (dosing days 7-19 of gestation) toxic signs similar to those in rats were seen at HD and in "a few" MD. (In a rangefinding study in pregnant rabbits, 4 of 5 animals given 12 mg/kg/day died). Food consumption was not measured. HD lost 3.8% of bodyweight during the treatment period, compared with a 0.5% loss in controls. There was a very slight and equivocal increase in resorptions at HD which the report attributes to maternal toxicity; however, a similar increase was not seen in the rangefinding study at doses causing more severe maternal toxicity. There were no drug effects on other reproductive parameters or fetal exams.

In the segment III studies (dosing day 15 of gestation through day 21 PP, and day 17 of gestation through day 21 PP, respectively) characteristic toxic signs were seen at MD and HD (+ miosis at LD in the second study). (In the second study, it was stated that transient "quadriplegia accompanied

with generalized asthenia was also seen "occasionally" at HD; it is not clear if this is the same as "flattened body position" as reported in the first study). Dam food consumption was decreased at MD and HD, primarily during the gestation period (mean value at HD ~ 70-75% of control during this period). Dam bodyweight gain was slightly decreased at HD during the gestation period only. Duration of gestation was slightly increased at HD in both studies. There was no clear drug effect on stillborn pups or pup survival; the claim of slightly decreased pup survival at HD in the first study, said to be statistically significant, is not convincing. (Overall survival at HD= 93% VS 98% in controls). There were no drug effects on pre-weaning developmental milestones. Both studies showed decreased pup weights at MD and HD throughout the 21 day postpartum period; maximum difference was ~10% below control. (In the second study, it was stated that "occasional [MD and HD] dams failed to exhibit maternal nursing behavior while displaying signs of reaction to treatment"; it is not clear if this had a role in the decreased pup weights). Weights of male, but not female pups, remained below controls at MD and HD during the post-weaning evaluations. These evaluations, done in the second study only and included open field behavior, rotarod performance, learning ability, and reproductive performance, showed no drug effects.

E) Genotoxicity Studies

The following studies were performed:

1) Ames Test (3 separate studies, + study of metabolite 226-90)
2) HGPRT test in V79 Chinese hamster cells
3) Unscheduled DNA synthesis in rat hepatocyte culture
4) Chromosomal aberrations in V79 Chinese hamster cells in vitro
5) Chromosomal aberrations in human lymphocytes in vitro
6) Mouse micronucleus test

No drug-induced genotoxicity was seen in #1-3 and 6, above. An increase in chromosomal aberrations was seen in both V79 Chinese hamster cells and in human lymphocytes, in vitro. In both of these assays the increase was seen only in the presence of a rat liver S9 preparation; however it is noted that in the human lymphocyte study, the original protocol stated that if the initial experiment was negative, additional experiments were to have been performed (using longer treatment times or shorter treatment times at higher doses); these additional experiments were not conducted "at the request of the Sponsor," and thus perhaps this study did not adequately evaluate the drug in the absence of metabolic activation.

In the V79 study, the largest increase in aberrations was seen at the middle concentration (3000 ug/ml) of the three concentrations studied; the smaller increases at the other two concentrations (1000 and 5000 ug/ml) were apparently not considered drug-related, although the reasons for this were not discussed. It is difficult to determine the presence or degree of cytotoxicity associated with

doses which did or did not increase chromosomal aberrations in this study, due to unexplained inconsistencies in the cytotoxicity results as discussed earlier. For example, in the absence of S9, where no increase in chromosomal aberrations were seen up to 3000 ug/ml, preliminary toxicity studies gave equivocal results, i.e. 1 study showed cell survival of $\leq$ 50% at 1155 ug/ml and above, with ~10% survival at 3000 ug/ml, whereas a second study showed a relatively flat D-R curve with 78% cell survival at the highest concentration tested of 2000 ug/ml. (Also, the relevance of these preliminary studies in the absence of S9 may be questioned since they used a 24 hour treatment period, whereas the chromosomal aberration assay used 3 and 18.75 hour treatment periods). The mitotic index data (presumably obtained in the main chromosomal aberration study) in the absence of S9 indicated no effect with 3 hours' treatment up to the highest concentration used (3000 ug/ml) (although a large effect with 18.75 hours' treatment was seen at this and lower doses). In the presence of S9, where an increase in chromosomal aberrations was seen, the single preliminary toxicity study indicated a cell survival of ~50% at the concentrations subsequently used in the chromosomal aberration assay; the mitotic index data (again, presumably obtained in the main chromosomal aberration assay) were somewhat contradictory; showing either no effect or a relatively small non D-R decrease.

In the human lymphocyte study, increases in aberrations were seen at 687 ug/ml and above; a statistically significant increase at the lowest dose, 447 ug/ml, was not considered drug related since values were within the historical control range. Increases in aberrations were not dose-related. An increase in polyploidy was also seen, in the presence of S9 only, at all but the lowest dose level, again not clearly dose-related. (Nothing was mentioned regarding evaluation of numerical aberrations in the V79 study). Varying degrees of mitotic index depression were seen at these doses, which were also not dose-related. It is noted that the lymphocyte sample in this study was from a single male donor; the original protocol also called for a female donor but the study was apparently "abandoned" by the sponsor prior to this.

EVALUATION:

This NDA is approvable. Adequately conducted toxicity studies included 6 month and 1 year studies in rats and dogs, 2 year carcinogenicity studies in rats and mice, and standard reproduction and genotoxicity batteries.

Various toxic signs, several of which presumably represent exaggerated pharmacological effects (acetylcholinesterase inhibition) of the drug, were seen in all species. The most consistent and prominent organ pathological effect in these studies was various G.I. lesions in dogs, consistent with the prominent G.I. toxic signs (emesis, diarrhea, bloody stool, etc.) seen in this species. Such lesions included serosal lesions (nodules, hemorrhage, granulation tissue, etc.) primarily in large intestine, intussusception of the ileum, mucosal and muscularis congestion/hemorrhage in small and large intestine, and muscularis degeneration in small intestine. These lesions were seen at high doses causing prominent G.I. signs. Some lesions were seen after relatively short term treatment, often in animals prematurely sacrificed in moribund condition. Some lesions (including serosal nodules and degeneration/granulation tissue in muscularis of large intestine) were also seen in a 2 week i.v. study, indicating that these effects are not necessarily due to a local action. The ability of this drug to produce these types of lesions in humans must be considered at least a possibility. G.I. effects are a prominent feature of the side effect profile in humans. The attached discussion (p. 46a) of the serosal lesions (especially the 2nd paragraph), taken from the NDA, discusses a possible anatomical basis for the possibility of the production of a similar lesion in humans. (The likelihood that these lesions are related to the pharmacological activity of the drug is increased by the fact that apparently similar lesions were produced in dog by another acetylcholinesterase inhibitor, velnacrine [Mentane]; see my review of 10/27/92 for NDA 20-282.

As noted earlier, there was a borderline/equivocal increase in mammary adenocarcinomas in high dose females in the mouse carcinogenicity study. (0/70, 1/70, 1/71, 0/70, and 4/70 in control-1, control-2, LD, MD, and HD, resp). According to the sponsor's analysis the incidence in HD F was statistically significantly greater than the combined, but not the individual, control groups. It is difficult to conclude that this is drug- related in view of the multiple comparisons made in this type of study and for the following additional reasons:

1) The incidences of hyperplasia and adenoma were not increased. (Attached 2 pages [p. 46b-c] taken from the hispathology summary table show all mammary gland results). In fact, the results appear even less impressive when combining adenomas and carcinomas: 1/70, 2/70, 2/71, 0/70, 4/70 in control-1, control-2, LD, MD, and HD, resp.

2) There was no clear indication of an earlier latency for occurrence of the carcinomas at HD. (One each found in HD animals sacrificed in moribund condition on days 449 and 579, resp; one each found in 2 HD animals at terminal sacrifice [~day 728]. The one carcinoma seen in controls was in an animal sacrificed in moribund condition on day 643, and the one seen at LD was in an animal found dead on day 609).

3) The HFD group had a (slightly) greater survival than controls.

4) The incidence at HD was in the published historical control range for this strain of mice and within the control range in studies performed by the sponsor. (The latter information was requested of the sponsor during the course of this review; the response, submitted 7/16/97, is attached [p. 46d-e]. Note that the studies summarized were conducted at 2 separate facilities as described).

5) There was no drug effect on mammary gland tumors in the rat carcinogenicity study.

From 1 YEAR DOG TOX. STUDY

6.15.2 Relationships Between Pharmacologic Effects of ENA 713 and Microscopic Lesions

Three morphologically unique lesions were noted in the intestinal tract of this study; a) an intussusception, b) subserosal smooth muscle degeneration, and c) subserosal nodules or foci of hemorrhage and granulation tissue. While the pathogenesis of these lesions is not clear, it is most probable that they reflect variable tissue responses to the exaggerated pharmacologic affects of ENA 713 at high doses. Clearly, the intussusception can be attributed to intestinal hypermobility and diarrhea caused by the pharmacologic affects of ENA 713. The relationship between the other lesions and the pharmacologic effects of ENA 713 is more tenuous, but there is precedent for this hypothesis in the literature. For example, minoxidil and several chemically unrelated hypotensive drugs all cause right atrial hemorrhage and fibrovascular proliferation (granulation tissue) in dogs[5]; a lesion morphologically very similar to the subserosal intestinal nodules noted with ENA 713. A common pathway of vascular injury and hemorrhage with subsequent repair was postulated for these chemically diverse but pharmacologically-related hypotensive drugs.[5] Further, the lesions were site specific in different species and species specific variations in vascular perfusion and the pharmacologic responses may determine the incidence and location of the lesions[5]. For example, in the pig the left atrium is the preferred site[5]. These hypotensive drug-related lesions are not known to occur in man[6].

The ENA 713 induced granulation tissue appeared to be site specific in this study; being limited to the subserosa of the colon approximately 15 cm from the anus. This region of the colon is perfused by the highly variable terminal anastomoses of the ileocolic artery and left colic arteries in the dog[7] in a manner much like the human[8]. The tenuous vascular supply to this region of the colon is fundamental in the pathogenesis of ischemic bowel disease in man, and the limited vascular supply to the rectum is a critical in the pathogenesis of rectal strictures in swine[9]. It is plausible that the intestinal hypermobility and other local pharmacologic effects of ENA 713 could both increase the metabolic activity of the tissues and alter tissue perfusion, resulting in focal loss of vascular integrity, hemorrhage and fibrovascular repair or, as noted in the moribund sacrifice, smooth muscle degeneration.

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[5]Pathogenesis of Cardiovascular Alterations in Dogs Treated with Minoxidil. G.M. Mesfin, et al., Toxicologic Pathology, 17:164-181, 1989.

[6]Review of Cardiovascular Findings in Humans Treated with Minoxidil. Joseph T. Sobota, Toxicologic Pathology, 17:193-202, 1989.

[7]Miller's Anatomy of the Dog. Chapter 11. The Heart and Arteries. H.E. Evans and G.C. Christensen, W.B. Sanders Company 1979.

[8]The Large Intestine, J.R. Jass, in: Systemic Pathology, Volume 3, edited by B.C. Moroson. Churchhill Livingstone (1987). Report #T-2-8/1/90.

[9]The Pathogenesis of Porcine Rectal Stricture. II. Experimental Somonellosis and Ischemic Proctitis B.P. Wilcock and H.J. Olander. Veterinary Pathology 14:43-55 (1977).

Page 46a

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

Raw Data

2 pages

SPONSOR'S HISTORICAL CONTROL

QUESTION: Please provide historical data for mammary adenomas and adenocarcinomas in CD1 mice from the same facility that performed the carcinogenicity study which is included in the NDA submission.

RESPONSE: Historical data for mammary adenomas/adenocarcinomas were collected for studies conducted at . The data from the e are provided due the small number of carcinogenicity studies which have been conducted at East Hanover and does not provide an adequate historical base. This was due to a new building being built in , and therefore no carcinogenicity studies were started between 1992 and 1996 at that site. Data from the two facilities are presented in the attached tables, separated by site.

TABLE 1: Historical Data from Studies in CD1 Mice Conducted at the

Study (Dosing start)	Mammary Adenoma (##/## examined)	Mammary Adenocarcinoma (##/## examined)
Study A (Aug, 86)		
Control 1	0/70	2/70
Control 2	0/70	1/70
Study B (Jun 87)		
Control 1	0/61	0/61
Control 2	0/67	2/67*
Study C (Nov 90)		
Control 1	0/78	0/78
Control 2	0/79	0/79

* Listed as carcinoma in report. A contract pathologist was used to read this study and used NTP critena, therefore, adenocarcinoma and carcinomas were not listed separately but were lumped together. Histopathology review of slides completed for this listing indicates both tumors were adenocarcinomas.

CONT'D

SPONSOR'S HISTORICAL CONTROL (CONT'D)

TABLE 2: Historical Data from Studies in CD1 Mice Conducted a'

Study	Mammary Adenomas (##examined)	Mammary Adenocarcinomas (## examined)
Study A (Jan., 1984)	0/79	1/79
Study B (Jun., 1984)	0/59	0/59
Study C (Oct., 1984)	0/59	1/59
Study D (Feb., 1985)	0/59	2/59
Study E (Mar., 1986)	0/57	3/57
Study F (Apr., 1987)	0/56	3/56
Study G (Oct., 1988)	0/70	0/70
Study H (Dec., 1990)	0/60	0/60
Study I (Mar., 1991)	0/67	2/67
Study J (Feb., 1993)	0/54	1/54
Study K (Mar., 1993)	0/64	0/64
Study L (Mar., 1995)	1/67	4/67

Page 46 e

LABELING:

A) In the Description and Clinical Pharmacology sections it is stated that Exelon is a "brain region selective" inhibitor of acetylcholinesterase. The latter section also states that the drug "selectively increases the availability of acetylcholine in the cortex and hippocampus." These statements are made based on various studies in rats. The brain regions studied were generally cortex, hippocampus, striatum, and pons/medulla. An in vitro study showed no regional selectivity regarding enzyme inhibition. (Incidentally, neither this nor the following studies distinguished between acetylcholinesterase and butyrylcholinesterase). Several ex vivo studies on enzyme inhibition using various routes of administration were performed; in general these studies did show greater enzyme inhibition in cortex and hippocampus compared to that in other regions, although the differences were not pronounced and were not always seen. These studies used acute dosing; notably, in a study in which drug was given continuously (s.c. via mini-pump) for 14 days, the selectivity for cortex and hippocampus was less that seen after acute dosing. Studies on increases in acetylcholine (Ach) levels were less clear regarding selectivity. In one study, using p.o. dosing, effects were greatest in cortex, slightly less in hippocampus and striatum (which were approximately equal to each other), and absent in pons/medulla. However a second study (not mentioned in sponsor's Summary; see volume 1.19, p. 5-941 through 5-942) showed similar effects in cortex, hippocampus, and striatum (again, with no effects in pons/medulla) after p.o. dosing either acutely or for 9 days. Finally, in a study of Ach turnover (also not in sponsor's Summary; see vol. 1.19, pp. 5-924 - 5-926), the drug decreased turnover to the greatest extent in cortex; decreases in hippocampus and striatum were equal to each other. (Decreased Ach turnover is likely a reactive effect to increased Ach levels). In summary, while there is some evidence for cortical/hippocampal selectivity of enzyme inhibition in rats, this selectively was not large, and appeared to decrease with subacute treatment. Furthermore, increases in Ach levels did not show the same pattern of selectivity, with one study (+ the turnover study) showing greatest effect is cortex but no difference between hippocampus and striatum, and another showing no differences between these 3 regions. (Note that increase in Ach level is a more distal event than inhibition of cholinesterase and is probably more relevant to the therapeutic effect of the drug, at least according to current theories).

It is thus recommended that mention of regional selectivity be omitted from the labeling. In addition to not being clearly established in rats as discussed above, I am not aware if there are any data in humans on this point. Also, including this in the labeling might imply a therapeutic advantage (e.g. fewer "non-specific" side effects) which has not necessarily been proven in clinical trials. (It is also noted that the proposed statements regarding brain region selectivity might be also read as indicating selectivity for brain vis a vis for the peripheral nervous system, with the implication of fewer side effects.).

B) In the Description section it is stated that the drug is a "pseudo-irreversible inhibitor" of acetylcholinesterase, and in the Clinical Pharmacology section it is stated that the drug "interacts with its target enzymes by forming a covalently bound complex that temporarily inactivates the

enzyme." Exelon is a structural analog of neostigmine and physostigmine, drugs which are believed to act in this way, however I did not locate any data to support this as the mechanism for Exelon. The sponsor should be requested to submit or indicate the location of this data.

C) In the Clinical Pharmacokinetics section, under Metabolism, it is stated [redacted] However, as discussed elsewhere in this review (see "Summary" section, discussion of ADME/PK data), according to the study report a quantitative comparison between this metabolite and parent drug could not be adequately made due to mechanistic differences. In one "arbitrary" assay the IC 50 of the metabolite was about 7 times greater than that of the parent. However, as noted earlier, it is not clear if these 2 compounds were compared in the same assay; it was also noted that the assay does not distinguish between acetyl- and butyrylcholinesterase. Furthermore, the enzyme source used was rat striatum, a region theoretically not primarily involved in the therapeutic action of the drug. Finally, the role of the metabolite in inhibiting cholinesterase in humans also depends on its levels in blood and tissues compared to that of the parent compound. At least in the animal studies, circulating levels of this metabolite were much higher than those of the parent compound, often by more than the 7-fold difference in potency noted above.

It is thus recommended that the proposed statement in the labeling be omitted or modified.

D) The Carcinogenesis, Mutagenesis, Impairment of Fertility section is adequate, with the following exceptions:

1) T[redacted]

2) It is stated that the drug had no effect on "fertility, reproductive performance, in utero and postnatal growth and development . . . ." However, whereas the Segment I study showed no drug effects on fertility, as noted earlier in this review decreased fetal and/or pup weights were seen in the segment I and other reproduction studies. Perhaps this section should just mention the lack of effect on fertility; the decreased fetal/pup weights can be mentioned in the Pregnancy section.

3) Whereas the sponsor's statement regarding the relative animal/human mg/m^2 doses are approximately correct, the exact values, obtained by my calculations using a 60 kg human, are 0.65x human for mouse and 0.89x human for rat; a decision on whether to use these more exact values can be made at a later time.

E) Pregnancy Section

1) Although the studies did not reveal any teratological effects, as noted above several

studies showed decreased fetal and/or pup weights; this should be mentioned in this section. (The dose at which this occurred varied across studies; in the Segment I study it was seen at the lowest dose tested [0.1 mg/kg free base]).

2) The sponsor has proposed using pregnancy category "C" although the reason for doing this is unclear since no adverse reproduction effects are mentioned. As noted above, decreases in fetal/pup weights were seen, although in view of the facts that (a) these were relatively slight and usually associated with maternal toxicity and (b) there were no drug-related teratological effects and no drug effects on fetal deaths or pup survival, the most appropriate category is "B".

3) The multiple of the human dose on a mg/m^2 basis for rabbit is closer to 4, rather than 2 as specified by the sponsor. (3.7 by my calculations for a 60 kg human).

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This section also states that tertiary anticholinergics such as atropine may be used for Exelon overdosage. It is not clear if this recommendation is based on human data. A brief summary of a dog study did state that atropine reversed several "muscarinic" signs of Exelon in dogs, although muscle fasciculation, a nicotinic sign, was not reversed. It is also possible, at least on theoretical grounds, that atropine could exacerbate some nicotinic effects (cardiovascular?) which might be produced by high doses of Exelon . Perhaps this section could be modified to mention these considerations.

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDATIONS:

This NDA is approvable. Comments on the proposed labeling are made above. The sponsor should submit, or indicate the location of, data supporting the statements in the proposed labeling that the drug is a "pseudo-irreversible inhibitor" of acetylcholinesterase and that it "interacts with its target enzymes by forming a covalently bound complex that temporarily inactivates the enzyme".

/s/

Barry N. Rosloff, Ph.D.

Attachments (3)

cc: NDA 20-823, original jacket + division file
Rosloff
Fitzgerald
Nighswander

APPEARS THIS WAY
ON ORIGINAL

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

40 pages

IND STUDY

5. NONCLINICAL PHARMACOLOGY AND TOXICOLOGY SUMMARY

A. PHARMACOLOGY STUDIES SUMMARY

1. PREFACE AND SUMMARY TABLES

Alzheimer's disease (AD) is a degenerative disorder of the CNS with severe consequences. The existence of an underactive central cholinergic system measured postmortem in AD brains has led to the so called "cholinergic deficit hypotheses" and further to clinical trials of cholinergic drugs to attempt to reverse the deficit. The rationale underlying utilization of acetylcholinesterase (AChE) inhibitors in treatment of AD is based on the assumption that inhibition of AChE results in reduced rates of removal of acetylcholine (ACh) from the synaptic cleft, thereby increasing the probability for effective encounter between neurotransmitter and the reduced numbers of muscarinic receptors in this disease. In the history of experimental clinical trials, compounds exerting different mechanisms of enzyme inhibition were investigated. Following the approval of tacrine by the FDA, based on studies where palliative improvement in AD patients was shown, hope arises to discover AChE inhibitors exerting more efficacy and less toxic effects.

The miotine derivative, called SDZ ENA 713, a carbamate, is a potent preferentially centrally acting acetylcholinesterase inhibitor (AChE-I). The compound was chosen based on criteria of brain selectivity, long-lasting activity in vivo and good tolerability.

The in vitro profile of SDZ ENA 713 is summarized in the Summary Table I and the pharmacological properties derived from in vivo experiments are summarized in Summary Table II.

Most attention was given regarding the main action of the drug: acetylcholinesterase inhibition and acetylcholine accumulation as well as cholinergic effects.

Following systemic administration of SDZ ENA 713 the AChE activity measured ex vivo was inhibited in several brain regions. The effect of SDZ ENA 713 on the enzyme was more pronounced in the hippocampus and cortex, areas known to be particularly affected in Alzheimer's disease, than in the striatum and pons/medulla. In contrast, physostigmine inhibited AChE to an equal degree in all brain regions.
A similar regional selectivity was also seen with regard to the enhancement of ACh levels following administration of SDZ ENA 713.

SDZ ENA 713 synchronized rhythmical slow wave activity (RSA, theta waves) in the hippocampal EEG in rats at a threshold dose of 75 µg/kg both i.p. and p.o. The activation of the slow-rhythmic hippocampal EEG by SDZ ENA 713 is a reflection of increased muscarinic activity in the hippocampus. In contrast to physostigmine and Tacrine, the influence of SDZ ENA 713 on the EEG was long-lasting after a single application.
No clear peripheral side effects, such as salivation or diarrhoea were seen with doses which inhibited central AChE activity by 40 to 50%.

SDZ ENA 713, in a dose range of 0-1.5 mg/kg i.v. had no effect on circulatory

parameters in the anaesthetized cat., in contrast to physostigmine. SDZ ENA 713, at the dose of 0.75 mg/kg i.v. clearly expressed signs of central cholinergic stimulation.

Although SDZ ENA 713 itself did not evoke bronchospasm in the guinea-pig at a dose of up to 1 mg/kg i.v., it potentiated ACh induced bronchospasm in vivo when given at a dose of 0.1 mg/kg i.v. For this reason the drug will be contraindicated in asthmatic patients, like other cholinomimetic agents.

In conclusion, SDZ ENA 713 is an AChE-I of the carbamate type. It's main preclinical properties are:

-high central to peripheral cholinergic activity ratio,
-selectivity for cortical and hippocampal brain regions,
-prolonged duration of action and
- low activity on cardiovascular system at centrally active doses.

The CNS selectivity of SDZ ENA 713 relative to other AChE inhibitors, suggests that the compound might have a minimal liability in regard to peripheral cholinergic effects and toxicity.

PHARMACOLOGY SUMMARY TABLE I
STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF SDZ ENA 713

PARAMETER	IC50	RELATIVE POTENCY (PHYSOSTIGMINE=1)
A.)<u>In vitro Effects of SDZ ENA 713</u>		
1.) Binding Striatal Membranes:		
Monoaminergic Receptors	inactive (IC50 > 10000 nM)	
Opiate receptors	inactive (IC50 > 10000 nM)	
Muscarinic receptors (non selective)	inactive (IC50 > 10000 nM)	
M1 (Pirenzepine)	inactive (IC50 ~5800 nM)	
2.) ACh Release Inhibiton		
relative Potency		0.01
3.) AChE Inhibition		
relative potency		
Rat		
Striatum		0.002
Cortex		0.002
Hippocampus		0.002
Pons/Medulla		0.002
3.) Mechanism of AChE Inhibiton	Active-Site-Directed, Pseudoirreversible Inhibition Mechanism (carbamate type, Time dependent).	

PHARMACOLOGY SUMMARY TABLE I (Continued)
STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF SDZ ENA 713

PARAMETER	TRESHOLD DOSE	ED50 or IC50
B. In vivo Effects of SDZ ENA713		
1. Behavioral effects		
Pimary Observation Test Rat (POT)		
acute		
Inhibition of Motor Activity	0.32 mg/kg p.o.	
Pupil Diameter	1 mg/kg p.o.	
Miosis	> 1mg/kg p.o.	
subchronic		
	similar as acute, Drug shows no cumulative nor additive effects on POT parameters	
Alert-Non-Mobile-Behavior in Mice (ANMB)		
SDZ ENA 713		
	2 mg/kg s.c.	
	0.5 mg/kg i.p.	
	0.5 mg/kg p.o.	
Physostigmine		
	0.06 mg/kg s.c	
	0.08 mg/kg i.p.	
	0.5 mg/kg p.o.	
Tacrine		
	1.6 mg/kg s.c	
	3.2 mg/kg i.p.	
	8 mg/kg p.o.	

PHARMACOLOGY SUMMARY TABLE I (Continued)
STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF SDZ ENA 713

PARAMETER	TRESHOLD DOSE	ED50 OR IC50
2.) Hippocampal EEG		
SDZ ENA 713		
Synchronization of Theta waves (Induction of slow rhythmic activity)	0.075 mg/kg p.o.	
Duration of Effect	> 6 hours	
3.) Biochemical effects		
AChE Inhibition ex vivo in Rat Brain		IC50
Cortex		0.5 mg/kg p.o.
Hippocampus		1.0 mg/kg p.o
PonsMedulla		2.0 mg/kg p.o.
Striatum		1.75 mg/kg p.o.
(Heart)		(8.75 mg/kg p.o.)
ACh Accumulation in Rat Brain		ED25
SDZ ENA 713: Cortex		0.7 mg/kg p.o.
Hippocampus		1.6 mg/kg p.o
Striatum		1.8 mg/kg p.o
Physostigmine: Cortex		0.3 mg/kg s.c.
Hippocampus		0.3 mg/kg s.c.
Striatum		0.3 mg/kg s.c
Tacrine:: Cortex		19 mg/kg p.o.
Hippocampus		17 mg/kg p.o.
Striatum		16 mg/kg p.o.

PHARMACOLOGY SUMMARY TABLE II
OTHER PHARMACOLOGIC EFFECTS OF SDZ ENA 713

	DOSE	BLOOD PRESSURE Change in %	HEART RATE Change in %	OBSERVATIONS
1.) Cardiovascular Effects				
A.) Anaesthetised Cat	0.01 mg/kg i.v. 0.3 mg/kg i.v. 1.5 mg/kg i.v.	+1 +5 +4	+1 0 0	 strong central effects tremor at 0.75 mg/kg
B.) Normotensive Awake Rat	1.9 mg/kg p.o. 5.6 mg/kg p.o.	+8 +29	-14 -17	
C.) Normotensive Squirrel Monkey	0.1 mg/kg p.o. 0.3 mg/kg p.o. 1.0 mg/kg p.o.	1hr 5hrs -5 -4 -5 -12 +14		SlightVomiting/Tremor Vomiting/Tremor Vomiting/Tremor
2. Pulmonary Effects	DOSE RANGE		OBSERVATIONS	
Airway Resistance SDZ ENA 713 alone SDZ ENA 713 added to ACh	0.001 - 1 mg/kg i.v 0.1 mg/kg i.v. + ACh 3.2 - 10 µg/kg i.v.		No Influence on Airway Resistance ACh Effect potentiated	

2. PHARMACOLOGY STUDIES RELEVANT TO THE THERAPEUTIC ACTIVITY OF SDZ ENA 713.

2.1 IN VITRO PROFILE

In this section the pharmacological in vitro profile of SDZ ENA 713 is described: the interaction with various receptors, the effects in functional tests such as inhibition of ACh-release evoked by electrical stimulation and its properties as an AChE-I

2.1.1 RADIOLIGAND BINDING STUDIES. [Doc. # 103-107]

2.1.1.1 Methods

Ligand binding studies were carried out using membranes of rat brain (Sandoz OFA-strain, 150-200 g) or calf caudate. Preparations of membrane and ligand binding experiments were performed as described previously (Closse et al. Naunyn Schmiedeberg's Arch.Pharmacol.327, 95-101,1984; Palacios et al. Europ.J,Pharmacol. 125, 45-62, 1986; Urwyler S. and Markstein R., J.Neurochem. 46, 1058-1067,1986; Billard W. et al. Life Sci. 35, 1885-1893, 1984; Closse A. et al. J.Neural Transm. 62,231-248, 1984; Urwyler S. and Coward D., Naunyn-Schmiedeberg's Arch.Pharmacol. 335, 115-122, 1987; Pert C.B. and Snyder S.H., Science 179, 1011-1014, 1973.).

2.1.1.2 Results

The compound SDZ ENA 713 shows no interaction with muscarinic receptors in vitro. This is concluded from the data obtained in binding studies using two different muscarinic ligands : the M1 selective antagonist 3H-pirenzepine (3H-Pir) and the nonselective agonist 3H-cis-methyldioxalan (3H-CD). Neither the binding of 3H-Pir nor that of 3H-CD could be displaced by SDZ ENA 713. Radioligand studies performed with α-and β-adrenergic, dopaminergic, serotoninergic and opiate ligands gave no indication of an interaction with monoaminergic or opiate recognition sites. In contrast to SDZ ENA 713, Tacrine shows a moderate affinity to the two muscarinic binding sites (Table A-1).

TABLE A-1

INTERACTION OF SDZ ENA 713 WITH VARIOUS RECEPTORS IN VITRO

^{3}H-Ligand	Receptor	IC_{50} nM
PIRENZEPINE	Muscarine M1	5'800
cis-METHYLDIOXALAN	Muscarine (nonselective)	> 10'000
PRAZOSIN	α-1	> 10'000
CLONIDINE	α-2	> 10'000
CGP 12177	β-1/-2 (Cortex/Cerebellum)	> 10'000
8-OH-DPAT	5-HT-1A	> 10'000
MESULERGINE	5-HT-1C	> 10'000
KETANSERINE	5-HT-2	> 10'000
ADTN	Dopamine D1	> 10'000
SCH 23390	Dopamine D1	> 10'000
SDZ 205-501	Dopamine D2	> 10'000
SPIPERONE	Dopamine D2	> 10'000
NALOXONE	Opiate	> 10'000

(Data from Screening Report, S. Urwyler, November 20. 1987)

2.1.2 EFFECT OF SDZ ENA 713 ON ELECTRICALLY EVOKED 3H-ACh RELEASE FROM RAT HIPPOCAMPUS SLICES. [Docs. # 103-107]

2.1.2.1 Methods.

Superfusion experiments were performed as described by Supavilai and Karobath (Life Sci.,36,417-426,1985). Rat hippocampal slices (SANDOZ Wistar strain, 180-200g) were loaded with 3H-ACh by incubation with 3H-choline. Two periods of electrical stimulation (2 Hz rectangular pulses 2 msec, 10 mA, 2 min) were applied after 70 min (S1) and after 125 min (S2) of superfusion. Drug effects on stimulation evoked tritium outflow are expressed as the ratios S2/S1.

2.1.2.2 Results

Electrically evoked 3H-ACh release from rat hippocampal slices is an in vitro functional model which can be used to investigate presynaptic muscarinic autoreceptor agonists and antagonists. In addition this model can also be used as an indirect method to evaluate drugs which inhibit AChE. Inhibition of AChE activity leads to the accumulation of endogenous ACh which then interacts with presynaptic muscarinic autoreceptors and inhibits further release of 3H-ACh. SDZ ENA 713 (100 µM) inhibits electrically evoked 3H-ACh release from

hippocampal slices by approximately 40 % (Table A-2). The inhibitory effects of SDZ ENA 713, like that of physostigmine or Tacrine, can be antagonized by atropine. These results are compatible with the proposed action of SDZ ENA 713 as an AChE-I. In this model SDZ ENA 713 was approximately 100 times less potent than physostigmine, and 10 times less potent than Tacrine.

TABLE A-2

INFLUENCE OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE ON BASAL AND ELECTRICALLY EVOKED ACh RELEASE IN RAT HIPPOCAMPAL SLICES

Drug	Basal ACh release(% of control)	electr.evoked ACh release(% of control)	n
Control	100 ±6.8	100 ±5.8	18
Atropine 1 µM	99.4±1.9	116.5±2.7	8
SDZ ENA 713			
100 µM	93.4±1.9	59.6±2.1	4
SDZ ENA 713 100 µM + 1 µM Atropine	93.1±2.1	104.6±7.8	4
Physostigmine			
0.1 µM	94.5±0.5	67.0±4.5	4
1.0 µM	90.4±1.8	45.7±2.6	14
10.0 µM	83.7±2.1	37.4±3.1	4
Phys+Atropine 1 µM+1 µM	103.0±2.0	122.4±3.1	4
Tacrine			
0.1 µM	103.7±1.3	81.6±2.2	4
1.0 µM	93.3±4.8	62.8±2.3	4
10.0µM	85.4±1.8	53.3±5.7	4
Tacrine+ Atropine 10 µM+ 1 µM	87.8±2.7	114.1±7.4	4

Values in % of control ±SEM

2.1.3 AChE INHIBITION IN DIFFERENT ENZYME PREPARATIONS FROM RAT BRAIN IN VITRO [Doc. # 103-107]

The mechanism of hydrolysis of ACh into choline and acetic acid by AChE is well established. The enzyme interacts with its substrate on two distinct sites. One site is responsible for recognition and fixation of ACh (aniqnic site), whereas the other so called catalytic or esteratic site is responsible for the degradation of ACh. Hence, different sites for the inhibitory action of cholinesterase inhibitors are possible: SDZ ENA 713 and physostigmine, as carbamates, interact directly with the catalytic site. They react chemically with this site resulting in a carbamylated enzyme, which slowly breaks down to regenerate free enzyme. In contrast our second reference compound Tacrine interacts with the recognition (anionic) site in a mixed non competitive-competitive manner (Berman and Leonard, Mol.Pharmacol. 41:412-418,1992).

2.1.3.1 Method

The activity of AChE was measured according to the method described by Ellman (Arch.Biochem.Biophys. 82,70,1959). Briefly rat brain tissue was homogenized in cold phosphate buffer pH 7.3 0.25 mM containing 0.1 % of Triton X-100. After centrifugation aliquots of the clear supernatant were used as enzyme source. The enzyme was preincubated with different concentrations of inhibitor. After different times substrate (acetylthiocholine jodide 0.5 mM) was added and the remaining activity determined.

2.1.3.2 Results

The IC_{50} values were arbitrarily measured after 15 minutes of enzyme preincubation in presence of the different inhibitors. No statistically significant differences in IC_{50} values of SDZ ENA 713 were found using AChE preparations of different rat brain regions (Table A-3). The values for cortex and hippocampus were slightly lower compared to that for striatum and pons/medulla. Physostigmine acts about 600 to 700 times more potently in the same enzyme preparations.

TABLE A-3

AChE-INHIBITION BY SDZ ENA 713 AND PHYSOSTIGMINE IN DIFFERENT RAT BRAIN REGIONS IN VITRO

Enzyme Source (Rat Brain)	SDZ ENA 713 $IC_{50} \times 10^{-5}M$	Physostigmine $IC_{50} \times 10^{-8}M$	Tacrine $IC_{50} \times 10^{-7}M$
Cortex	1.7	3.3	1.3
Hippocampus	1.5	2.7	1.5
Striatum	2.0	3.3	1.0
Pons/medulla	2.0	3.1	1.2

IC_{50} values after 15 min preincubation. Supernatant of fresh tissue homogenates (1% Triton X-100) were used as enzyme source (N=5).

The values for Tacrine cannot be compared directly with those for SDZ ENA 713 or physostigmine because of the different mechanism of action, but it inhibited AChE activity to at least a 100 times higher degree measured under identical experimental conditions.

2.2 IN VIVO STUDIES TO DEFINE THE ACTIVITY OF SDZ ENA 713

All in vivo experiments described hereafter were performed in accordance with the Swiss animal protection law. The studies employed mice, rats, guinea-pigs, rabbits, cats and monkeys bred at SANDOZ Ltd. Basle or purchased from licensed , commercial breeders. The experiments were performed with the hydrogentartrate form of SDZ ENA 713. The drug solutions were freshly prepared before use in physiological (0.9%) saline or in distilled water. The doses are expressed in mg/kg of the free base. The pharmacological comparison of drug effects in terms of µmoles/kg is possible by multiplying the mg/kg dose by the following conversion factors : SDZ ENA 713=> 4; physostigmine=> 3.5 and Tacrine=> 5.

2.2.1 BEHAVIORAL EFFECTS Of SDZ ENA 713

2.2.1.1 PRIMARY OBSERVATION TEST (POT): ACUTE AND SUB-CHRONIC (5 DAYS) EFFECTS IN MICE [Doc. # 103-107]

2.2.1.1.1 Method

As an initial screening procedure groups of 3 male mice received single doses of SDZ 212-173 or reference compound p.o. or s.c. The mice were observed for one hour at 1, 7 and 22 hours after drug application and a symptom check-list completed according to methods based on those of Irwin (Psychopharmacologia,13,222-257,1968). Chronic effects were tested by treating groups of 4 mice with daily doses of drugs followed by the observation procedure after the first and 5th drug application.

2.2.1.1.2 Results

Acute effects

The results with SDZ ENA 713 and the standard drugs physostigmine and Tacrine are summarized in Table A-4. The profile observed with SDZ ENA 713 in the POT was qualitatively similar to that of physostigmine (Tables A-4 and A-5). Motor activity was inhibited by SDZ ENA 713 starting with 0.32 mg/kg orally. Physostigmine exerted similar effects with the same dose. The dose of 1 mg/kg p.o. SDZ- SDZ ENA 713 induced a slight increase of pupil diameter (+10%), whereas the higher dose produced the expected miosis (3.2 mg/kg -> -40%). The miotic effect of SDZ ENA 713 was, with the exception of the low dose effect,

comparable to that observed after physostigmine. SDZ ENA 713 and physostigmine induced hypothermia potently in mice. In summary, the influence of SDZ ENA 713 on POT parameters was about 2-3 times weaker compared to physostigmine. The acute influence of Tacrine on POT parameters was weaker compared to those of the carbamates: 30 times less potent than physostigmine and about 70 times less active than SDZ ENA 713.

TABLE A-4

ACUTE EFFECTS OF SDZ ENA 713 AND REFERENCE COMPOUNDS IN THE MOUSE POT

Drug	Parameter	mg/kg p.o. 0.1	0.32	1.0	3.2	10	32
SDZ ENA 713 p.o.	Motor activity		↙	↙↙	↙↙↙		
	Locomotion		0	↙↙	↙↙		
	Reactivity		0	(↙)	↙↙		
	% Pupil diameter		0	+10	-40		
	°C body temp.		-0.4	-2.1	-9.9		
Physo-stigmine s.c.	Motor activity	0	(↙)	↙	lethal		
	Locomotion	0	(↙)	↙			
	Reactivity	0	(↙)	(↙)			
	% Pupil diameter	-44	-26	-59			
	°C body temp.	-0.7	-0.7	-5.0			
Tacrine p.o.	Motor activity				(↗)	(↗)	↗↗
	Locomotion				0	(↗)	↗
	Reactivity				(↗)	0	↗
	% Pupil diameter				-18	-55	
	°C body temp.				-0.1	-0.2	-5.4

(↗) = Trend , ↗ = Clear effect ↗↗ = Moderate effect
↗↗↗ = Marked effect (direction indicated)

Subchronic effects

The POT parameters were determined after subchronic administration of the different AChE inhibitors to determine whether tolerance to the effects seen after acute administration would occur, or whether enhanced drug effects would be seen due to accumulation. The results with SDZ ENA 713 and the standard drugs physostigmine and Tacrine are summarized in Table A-5. SDZ ENA 713 with the same dose 0.32, 1 and 3.2 mg/kg p.o. exerted the same profile in the POT as described above. With the higher doses, 1 and 3.2 mg/kg, SDZ ENA 713 inhibited motor activity, and elicited intention tremor after the first as well as the fifth administration (Table A-5). Simultaneously, hypothermia was recorded (ED -3°C about 1.25 mg/kg). The effect of SDZ ENA 713 on body temperature was not altered after its fifth application. Physostigmine induced a small reduction of motor activity with the dose of 1 mg/kg p.o. after the first administration, whereas Tacrine exerted comparable effects with the dose of 10 mg/kg p.o. After 5 days of

treatment no influence on this parameter with either physostigmine nor Tacrine was observed. The influence on body temperature by these drugs was small compared to SDZ ENA 713 at the doses tested. From these experiments, the conclusion can be drawn that SDZ ENA 713 shows no cumulative or additive effects on POT parameters. A possible tolerance, similar to that seen with physostigmine and Tacrine to the behavioral effects cannot be excluded. No tolerance to the hypothermic response to SDZ ENA 713 was seen after 5 days of administration.

TABLE A-5

SUBCHRONIC EFFECTS OF SDZ ENA 713 AND REFERENCE COMPOUNDS IN THE MOUSE POT

Drug	Parameter	60 min after 1.application	60 min after 5.application
SDZ ENA 713 p.o.	Dose mg/kg	0.32 1.0 3.2	0.32 1.0 3.2
	Motor activity	0 ↙ ↙↙	0 (↙) ↙↙
	Behavior	0 (↙) ↙↙	0 0 ↙
	Muscle tonus	0 (↙) (↙)	0 0 ↙
	% Pupil diameter	0 0 +33	+8 -25 0
	C body temp.	-0.1 -2.2 -7.5	+0.1 -1.8 -6.9
Physo-stigmine s.c.	Dose mg/kg	0.28 1	0.28 1
	Motor activity	0 ↙	0 0
	Behavior	0 0	0 0
	Muscle tonus	0 0	0 0
	% Pupil diameter	-15 -23	-33 -7
	C body temp.	-0.3 -1.3	-0.1 -0.7
Tacrine p.o.	Dose mg/kg	3.2 10	3.2 10
	Motor activity	0 ↙	0 0
	Behavior	0 0	0 0
	Muscle tonus	0 0	0 0
	% Pupil diameter	-15 -31	-27 -47
	C body temp.	-0.3 -0.3	0 0

(↙) = Trend , ↙ = Clear effect ↙↙ = Moderate effect
↙↙↙ = Marked effect (direction indicated)

2.2.1.2 EFFECTS OF SDZ ENA 713 ON ALERT-NON-MOBILE-BEHAVIOR IN MICE [Doc. # 103-107]

2.2.1.2.1 Method

The effects of the administration of AChE-I on several components of the locomotor and exploratory behavior of the mouse were examined and quantified

using the open-field system described by Palacios et al. (Europ.J,Pharmacol. 125, 45-62, 1986). One parameter, the induction of episodes of the so-called "alert-non-mobile- behavior (resting time) " according to Karczmar was chosen as an index of central cholinergic stimulation (Karczmar, A.G. In:D.J. Jenden (Ed.), Cholinergic Mechanism and Psychopharmacology, Plenum Press, New York, 1978, pp 679-708.).

2.2.1.2.2- Results

Centrally acting cholinergic agents induce stimulation of the ANMB. Orally administered SDZ ENA 713 was found to be equipotent with physostigmine (p.o.) in inducing this behavior (Table A-6). The ED_{50} for SDZ ENA 713 was 0.5 mg/kg p.o., this being 16 times lower than that for Tacrine, 8 mg/kg p.o. SDZ ENA 713 was similarly active after i.p. application and about 4 times weaker after s.c. administration. A comparison of SDZ ENA 713 with the muscarinic agonists RS 86 and SDZ 210-086 shows a similar effect for RS 86, whereas SDZ 210-086 was about 6 times more active in stimulation of ANMB.

TABLE A-6

COMPARISON OF POTENCIES OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE IN INDUCTION OF ALERT-NON-MOBILE BEHAVIOR IN MICE

Drug	ED_{50} mg/kg for ANMB		
	s.c.	i.p.	p.o.
SDZ ENA 713	2	0.5	0.5
Physostigmine	0.06	0.08	0.5
Tacrine	1.6	3.2	8.0
RS 86* SDZ 210-086*		0.38 0.08	

ANMB=Alert-Non-Mobile-Behavior (resting time)Time of observation after drug administration :15 min. (*: non-selective muscarinic agonists)

2.2.1.3 EFFECTS ON EXPLORATION BEHAVIOR DISTURBED BY SCOPOLAMINE [Doc. # 103-157]

The abilities of SDZ ENA 713 and tacrine to reverse scopolamine-induced disruption of exploration pattern was examined in a test where rats explored a circular swimming pool (Kelly and Malanowski, Can J Physiol Pharmacol, 71, 352-364,1993). This test was chosen since disturbances of everyday activities are distressing in Alzheimer's disease and the test can be considered as a model where ability to normalize an "everyday activity", exploration, disrupted by hypocholinergic function, is measured. The various scopolamine-induced alterations can also be interpreted as due to impairments in memory functions

(Kelly and Malanowski, Can.J.Physiol Pharmacol, 71, 352-364,1993).

2.2.1.3.1 Methods

Rats were placed in a circular, black swimming pool in a dimly-lit room and the exploration pattern quantitated as described in detail elsewhere (Kelly and Malanowski, 1993). All compounds were dissolved in saline and administered subcutaneously. Saline (1 ml/kg) or scopolamine (0.3 mg/kg) was injected 30 minutes before the first swim, and 15 minutes later saline-treated animals received a further injection of saline, while scopolamine-treated animals received either saline, SDZ ENA 713 (1, 3 or 10 mg/kg, see figure key/legend) or tacrine (3,10 or 30 mg/kg, see figure key/legend). Rats were placed in the pool for a second swim 3 days later.

2.2.1.3.2 Results

In the first swim habituation of perimeter preference was partly normalized by both SDZ ENA 713 and tacrine. SDZ ENA 713 was about three times more potent than tacrine (Figure A-1). Neither compound significantly normalized habituation of swimming speed in the first swim, nor the between-swim habituation of perimeter preference. The results indicate that SDZ ENA 713 is more potent than tacrine in normalizing certain behavioral disturbances resulting from cholinergic hypofunction.

FIGURE A-1

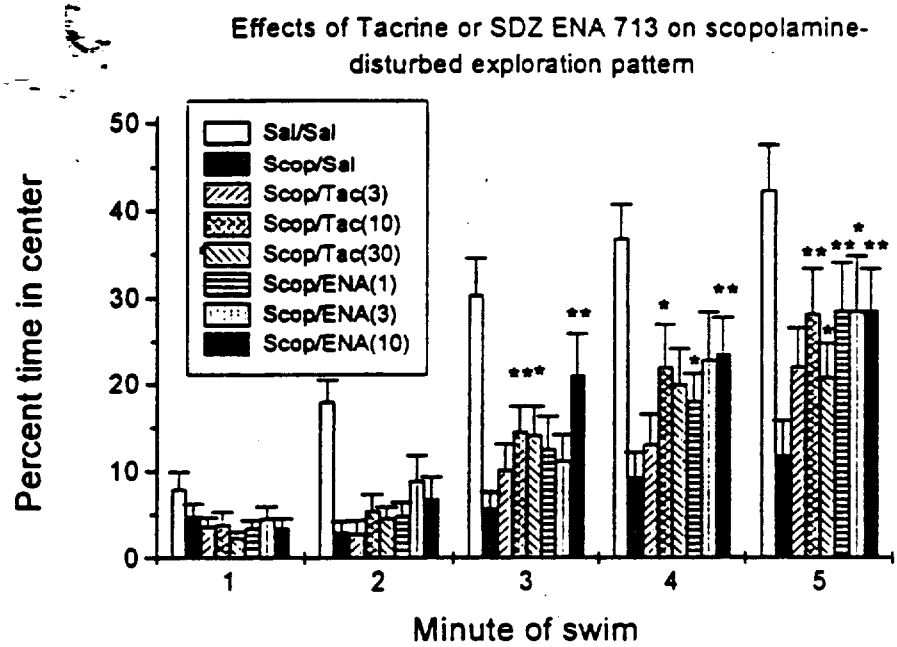

Figure A-1. Percentage of time spent in the central region for each minute of the first swim. Mean ± SEM for groups of 18-22 animals which received the indicated treatments are shown. Scopolamine hydrochloride (0.3 mg/kg s.c.) or saline vehicle was administered 30 min before the swim test, and tacrine, SDZ ENA 713 or saline vehicle 15 min before the swim test (doses indicated in parentheses in the key box of the figure are in mg/kg s.c.). * $p<0.05$, ** $p<0.01$ vs Scop/Sal (one-tail Mann-Whitney U-tests).

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2.2.2 EFFECTS OF SDZ ENA 713 ON HIPPOCAMPAL ELECTROENCEPHALOGRAM IN RAT [Docs. #103-107]

A characteristic property of a central muscarinic stimulation either by muscarinic agonists or by ACh is the induction of slow rhythmic activity in the hippocampal EEG (synchronization of theta-waves).

2.2.2.1 Method.

Experiments were performed according the methods described by P.Bevan in Br.J.Pharmac. 82,431-440,1984.

2.2.2.2 Results

After the i.p. and p.o. administration of SDZ ENA 713, an initiation of theta waves (0.2 - 4.2 Hz) and an increase in their amplitude was found. This synchronization of the hippocampal EEG was obtained with a threshold dose of 0.075mg/kg i.p. and p.o., which is similar to that for physostigmine (measured only after i.p. administration) and about 3 times lower than that of Tacrine. The activation of the energy in the theta-frequency band was dose dependent (Figures A-2a, A2b) and completely blocked by pretreatment with scopolamine, but not by the peripheral muscarinic receptor blocker N- methylscopolamine.The effects were accompanied by an increase in blood pressure (max.25%). The pattern of EEG synchronization is different from that expressed by muscarinic agonists. SDZ ENA 713 and physostigmine at doses higher than 0.75 and 0.28 mg/kg i.p. respectively were lethal. The influence of SDZ ENA 713 on theta waves in rat hippocampus after oral administration (0.75 mg/kg) was long-lasting (hours) in comparison with Tacrine (minutes) (up to 4 mg/kg p.o).

FIGURE A-2a
MAXIMAL AXCTIVATION OF RAT HIPPOCAMPAL EEG (theta-rhythm) AFTER i.p. ADMINISTRATION OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE

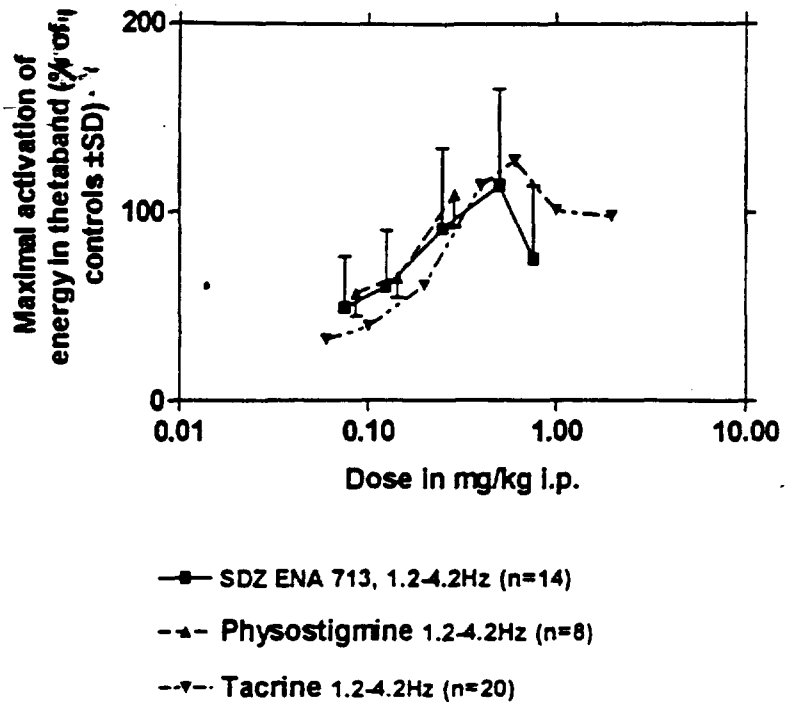
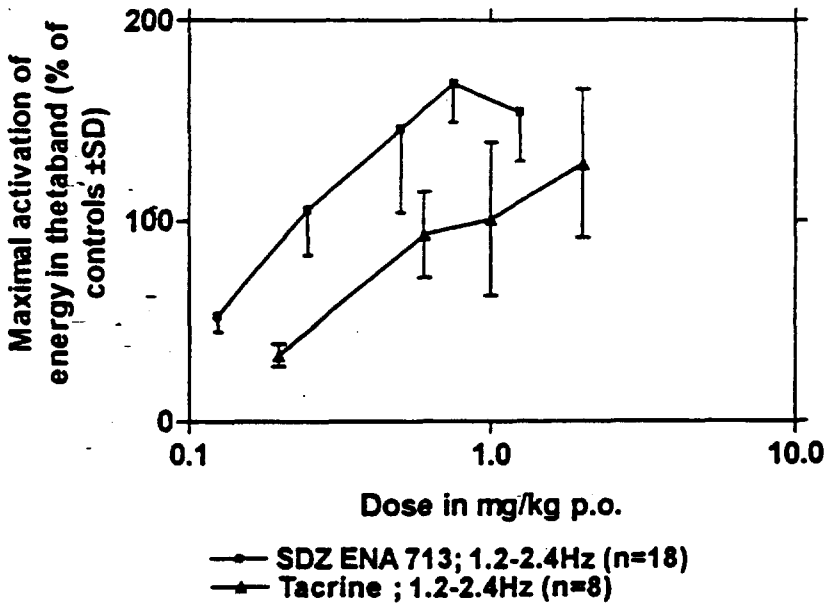


FiGURE A-2b

MAXIMAL AXCTIVATION OF RAT HIPPOCAMPAL EEG (theta-rhythm) AFTER p.o. ADMINISTRATION OF SDZ ENA 713 AND TACRINE



2.2.3 PERIPHERAL EFFECTS OF SDZ ENA 713

2.2.3.1 SALIVATION IN ANAESTHETIZED MICE [Doc. #103-107]

2.2.3.1.1 Method

Male OF1 mice were anaesthetized with pentobarbital (50 mg/kg i.p.) and placed prone with their mouths on a preweighed filter paper. The saliva produced following 30 (15) minutes after administration was absorbed on the paper and weighed. The weight of the secreted saliva was corrected for the normal vaporization.

2.2.3.1.2 Results

The administration of SDZ ENA 713 caused no increase in salivation in anaesthetized OF-1 mice at the dose of 1.5 mg/kg i.p. (Table A-7). This dose is well above that at which central effects in the ANMB and POT tests were seen. Although moderately enhanced salivation was measured with the dose of 6.25 mg/kg i.p., additive interactions with the anaesthetic led to increased lethality at this dose. Tacrine in the dose of 1.6 mg/kg s.c. and physostigmine at 0.06 mg/kg s.c. also have influence on salivation under the chosen conditions, whereas most direct acting muscarinic agonists like RS86 or 210-086 induce such peripheral cholinergic signs with relatively lower doses.

TABLE A-7

COMPARISON OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE IN INDUCTION OF SALIVATION IN MICE

Drug	Dose mg/kg i.p.	Salivation saliva mg/30 min, Mean±SEM (n)
Saline	0	0.3 ±0.3 (12)
SDA ENA 713	1.55	0.5 ±0.3 (6)
	6.25	67 ±15 (6)
Physostigmine	0.07	0.2 ±0.2 (6)
	0.19	80 ±21 (6)
Tacrine	1.56	0 (6)
RS86	0.38	304 ±67 (6)*
SDZ 210-086	0.08	67 ±22 (6)*

* The production of saliva induced by the two muscarinic agonists was measured between 0 and 15 minutes after drug administration.

2.2.4 BIOCHEMICAL EFFECTS OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE

2.2.4.1 AChE INHIBITION EX VIVO BY SDZ ENA 713 IN DIFFERENT RAT BRAIN REGIONS [Doc. #103-107]

The pseudo-irreversible mechanism of AChE inhibition by the carbamates, SDZ ENA 713 and physostigmine (for details see Appendix) allows the measurement of inhibitory drug effects ex vivo: the remaining enzyme activity is a measure for the in vivo drug action.

2.2.4.1.1 Method

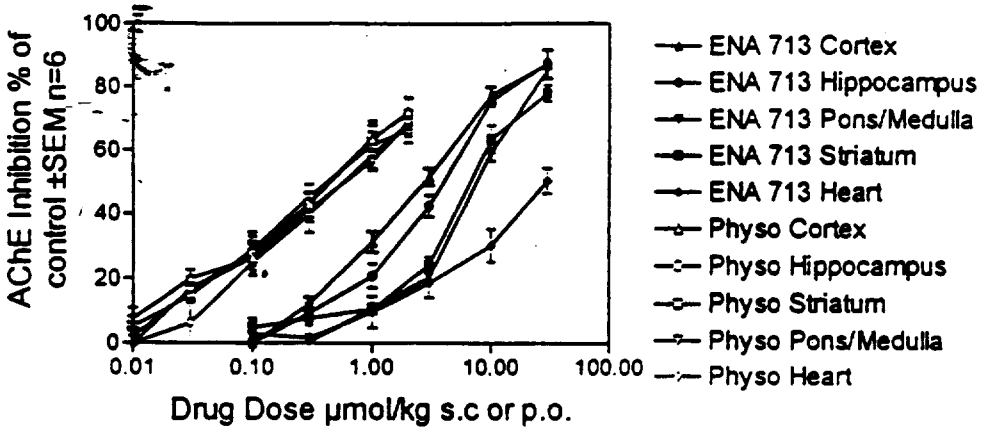
Rats received various single doses of SDZ ENA 713 and physostigmine p.o. and s.c. respectively 30 minutes before killing by decapitation. The AChE activity in the different brain regions was measured in supernatant aliquotes of 1% Triton X-100 extracts as described in Methods of part 2.1.3.1

2.2.4.1.2 Results

30 minutes after administration of different doses of SDZ ENA 713,the AChE activity in different rat brain regions was measured ex vivo. The IC_{50} values found were 2 mg/kg p.o. in pons/medulla, 1.75 mg/kg p.o. in striatum, 1 mg/kg p.o. in hippocampus and 0.5 mg/kg p.o. in cortex. In contrast, the potency of SDZ ENA 713 in inhibiting AChE in the heart, also measured 30 minutes after acute oral administration was considerably less: IC50 8.75 mg/kg p.o. (Figure A-3). Six hours after administration of SDZ ENA 713 (2.5 mg/kg p.o.) the AChE in striatum was still inhibited by 16± 6% and 20±6% in pons/medulla, whereas at the same time the activities in cortex and hippocampus were inhibited by 39±9% and 44±6% respectively. No inhibition was detected in the heart six hours after administration of the same dose (2.5 mg/kg p.o.) of SDZ ENA 713. It is of interest to note that no salivation was observed in animals receiving doses of SDZ ENA 713 of up to 1 mg/kg p.o.The corresponding IC50 values for physostigmine determined after s.c. administration in the same rat brain regions were: 0.27 mg/kg for pons/medulla; 0.28 mg/kg for striatum; 0.27 mg/kg for hippocampus and 0.22 mg/kg for cortex. Because of the different mechanism of enzyme inhibition, the ex vivo measurement of AChE inhibition after administration of Tacrine was not possible.

FIGURE A-3

ACETYLCHOLINESTERASE INHIBITION BY SDZ ENA 713 AND PHYSOSTIGMINE IN RAT BRAIN AND HEART *ex vivo*



2.2.4.2 ACHE ACTIVITY AFTER 14 DAYS CONTINUOUS TREATMENT WITH SDZ ENA 713 RELEASED BY MINI-PUMPS.[Doc. #103-107]

To determine the influence of continuous administration of SDZ ENA 713 for 14 days, mini-pumps were placed under the skin of rats and at the end of the experiments the activity of AChE measured in the different rat brain regions as well as in heart and blood. In addition, the effect of a challenge dose of SDZ ENA 713 s.c. administered on day 14 was measured.

2.2.4.2.1 Methods.

Alza-mini-pumps Nr. 1002 were filled with a solution of SDZ ENA 713 or saline and implanted under the skin of rats under halothane anaesthesia. The mini-pumps released 0.75 mg/kg per 24 hours continuously. At the end the , rats were grouped and injected with a challenge dose of 0.75 mg/kg s.c. SDZ ENA 713 or placebo 30 min before sacrificing by decapitation . AChE activity was determined as described under 2.1.3.1. The remaining solution was checked by thin-layer chromatography.

2.2.4.2.2 Results

The continuous release of SDZ ENA 713 (0.75 mg/kg per day, 0.46 l/hour) induced at the end of the experiment a marked inhibition of AChE in all brain regions (TableA-8). The inhibition was in the same range as after administration of a similar acute dose. The administration of a challenge dose to the chronic treated

animals led to a further decrease in AChE activity in all organs measured, indicating that neither hypo- nor hypersensitivity occurred. It is noted that brain region selectivity diminished after continuous administration of SDZ ENA 713. Furthermore selectivity of the drug for inhibition of AChE in brain rather than heart was lost after this application route.

TABLE A-8

AChE ACTIVITY IN VARIOUS BRAIN REGIONS, HEART AND PLASMA OF THE RAT AFTER CONTINUOUS TREATMENT WITH SDZ ENA 713 MINI-PUMPS 0.75 MG/KG PER DAY (14 DAYS)

AChE activity in % of controls SD n=7				
TREATMENT chronic => challenge => REGION	Placebo Placebo	Placebo SDZ ENA 713	SDZ ENA 713 Placebo	SDZ ENA 713 SDZ ENA 713
Striatum	100 ±6.4	78.7±7.2	64.9±3.3	28.6±7.9
Cortex	100 ±6.3	36.9±4.8	48.9±6.6	17.4±1.5
Hippocampus	100 ±7.1	45.3±9.3	52.8±4.1	19.7±1.6
Pons/Medulla	100 ±4.0	50.7±8.6	60.1±4.3	24.3±2.5
Heart	100 ±7.7	55.2±5.2	48.8±6.5	30.2±3.6
Blood	100±24	34.0±5.4	60.6±8.2	22.3±4.5

Control values (nmole/mg x min SD n=7) :
Cortex: 3.33 ± 0.21 Hippocampus: 4.92 ± 0.35 Striatum: 31.4 ± 2.01
Pons/Medulla: 8.52 ± 0.34 Heart: 2.48± 0.19 Plasma : 286.5 ± 69.0

2.2.4.3 ACH LEVELS IN VARIOUS RAT BRAIN REGIONS FOLLOWING SDZ ENA 713, PHYSOSTIGMINE AND TACRINE [Docs. #103-107]

2.2.4.3.1 Method.

Male OFA rats were used. The animals were killed after administration of the drugs by microwave-irradiation as described in Palacios et al. (Europ.J.Pharmacol.,125,45-62,1986). The levels of ACh in the different brain parts were analyzed by mass-fragmentography according to the method of Jenden et al. (Anal.Biochem. 55, 438-448, 1973).

2.2.4.3.2 Results

The effects of SDZ ENA 713, physostigmine and of Tacrine as AChE inhibitors in vivo was determined by measuring the levels of ACh in different regions of rat brain at various times after drug administration. A single application of SDZ ENA 713 (6.25 mg/kg p.o.) increased ACh concentrations in striatum, cortex and hippocampus, but had no effect on the levels in pons/medulla. The maximal effect was achieved about 30 min. after oral application and declined during the next 3-4 hours. In cortex and hippocampus the ACh levels were still significantly higher at 4 hours compared to controls. Physostigmine and Tacrine at the dose administered (0.28 mg/kg s.c. and 20 mg/kg p.o. respectively) enhanced brain ACh concentrations to a similar extent, and like SDZ ENA 713 did not affect the ACh content in pons/medulla. The effects of SDZ ENA 713, physostigmine and Tacrine were dose dependent as demonstrated in Figure A-4. With SDZ ENA 713 the highest accumulation was found in cortex with a threshold dose of 0.15 mg/kg p.o., followed by the effects in hippocampus and striatum (threshold doses approx. 10 times higher). Again, no influence on ACh content in pons/medulla could be detected at up to the highest dose tested (6.25 mg/kg p.o.). As shown in Figure A-4, physostigmine (s.c. injection) was the most active compound, followed by SDZ ENA 713 and Tacrine (p.o. administration). Interestingly, a regional selectivity was again found for SDZ ENA 713, in that it preferentially increased the ACh levels in the cortex after acute administration.