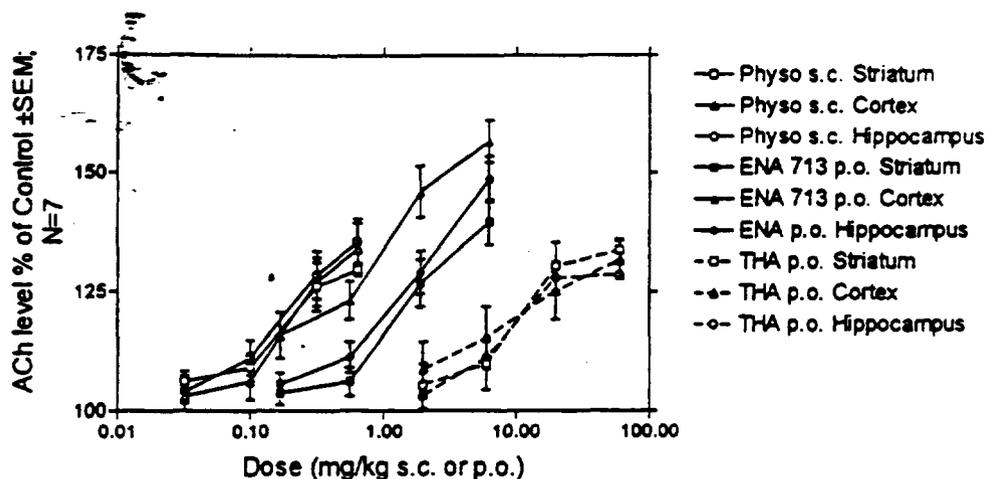


FIGURE A-4

ACETYLCHOLINE LEVELS IN RAT BRAIN 60 MIN AFTER ADMINISTRATION OF AChE INHIBITORS



2.2.4.4 INFLUENCE OF THE MAJOR METABOLITE OF SDZ ENA 713, SDZ 226-90, ON AChE ACTIVITY IN RAT BRAIN IN VITRO AND EX VIVO [Doc. #103-307]

2.2.4.4.1 Method

Male OFA rats (200 g) obtained from _____, were used. AChE activities in rat brain regions were determined ex vivo following p.o. administration of either SDZ 226-90 and SDZ ENA 713 alone and after co-administration of both compounds. 48 rats were randomized into 8 groups, treated and sacrificed as indicated in Table A-9.

TABLE A-9

EXPERIMENTAL DESIGN: ALLOCATION TO TREATMENT

Group/Treatment	226-90 $\mu\text{mol/kg}$ Time min	ENA $\mu\text{mol/kg}$ Time min	Sacrificing after min
1 Control	Placebo	Placebo	60
2 226-90	100 / 0 min		90
3 226-90	300 / 0 min		90
4 226-90+ENA	100 / 0 min	8 / 60 min	90
5 226-90+ENA	300 / 0 min	8 / 60 min	90
6 ENA		8 / 60 min	30
7 ENA+226-90	100 / 30 min	8 / 60 min	60
8 ENA+226-90	300 / 30 min	8 / 60 min	60

The brains were dissected after decapitation according the method described by Glowinski and Iversen (J. Neurochem. 13, 655-669, 1966) and immediately frozen on dry ice. The dissected brains were stored at -70°C until analysis. The frozen tissue was homogenized and AChE activities measured as described in section 2.1.3.1.

2.2.4.4.2 Results

1.) Influence of 226-90 on AChE activity in vitro:

226-90 inhibits AChE activity isolated from rat striatum. In enzyme kinetics experiments non time dependent inhibition was found in contrast to the inhibition by the parent drug SDZ ENA 713. The results suggests a linear mixed type of inhibition for this compound, indicating both competitive and non competitive AChE inhibition. The apparent K_i in rat striatal enzyme was determined to be approximately 1.8×10^{-5} M. Based on the different mechanisms of inhibition by SDZ ENA 713 and 226-90 a direct comparison of K_i values can not be made (time dependent versus non-time dependent inhibition). Due to the competitive mechanism of inhibition by 226-90 the range of the IC_{50} 's is between _____

_____ depending on the substrate concentration used. The arbitrary observed IC_{50} following preincubation of the enzyme for 15 min with both compounds and at maximal substrate concentration are as follows:

SDZ ENA 713 : IC_{50} : $\sim 1.7 \times 10^{-5}$ M and 226-90 : IC_{50} : $\sim 12 \times 10^{-5}$ M.

Influence of 226-90 on AChE activity ex vivo:

226-90 at the doses of 100 and 300 μ mol/kg p.o. (16.7 and 50.1 mg/kg) does not inhibit AChE activity in any rat brain regions determined ex vivo. Due to the competitive part of AChE inhibition determined in vitro, it seems reasonable to assume that a high dose of 226-90 administered before SDZ ENA 713 could prevent the carbamylation of the enzyme and therefore reduce the inhibitory potency of SDZ ENA 713. As shown in Table A-10, 226-90 administered p.o., 100 and 300 μ mol/kg 60 min before 8 μ mol/kg p.o. SDZ ENA 713 showed a small additional increase rather than decrease in AChE inhibition. Although the effects were small they reached statistical significance in all brain regions examined except the striatum due to the larger variability. The corresponding SDZ ENA 713 control exerted the expected inhibition in all brain regions. However, when 226-90 was administered p.o. after SDZ ENA 713 no additional inhibition of AChE was seen.

TABLE A-10

AChE ACTIVITY IN DIFFERENT RAT BRAIN REGIONS FOLLOWING ADMINISTRATION OF SDZ 226-90 AND SDZ ENA 713

Treatment	Striatum		Cortex		Hippocampus		Pons/medulla	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Control	41.75	3.31	4.59	0.62	6.31	0.40	10.83	1.32
226-90 100µmol/kg	40.29	3.45	4.41	0.29	5.70	0.44	11.48	0.46
226-90 300µmol/kg	41.38	3.22	4.74	0.42	5.88	0.24	11.07	0.84
ENA 8µmol/kg	20.56	3.61	2.48	0.27	2.61	0.29	7.02	0.84
226-90 100µmol/kg+ENA 8µmol/kg	16.99	3.43	2.04	0.33*	2.02	0.30*	5.89	0.68*
226-90 300µmol/kg+ENA 8µmol/kg	16.35	2.87	1.92	0.14*	1.94	0.21*	5.79	0.44*
ENA 8µmol/kg+226-90 100µmol/kg	20.75	2.47	2.25	0.13	2.39	0.08	6.69	0.37
ENA 8µmol/kg+226-90 300µmol/kg	19.03	3.60	2.27	0.16	2.29	0.18	6.69	0.37

Mean values in nmol x min⁻¹ x mg⁻¹ tissue ±SD; n=6; * significantly different from SDZ ENA 713 alone p<0.05, Dunnet's test. (Significances (*) derived from Figure 3 of Doc.#103-307).

2.2.4.4 MUSCARINIC AND NICOTINIC EFFECTS OF SDZ ENA 713, PHYSOSTIGMINE AND TACRINE ON BRAIN DEOXYGLUCOSE UTILISATION (DOG). [Doc. #103-107]

Changes in the functional activity of the CNS are associated with altered deoxyglucose (DOG) utilization in the brain which can be visualized simultaneously in several brain regions using the autoradiographic method of Sokoloff et al., 1977. The administration of cholinergic drugs induces in this model of DOG a characteristic "fingerprint" pattern by modifying regional glucose metabolism.

2.2.4.4.1 Method

Male Wistar rats (150-200g) were used. Drugs were administered at various doses and by different routes (iv, po, ip) to animals. [14C]-2-deoxyglucose (125 μ Ci/kg) was injected 45 min before the animals were sacrificed. The brains were immediately excised, frozen at -80C and subsequently cut into slices with a thickness of 20 μ m. The optical densities of the radiographic images were measured as described by Sokoloff et al., (J. Neurochem., 28, 897-916, 1977) and modified by Palacios and Wiederhold (Brain Res. 327, 390-394, 1985).

2.2.4.4.2 Results

After p.o. application of SDZ ENA 713, 1.88mg/kg p.o.) significant changes in DOG utilization in the rat brain were observed. The most marked changes were found in the visual regions and the anteroventral thalamus and also in the lateral habenula nucleus (Table A-11). A similar pattern of effects was obtained with physostigmine (0.25 and 0.5 mg/kg s.c.). Tacrine was investigated in a higher dose range (5, 10 and 20 mg/kg s.c.) and induced dose dependent DOG changes in the same brain regions. With this AChE-I cortical and hypothalamic regions were relatively more strongly influenced. Preadministration of mecamylamine, which blocks nicotinic receptors, suppressed the influence of all AChE-I's in visual regions such as superior colliculus and simultaneously potentiated effects in the other regions.

TABLE A-11
RELATIVE CHANGES IN DEOXYGLUCOSE-UPTAKE IN RAT BRAIN AFTER AChE-I

Brain area	SDZ ENA 713 1.88 mg/kg p.o.	Physostigmine 0.25 mg/kg s.c.	Tacrine 10 mg/kg s.c.
caudate putamen	-4	-6	-8
ant cingulate cortex	-17*	-11*	-6
frontopariet.cortex mot. area	-8	-10	19*
frontopariet.cortex som.sens.	7	1	18*
anteromed thalamic nu	-20	8	-15*
anteroventral thalamic nu	31*	26*	44*
lat habenular nu	18*	9	27*
dors lat geniculate nu	17*	49*	52*
laterodorsal thalamic nu	-4	-2	38*
ventroposterior thalamic nu	3	-10	13
basolateral amygdaloid nu	-2	-14	-14
subthalamic nu	-9	-3	-7
ant pretecal area	0	-7	9
<u>sup coll superf. gray layer</u>	71*	92*	65*
hippocamp.stratum radiatum	-7	6	-11
substantia nigra reticular	-5	6	-7
<u>med term. nu optic tract</u>	42*	26*	14

Values are changes in relative deoxyglucose uptake in % difference of control animals. Administration of SDZ ENA 713, physostigmine 10 min., Tacrine 60 min., before deoxyglucose injection. Underline = nicotinic effects; * : p < 0.05, n=5.

3 OTHER PHARMACOLOGICAL ACTIVITIES OF SDZ ENA 713

3.1 CARDIOVASCULAR EFFECTS

3.1.1 EFFECTS OF SDZ ENA 713 IN THE ANAESTHETIZED CAT [Doc. #103-107]

3.1.1.1 Method

Male and female cats, (ca 2 kg), were anaesthetized with chloralose (43mg/kg i.m.) and urethane (430 mg/kg i.m.). Cannulae were implanted in the femoral artery, the femoral vein and the trachea. After a period of adaptation, stable control values were recorded for the vasopressor response to noradrenaline (1 - 8 g/kg i.v.), the effect of isoprenaline (0.25 - 2g/kg i.v.) on blood pressure and heart rate, and the pressor effect induced by occluding the carotid arteries for 30 seconds. SDZ ENA 713 was administered by infusion over a period of 30 min at a rate of 0.00128 - 0.16 M/kg per min. The total doses administered were 0.0384 - 6 mol/kg i.v. (ca 0.01 to 1.5 mg/kg i.v.). The volume infused was kept constant at 0.2 ml/min for the 2 hours of the test. Noradrenaline was administered 20 minutes, isoprenaline 25 min, and the carotid arteries were occluded 30 minutes after the onset of each infusion.

3.1.1.2 Results

SDZ ENA 713 tested in the dose range 0 - 1.5 mg/kg i.v., exerted only minimal effects on the circulatory parameters (Table A-12). At the dose of 0.75 mg/kg i.v. SDZ ENA 713 induced central effects manifested by strong tremor or slight cramps. Such central effects occurred after physostigmine at the dose of 0.14 mg/kg i.v., but in comparison to SDZ ENA 713 its influence on circulatory parameters was also more potent and was seen after 0.33 mg/kg i.v.. From these results it was concluded, that SDZ ENA 713 is considerably better tolerated than physostigmine, as regards the cardiovascular system.

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TABLE A-12

CARDIOVASCULAR EFFECTS OF SDZ ENA 713 AND PHYSOSTIGMINE IN
THE ANAESTHETIZED CAT

Drug	Cumulative dose mg/kg i.v.	BP %	HR %	NABP %	ISPBP %	OCA %	NT	Lethal dose mg/kg i.v.
ENA 713 (n=4)	0.01	+1	+1	-3	-7	-11	-7	>1.5
	0.06	+2	+1	+2	-6	-1	-7	
	0.30	+5	0	-19	-13	+3	-12	
	1.50	+4	0	-27	-38	-8	-10	
Physostigmine (n=3)	0.01	+1	0	-17	+5	-2	-5	>1.7
	0.07	+2	0	-33	-21	-2	-12	
	0.34	+4	-1	-59	-33	-46	-12	
	1.71	-40	-5	-61	-100	-75	+43	

BP = blood pressure (mm Hg) HR = heart rate (beats/min)

NABP = pressor response to noradrenaline

ISPBP = isoprenaline-induced fall in blood pressure

OCA = pressor response to carotid artery occlusion

NT = nictitating membrane tone

Effects are expressed as percentage changes compared with predrug values

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3.1.2 EFFECTS OF SDZ ENA 713 ON THE CARDIOVASCULAR SYSTEM OF THE NORMOTENSIVE AWAKE RAT. [Doc. #103-107]

Central cholinergic stimulation by muscarinic agonists induces an increase in blood pressure under certain experimental conditions in animals and man (Brezenoff et al. Ann.rev.Pharmacol.Toxicol. 22, 341-381, 1982). Experiments on awake rats led to the conclusion that besides an initial decrease in blood pressure and pulse frequency, the blood pressure increase seen after these drugs was centrally mediated by M2 receptors (Pazos et al. Europ.J.Pharmacol., 125, 63-70, 1986).

3.1.2.1 Methods.

The experiments were carried out using male Wistar rats and the drugs were administered orally (SDZ ENA 713) or by the intravenous route (N-methylscopolamine (NMS)). The method is described in detail by Pazos et al. (Europ.J.Pharmacol., 125, 63-70, 1986).

3.1.2.2 Results

Oral administration of SDZ ENA 713 induced only weak bradycardia in this model which was reversed by methyl-scopolamine. At the higher dose (5.6 mg/kg p.o.), SDZ ENA 713, significantly increased blood pressure (29%), this effect being antagonized by scopolamine but not by the peripheral blocker N-methylscopolamine (Table A-13) In contrast to certain muscarinic agonists, SDZ ENA 713 did not cause an initial drop in blood pressure.

**APPEARS THIS WAY
ON ORIGINAL**

TABLE A-13

MAXIMAL CARDIOVASCULAR EFFECTS OF SDZ 212-712 (p.o.) IN AWAKE RATS

Drug	Blood Pressure (mm Hg)				Heart Rate (bpm)			
	Control (Mean ±SEM)	Treatment (Mean ±SEM)	Difference (%)	Time (min)	Control (Mean ±SEM)	Treatment (Mean ±SEM)	Difference (%)	Time (min)
ENA 713 mg/kg p.o.	97 ±3	105 ±5	8	20	467 ±15	402 ±26	-14	20
	5.6 95 ±5	122 ±5	29*	17	475 ±7	395 ±7	-17	17
ENA 713 mg/kg p.o. 5.6 + NMS	109 ±2	139 ±11	28*	20				

* p<0.01 paired sample t-test

NMS = N-methylscopolamine 1 mg/kg i.v.

3.1.3 EFFECTS OF SDZ ENA 713 IN THE CONSCIOUS
NORMOTENSIVE SQUIRREL MONKEY. [Doc. #103-107]

3.1.3.1 Method.

Squirrel monkeys (0.65 - 1 kg) were used. The animals were restrained in a monkey seat and were placed in a cabin (2 m³ in capacity). Blood pressure was measured by means of a cuff around the tail. Cardiovascular effects and behavioral changes were determined 1 and 5 hours after drug administration. Each value represents the mean of at least 5 consecutive measurements of blood pressure.

3.1.3.2 Results

SDZ ENA 713, 0.1 mg/kg p.o. (n=2) induced in one animal slight vomiting and in both monkeys tested slight tremor was observed. No effect on respiration rate and blood sure 1 and 5 hours following drug administration could be detected (Table A-14). With the dose of 0.3 mg/kg p.o. both monkeys vomited and exhibited tremor. The small effect on blood pressure in one animal seems to be uncertain because this monkey also showed an slight increase under placebo (Table A-14: monkey no. 38). SDZ ENA 713 at the dose of 1 mg/kg p.o. induced strong vomiting and tremor in one squirrel monkey and as a consequence the animal showed disinterest toward the environment. At this dose a slight increase in blood pressure was seen after 1 hour. It was technically impossible to measure the blood pressure in this animal after 5 hours because of marked tremor.

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ON ORIGINAL**

TABLE A-14

BLOOD PRESSURE EFFECTS OF SDZ ENA 713 IN THE CONSCIOUS NORMOTENSIVE SQUIRREL MONKEY.

Drug	Animal No.	Control value mm Hg	1 hour after drug mm Hg	Δ BP	5 hours after drug mm Hg	Δ BP
SDZ ENA 713						
0.1 mg/kg p.o.	42	130 \pm 4	124 \pm 9	-6	125 \pm 6	-5
0.1 mg/kg p.o.	40	98 \pm 3	92 \pm 3	-6	97 \pm 2	-1
Placebo						
3 ml/kg p.o.	42	135 \pm 6	119 \pm 9	-16	127 \pm 5	-8
3 ml/kg p.o.	40	100 \pm 7	103 \pm 9	+3	99 \pm 4	-1
SDZ ENA 713						
0.3 mg/kg p.o.	41	104 \pm 2	99 \pm 3	-5	92 \pm 6	-12
0.3 mg/kg p.o.	8	101 \pm 3	115 \pm 4	+14	114 \pm 4	+13
Placebo						
3 ml/kg p.o.	41	100 \pm 5	92 \pm 7	-8	110 \pm 4	+10
3 ml/kg p.o.	38	97 \pm 3	102 \pm 3	+5	120 \pm 2	+23
SDZ ENA 713						
1 mg/kg p.o.	40	99 \pm 3	113 \pm 10	+14	not measured	
Placebo						
3 ml/kg p.o.	40	106 \pm 3	100 \pm 2	-6	101 \pm 3	-5

3.1.4 COMMENTS ON THE CARDIOVASCULAR INFLUENCE OF SDZ ENA 173

The effects of anticholinesterase agents on the cardiovascular system are complex. The primary effect produced by these drugs is a bradycardia with consequent decrease in cardiac output and blood pressure. SDZ ENA 713 tested in the rat, cat and monkey showed no influence on cardiovascular parameters at doses at which clear central effects could be demonstrated. Higher doses of this drug, inducing unwanted peripheral side effects also started to influence cardiovascular parameters.

3.2 PULMONARY EFFECTS

3.2.1 PULMONARY EFFECT OF SDZ ENA 713 IN THE VENTILATED GUINEA-PIG [Doc. #103-107]

ACh induces bronchospasm in this animal model expressed as a dose dependent increase in airway resistance.

3.2.1.1 Method

Guinea-pigs (male, 400-600 g) were anaesthetized with phenobarbital (100 mg/kg i.p. and pentobarbital (30 mg/kg i.p.) and paralysed with gallamine (10 mg/kg i.m.). Animals were ventilated via a tracheal cannula (10 ml/kg, 1 Hz). Blood pressure and heart rate were recorded from the carotid artery. Ventilation was monitored by a flow transducer in line with the inspiratory circuit. Coincident pressure changes within the thorax were monitored directly via an intrathoracic trochar, so that the pressure difference between the trachea and thorax could be measured and displayed. From these measurements of flow and differential pressure, both resistance (R_L) and compliance (C_{dyn}) were calculated (Buxco, model 6) at each inspiration.

3.2.1.2 Results

SDZ ENA 713 in the dose-range of 0.01 to 1 mg/kg i.v., injected at 10 minute intervals, showed no influence on airway resistance, whereas ACh, starting with the dose of 3.2 µg/kg i.v. dose dependently increased this resistance in the ventilated animals (Figure A-5).

FIGURE A-5

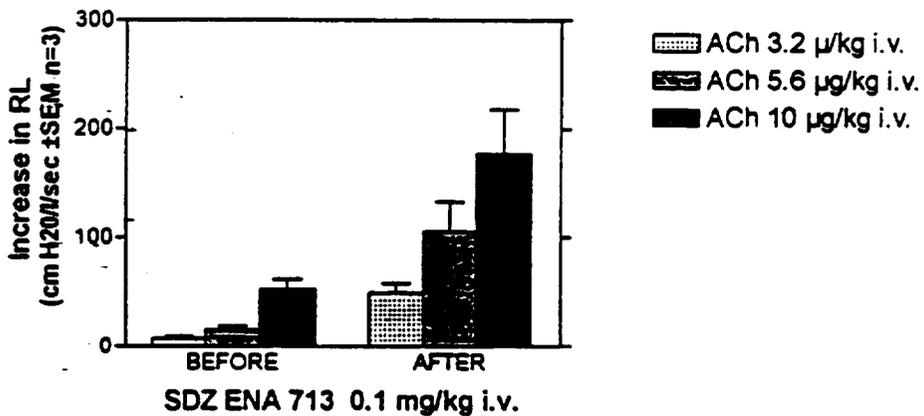
EFFECT OF ACETYLCHOLINE AND SDZ ENA 713 ON AIRWAY RESISTANCE IN VENTILATED GUINEA PIGS



Blood pressure and heart rate were not affected by SDZ ENA 713 until the highest dose used, 1 mg/kg i.v., where a drop in both parameters was observed. However, in animals pretreated with 0.1 mg/kg i.v. SDZ ENA 713 (15 min.), the effect of ACh on airway resistance at the doses of 3.2, 5.6 and 10 µg/kg i.v. was clearly potentiated, probably due to the inhibition of AChE by SDZ ENA 713 (Figure A-6). For this reason the drug may be contraindicated in asthmatic patients.

FIGURE A-6

EFFECT OF SDZ ENA 713 ON ACETYLCHOLINE-INDUCED BRONCHOSPASM IN VENTILATED GUINEA PIGS



3.3 DEPENDENCE LIABILITY STUDIES

3.3.1 STUDY OF POTENTIAL TO CAUSE PHYSICAL DEPENDENCE [Doc.# 103-197]

In a test of potential to cause physical dependence it was determined if signs of a withdrawal syndrome occur following cessation of prolonged application of the compound.

3.3.1.1 Method

Monkeys automatically received intravenous application of SDZ ENA 713 every four hours for 8 weeks in a series of increasing doses (2 weeks at 5.6 µg/kg/infusion, 2 weeks at 10 µg/kg/infusion, 4 weeks at 18 µg/kg/infusion) following a 2-week baseline period when the applied solution was saline. Application of substance was then terminated and replaced by saline in order to determine if signs of a withdrawal syndrome occurred. This schedule was then repeated with morphine as a positive control. Three times a week plasma samples were collected and assayed for butyrylcholinesterase by the method of Ellman et al. (Biochem Pharmacol, 7, 88-95, 1961). At least once a week, and on every workday in the first withdrawal week the occurrence of behavioral elements was noted in three separate 5-min observation periods, and reactions to offering the animal a peanut once per observation day were also recorded.

3.3.1.2 Results

During the final weeks of treatment with SDZ ENA 713 plasma butyrylcholinesterase values were reduced to 20-25% of control. After cessation of treatment values rapidly rose, reaching more than 90% of previous control levels in every animal within a week.

The frequency of occurrence of behavioral elements in the withdrawal week was compared to the corresponding frequency in the previous saline baseline and drug phases by paired t-tests. A weak significance level ($p < 0.05$, 2-tail) was accepted in order to increase the chance of detecting a withdrawal syndrome. After withdrawal from SDZ ENA 713 no behavioral element showed a significant increase compared to the previous saline and drug phases. After withdrawal from morphine elements showing a significant increase were: lying on stomach, body twitching, retching and piloerection. After morphine withdrawal, but not after withdrawal from SDZ ENA 713, several other elements such as cowering and lying on side showed quite large, but statistically non-significant, increases (Table A-15).

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Table A-15

Number of positive observation periods per observation day in various phases of the physical dependence experiment

Behavioral element	Saline baseline	EMA 713	Saline	Saline baseline	Morphine	Saline
Self-biting	0±0	0.05±0.05	0±0	0±0	0±0	0±0
Lying on stomach	0±0	0.05±0.05	0.05±0.05	0±0	0.09±0.09	<u>1.53±0.07</u>
Unusual movements	0.17±0.17	0.43±0.27	0.50±0.30	0.50±0.29	0.33±0.18	<u>0.07±0.07</u>
Vomiting	0±0	0.02±0.02	0±0	0±0	0±0	0.07±0.07
Yawning	0.08±0.08	0.05±0.03	0±0	0±0	0.06±0.03	0.13±0.07
Harness-biting	0.75±0.55	1.00±0.67	0.95±0.43	0.81±0.83	0.12±0.12	0.80±0.60
Covering	0.33±0.24	0±0	0.05±0.05	0±0	0±0	0.93±0.37
Locomotion	2.83±0.10	2.52±0.39	2.55±0.33	2.67±0.33	2.10±0.56	2.60±0.23
Motility	2.83±0.10	3.00±0.00	3.00±0.00	3.00±0.00	2.85±0.06	2.87±0.13
Twitching	0.17±0.10	0.02±0.02	0±0	0±0	0.03±0.03	1.73±0.13
Vocalization	0.25±0.16	0.32±0.17	0.55±0.24	1.50±0.50	0.61±0.27	<u>1.47±0.55</u>
Lying on side	0±0	0.09±0.04	0±0	0.17±0.17	0±0	1.27±0.33
Stretching	0±0	0.02±0.02	0.05±0.05	0±0	0±0	0±0
Retching	0±0	0.02±0.02	0±0	0±0	0±0	<u>0.40±0.00</u>
Trembling	0±0	0±0	0±0	0±0	0±0	<u>0.20±0.20</u>
Head-shake	1.00±0.41	1.05±0.17	1.10±0.30	1.67±0.44	0.40±0.35	1.20±0.40
Biting apparatus	0±0	0.11±0.09	0.10±0.10	0±0	0.03±0.03	0.20±0.20
Shaking cage floor	0±0	0.14±0.14	0.05±0.05	0±0	0±0	0±0
Scratching	2.00±0.49	2.32±0.30	2.40±0.32	2.83±0.17	2.24±0.24	1.20±0.42
Piloerection	0±0	0.05±0.05	0±0	0±0	0±0	<u>1.60±0.20</u>
Salivation	0±0	0±0	0±0	0±0	0±0	<u>0.07±0.07</u>
Red nose	0.58±0.25	0.80±0.35	0.90±0.31	2.17±0.17	2.36±0.45	2.73±0.07
Shows teeth	0.42±0.25	0.39±0.24	0.30±0.24	0.33±0.33	0.10±0.10	0.67±0.13
Strikes hand	0±0	0±0	0±0	0±0	0±0	0±0
Takes the peanut	0.58±0.21	0.75±0.25	0.75±0.25	0.67±0.33	0.67±0.33	0.87±0.13
Jumps forward	0.17±0.10	0.18±0.10	0.25±0.25	0.33±0.33	0.36±0.32	0.47±0.10
Jumps away	0.58±0.16	0.36±0.22	0.50±0.29	0.67±0.33	0.67±0.33	0.33±0.10
Vocalizes	0.33±0.24	0.11±0.09	0±0	0±0	0±0	0.27±0.10
Aggression	0±0	0±0	0±0	0±0	0.06±0.06	0.07±0.07
Fear	0±0	0.02±0.02	0±0	0±0	0±0	0±0

Shown are mean ± SEM of 3-4 subjects. The occurrence of the behavioral elements was determined in three observation periods per observation day, except for the reactions shown in the bottom eight lines of the table, which were assessed only once after offering a peanut before the first observation period. Values which differ significantly (paired t-test, $p < 0.05$) from those in both the preceding substance and saline baseline phases are shown underlined.

3.3.2 STUDY OF POTENTIAL TO CAUSE PSYCHOLOGICAL DEPENDENCE [Doc. #103-197]

3.3.2.1 Method

Five male rhesus monkeys had access to test solutions for 23 hr per day. Periods of availability of SDZ ENA 713 were separated by periods when only saline, the vehicle used to dissolve SDZ ENA 713, was available. Infusions through a chronic intravenous catheter were initiated automatically by pressing a lever in the test cage. To encourage sampling of new solutions each infusion during the first 20 minutes of the test day on the first four days of availability of the new solution was accompanied by a sugar pellet delivered into a container in the test cage. Since during such periods the self-administration of about a hundred infusions is not uncommon the maximum dose was set to be one hundredth of the dose (1 mg/kg i.v.) which produced a clear side-effect in a dose-finding study. Following the testing of three doses of SDZ ENA 713, morphine (0.1 mg/kg/infusion), as a positive control substance, was tested.

3.3.2.2 Results

The results shown in Table A-16 show that three animals, S419, S8841 and S309 failed to self-administer SDZ ENA 713, but self-administered the positive control substance, morphine, at rates significantly higher than those of saline. One animal, S487, did not self-administer either substance at rates significantly higher than those of saline. One animal, S8863 was atypical in that it developed an atypically high response rate as the experiment progressed. The decreased rate of self-administering the highest dose of SDZ ENA 713 by this animal compared to the previous rate of self-administering saline shows that this dose is capable of influencing behavior. In summary, the evidence suggests that SDZ ENA 713 has no potential to cause psychological dependence.

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Table A-16

Treatment available	Duration	Infusions/day Subject				
		S419	S487	S8863	S8841	S309
Saline + Pellets	4 days	62±2	126±12	179±39	186±17	33±7
Saline	3 days	5±3	13±4	51±9	126±15	5±2
Saline	1 week	5±1	20±2	76±9	148±11	4±1
ENA 713 (5.6) + Pell.	4 days	87±4	124±3	232±29	173±10	24±3
ENA 713 (5.6)	3 days	11±4	18±7	106±36	143±24	6±3
ENA 713 (5.6)	4 weeks	1±0*	4±1*	342±38*	125±13	4±1
Saline	1 week	2±1	3±0	362±34	111±7	1±0
Saline + Pellets	4 days	86±13	102±3	474±55	183±42	4±1
Saline	3 days	5±1	16±3	467±63	109±10	1±0
Saline	1 week	4±1	3±1	507±32	118±14	0±0
ENA 713 (3.2) + Pell.	4 days	58±9	109±3	437±16	133±10	2±1
ENA 713 (3.2)	3 days	1±1	16±1	677±29	36±5	1±1
ENA 713 (3.2)	4 weeks	7±3	3±0	731±26*	33±3*	0±0
Saline	1 week	6±1	3±2	548±45	14±3	1±0
Saline + Pellets	4 days	79±8	101±9	381±53	104±10	6±2
Saline	3 days	6±1	24±4	315±53	25±2	1±1
Saline	1 week	10±4	5±1	423±69	22±5	0±0
ENA 713 (10) + Pell.	4 days	74±13	81±12	169±16	53±14	1±0
ENA 713 (10)	3 days	48±25	21±8	194±21	19±7	0±0
ENA 713 (10)	4 weeks	12±1	5±1	191±12*	17±2	0±0
Saline	1 week	2±1	2±0	548±72	36±6	0±0
Saline + Pellets	4 days	75±14	66±3	414±29	87±2	5±1
Saline	3 days	12±1	3±1	248±51	21±9	1±0
Saline	1 week	9±2	3±1	258±23	48±8	0±0
Morphine + Pell.	4 days	26±9	57±7	80±7	29±3	8±2
Morphine	3 days	10±2	4±1	42±3	52±14	26±2
Morphine	4 weeks	63±6	2±0	112±8*	65±3*	22±2*
Saline	1 week	71±40	24±6	122±20	123±47	3±1

Daily rates of self-administered infusions (mean ± SEM) of individual subjects during consecutive phases of the self-administration experiment. During periods indicated by "pellets" ("pell.") each infusion during the first 20 min of the session was accompanied by delivery of a sugar pellet into a receptacle in the cage. The "4 weeks" values at the mean ± SEM of all the daily values in the final four weeks of availability of the tested dose. (i.e. each row corresponds to 4 rows of the tables 1a to 1e in Doc # 103-197). Doses in parentheses are in µg/kg/infusion. * p<0.05 vs last preceding week of saline availability (2-tailed t-test).

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3.4 Drug Interaction Studies

3.4.1 Interaction with Amitriptyline [Doc. # 103-344]

The possible interaction of SDZ ENA 713 with amitriptyline was examined in a test to assess antidepressant potential in mice (Porsolt et al. Arch Int Pharmacodyn, 229, 327-336, 1977).

3.4.1.1 Methods

The test was performed according to the method published by Porsolt et al, 1977. On the day before the experiment male CD1 mice were caged singly in the experimental room. After application of substances they were placed singly in a beaker (diameter 10 cm, height 18 cm) filled to a depth of 14 cm with water at room temperature (20 - 23°C). Using a stopwatch the duration of immobility in the final 4 minutes of a 6 minute test was measured. A mouse was judged to be immobile when it had stopped struggling and made only those movements necessary to keep its head above water.

SDZ ENA 713 and amitriptyline were dissolved in 0.9% saline. SDZ ENA 713 hydrogen tartrate was administered p.o. 90 minutes before the test in a volume of 10 ml/kg. Amitriptyline hydrochloride was administered p.o. 60 minutes before the test in a volume of 10 ml/kg. Doses of SDZ ENA 713 are expressed as those of the base and doses of amitriptyline are those of the salt.

3.4.1.2 Results

A range of doses of SDZ ENA 713 (0.1 - 1 mg/kg) was administered orally before amitriptyline (30 mg/kg p.o.) or vehicle in the Porsolt test of antidepressant potential. There was a dose-related increase of immobility time with increasing dose of SDZ ENA 713 in the amitriptyline group only. When a range of doses of amitriptyline was administered to mice treated with either SDZ ENA 713 (1 mg/kg p.o.) or vehicle analysis of variance indicated an effect of SDZ ENA 713 and of amitriptyline but no significant interaction of these treatments. These results indicate that high doses of SDZ ENA 713 may antagonize the effect of amitriptyline in this test. The present results indicate that SDZ ENA 713 antagonizes the reduction of immobility time associated with amitriptyline in this test conducted in animals with an intact cholinergic system. It is not clear whether this antagonism is due to elevation of immobility in all groups, including the group which received no amitriptyline, as suggested by the analysis of the second experiment or whether only amitriptyline effects are antagonized as suggested by the results of the first experiment. The predictive value of these results for humans, especially for patients with dementia who have an altered cholinergic system, is questionable.

SANDOZ PHARMACEUTICALS CORPORATION
59 ROUTE 10
EAST HANOVER, NEW JERSEY USA 07936

SANDOZ RESEARCH INSTITUTE

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ENA 713 NDA SUMMARY NONCLINICAL PHARMACOKINETICS (ADME)

Barbara A. Orwig

Barbara A. Orwig
Drug Metabolism and Pharmacokinetics Department

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Exelon™ (ENA 713) NDA, Section V

C. ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION (ADME) STUDIES

1 INTRODUCTION

This section of the ENA 713 New Drug Application contains all of the studies which investigated the absorption, distribution, metabolism, and excretion of ENA 713 in laboratory species. The majority of these studies were conducted using ³H- or ¹⁴C-labeled drug. Included in this summary are two sections describing experimental details and results. The first section, "General Methodology", describes the synthesis and characterization of the radiolabeled drug used in the studies, the synthesis of standards, the animal studies, and the methods used for characterization and analysis of ENA 713 and its metabolites in biological samples. The second section, "Comparative ADME", summarizes the results obtained in the laboratory animals and compares them with corresponding data in humans. This comparison supports the selection of the toxicity species and the extrapolation of safety data to humans.

2 GENERAL METHODOLOGY

2.1 Radiolabeled ENA 713

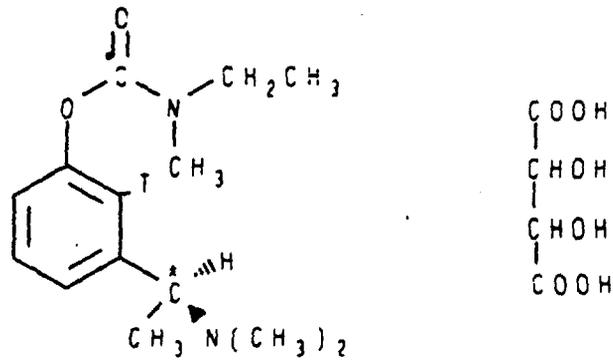
Three preparations of radiolabeled ENA 713 were synthesized by the _____, Sandoz Research Institute, East Hanover, New Jersey (1-3) The ENA 713 used in the animal studies was labeled with tritium in the 2-position of the phenyl ring, while the drug substance used in the human study and in an *in vitro* protein binding study was labeled with carbon-14 in the benzylic carbon position (Figure C-1).

The chemical identity of the product, ENA 713 hta (ENA 713 hydrogen tartrate salt), was confirmed by comparative infrared spectroscopy (IR), HPLC, thin-layer chromatography (TLC), and melting point (1,2) or differential scanning calorimetry (3) with a reference standard. The enantiomeric purity was determined by optical rotation measurement (1), HPLC using a chiral column (2), or chiral shift reagent proton NMR (nuclear magnetic resonance spectroscopy) (3). The radiochemical purity was established by radio-TLC and radio-HPLC to be >95% for all dilution batches. The tritium label was metabolically stable as indicated by minimal tritiated water formation (1.3-6.0%) in the species tested, except for rat. Following an intravenous dose to rat, ca. 15% tritiated water was formed; thus, the ¹⁴C-labeled drug (2) was prepared for human dosing.

2.2 Studies in Animals and Humans

_____ New Zealand White rabbits, and beagle dogs were housed in appropriate metabolism cages and were provided with food and water *ad libitum*. The animals received a designated range of oral doses or an intravenous reference dose as shown in Table C-1. These doses encompassed the dose ranges used and were prepared in the same form as in the toxicity trials for ENA 713. Relevant studies in humans, which are also described in Summary Section VI of this NDA, are included here for comparison purposes.

FIGURE C-1: ENA 713 LABELED WITH ^3H (T) OR ^{14}C (*)



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TABLE C-1: ENA 713 ADME STUDIES

Species	Radiotracer	Dose (mg/kg)	Dosage Form	Dose Route	Dose Duration ^a	Reference
Mouse	³ H	0.25	solution	i.v.	S	4
	³ H	0.25	suspension	p.o.	S	4
	³ H	1.56	suspension	p.o.	S	4
Rat	³ H	0.38	solution	i.v.	S	5
	³ H	0.38	suspension	p.o.	S	5
	³ H	3.75	suspension	p.o.	S	5
	³ H	3.75	suspension	p.o.	M	5
	- ^b	0.5	solution	i.v.	M	6
	- ^b	2.5	solution	i.v.	M	6
Rabbit	³ H	1.12	solution	i.v.	S	7
	³ H	1.12	suspension	p.o.	S	7
	³ H	1.12	suspension	p.o.	M	8 ^c
	³ H	1.12	suspension	p.o.	S	9 ^d

^a S = single dose; M = multiple dose

^b Nonradiolabeled

^c Placental transfer study

^d Passage into milk study

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TABLE C-1: ENA 713 ADME STUDIES - continued

Species	Radiotracer	Dose (mg/kg)	Dosage Form	Dose Route	Dose Duration ^a	Reference
Dog	³ H	0.038	solution	i.v.	S	10
	³ H	0.038	capsule	p.o.	S	10
	³ H	1.88	capsule	p.o.	S	10
	^b	0.11	capsule	p.o.	S,M	11
	^b	0.19	capsule	p.o.	S,M	11
	^b	0.26	capsule	p.o.	S,M	11
	^b	0.038	capsule	p.o.	S,M	12
	^b	0.38	capsule	p.o.	S,M	12
	^b	1.88 ^c	capsule	p.o.	S,M	12
	^b	0.19	capsule	p.o.	S,M	13
	^b	0.38	capsule	p.o.	S,M	13
	^b	1.31 ^d	capsule	p.o.	S,M	13
	^b	0.11	capsule	p.o.	S,M	14
	^b	0.45	capsule	p.o.	S,M	14
	^b	1.58	capsule	p.o.	S,M	14
	^b	0.15	solution	i.v.	S,M	15
	^b	0.75	solution	i.v.	S,M	15
Man	¹⁴ C	0.0132	solution	p.o.	S	16
	¹⁴ C	0.0310	solution	p.o.	S	16

^a S = single dose; M = multiple dose

^b Nonradiolabeled

^c Initial dose was 2.25 mg/kg; reduced on Day 2 to 1.88 mg/kg

^d Initial dose was 1.56 mg/kg; reduced on Day 4 to 1.31 mg/kg

In this section, all doses are expressed as the equivalent weights of the free base, ENA 713. Doses were prepared by weighing out the drug substance, which was the hydrogen tartrate salt of ENA 713 (see 2.1, this section). In order to convert from the hydrogen tartrate salt to the base equivalent, division by the factor 1.60 is necessary. In some studies, however, the incorrect factor of 1.65 was used, resulting in minor differences (less than 5%) among the reports in the *calculated* dose of ENA 713 free base. The doses expressed in this summary are the free base equivalent derived from the correct conversion factor.

After dosing, serial blood or plasma samples and quantitative urine and feces were collected from each species. Selected tissues and organs were obtained from mice and rats, and from the rabbits used for the placental transfer study. Bile samples were collected from a selected group of rats and milk samples were collected from rabbits.

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2.3 Analysis of Radioactivity

2.4 Analysis of ENA 713

2.5 Characterization, Isolation, and Structure Elucidation of Metabolites (26-33)

Metabolites were characterized and quantified by HPLC coupled with radioactivity monitoring (HPLC-RAM). Radioactive peaks were assigned Arabic numerals in order of their elution from the reversed phase chromatographic column and in chronological order of analysis of sample type and/or species. Metabolites were identified by co-elution with radiolabeled or nonradiolabeled standards, by comparison of their retention times with those of previously identified metabolites in another species, or by mass spectrometry and nuclear magnetic resonance spectroscopy following isolation. Phase II metabolites, e.g., sulfates or glucuronides, were also identified after treatment of the corresponding precursor with β -glucuronidase, sulfatase, and/or Glusulase® (enzyme preparation containing both β -glucuronidase and sulfatase activities).

3 COMPARATIVE ADME

3.1 Absorption

As shown in Table C-2, the absorption of orally administered ENA 713 (solution, suspension, capsule) was characterized by a rapid onset and rate in all species.

TABLE C-2: ABSORPTION PARAMETERS FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED ENA 713

Species	Dose (mg/kg)	Time to Peak (h) in Blood/Plasma ^a		% Dose Absorbed	Reference
		Radioactivity	ENA 713		
Mouse	0.25	1.0	1.0	62	4
	1.56	0.25	0.5	68	4
Rat	0.38	0.63	- ^c	100	5
	3.75	0.44	0.25	100	5
	3.75/day ^d	0.75	0.25	100	5
Rabbit	1.12	1.3	- ^c	98	7
Dog	0.038	1.3	- ^c	>90	10
	1.88	1.7	1.2	>90	10
	0.11	- ^e	0.5(M); 0.7(F) ^f	- ^b	11
	0.11/day ^d	- ^e	0.7(M); 0.5(F)	- ^b	11
	0.19	- ^e	0.7(M); 0.8(F)	- ^b	11
	0.19/day ^d	- ^e	0.7(M); 1.7(F)	- ^b	11
	0.29	- ^e	0.8(M); 0.5(F)	- ^b	11
	0.29/day ^d	- ^e	1.0(M); 0.7(F)	- ^b	11

^a Results in plasma are given in brackets, []

^b Not determined

^c Below the limit of quantification

^d Denotes steady state in multiple dose study

^e Nonradiolabeled dose

^f M = male; F = female

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TABLE C-2: ABSORPTION PARAMETERS FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED ENA 713 - continued

Species	Dose (mg/kg)	Time to Peak: (h) in Blood/Plasma ^a		% Dose Absorbed	Reference
		Radioactivity	ENA 713		
Dog - cont'd	0.038	- ^b	0.5(M); 1.0(F) ^f	- ^b	12
	0.038/day ^d	- ^b	1.0(M); 2.0(F)	- ^b	12
	0.38	- ^b	0.8(M); 0.7(F)	- ^b	12
	0.38/day ^d	- ^b	0.5(M); 1.0(F)	- ^b	12
	2.25	- ^b	1.0(M); 0.5(F)	- ^b	12
	1.88/day ^d	- ^b	0.5(M); 0.5(F)	- ^b	12
	0.11	- ^b	0.5(M); 0.7(F)	- ^b	14
	0.11/day ^d	- ^b	0.8(M); 0.7(F)	- ^b	14
	0.45	- ^b	1.0(M); 0.7(F)	- ^b	14
	0.45/day ^d	- ^b	1.7(M); 0.5(F)	- ^b	14
	1.58	- ^b	0.7(M); 0.7(F)	- ^b	14
	1.58/day ^d	- ^b	2.8(M); 1.2(F)	- ^b	14
	0.19	- ^b	0.6(M); 0.6(F)	- ^b	13
	0.19/day ^d	- ^b	0.8(M); 2.0(F)	- ^b	13
	0.38	- ^b	0.5(M); 1.0(F)	- ^b	13
	0.38/day ^d	- ^b	2.2(M); 2.2(F)	- ^b	13
	1.56	- ^b	0.6(M); 1.2(F)	- ^b	13
	1.31/day ^{d, ‡}	- ^b	2.4(M); 1.8(F)	- ^b	13
Human	0.0132	[0.79]	[0.67]	>96	16
	0.0310	[1.1]	[0.75]	>97	16

^a Results in plasma are given in brackets, []

^b Not determined

^d Denotes steady state in multiple dose study

^e Nonradiolabeled dose

^f M = male; F = female

[‡] Initial dose was 1.56 mg/kg; reduced on Day 4 to 1.31 mg/kg

including human. Peak concentrations of radioactivity and ENA 713 were generally achieved between 0.25 and 1.7 h postdose, except following chronic dosing in the dog, where peak times up to 2.4 h were observed. In all species but mouse, absorption was essentially complete (85-100%). The extent of absorption in the mouse was moderate, 62-68%. Absorption was independent of the dose in mouse, rat, and dog and of the frequency of administration (single vs repeated) in rat and dog.

3.2 Blood and Plasma Concentrations

To facilitate comparisons across species and dose levels, blood and plasma concentrations were normalized by the respective doses. The results, expressed in terms of peak concentration (C_{max}) and area under the concentration vs time curve (AUC), are shown in Table C-3 for total radioactivity and Table C-4 for ENA 713.

As shown in Table C-3, the systemic exposure to total radioactivity was roughly proportional to dose in all of the species except dog. In the dog, the dose-normalized C_{max} and AUC values appeared to decrease with increasing dose, a possible reflection of varying degrees of absorption in the wide dose range (a difference of ca. 50-fold) used in the radioactive study. In the mouse, rat, and human studies smaller ranges were employed (differences of ca. 2- to 10-fold). The systemic exposure of each species relative to humans is presented in Table C-5. Based on either C_{max} or AUC values derived from total radioactivity measurements, the human (dosed under fasting conditions) was exposed systemically to drug and drug-derived compounds to a greater extent than animals receiving an equivalent dose.

With respect to ENA 713, the AUC values showed a tendency to increase, disproportionately, with increasing oral doses (Table C-4). Human was exposed (using the results following the high dose) to higher plasma concentrations (AUC) of ENA 713 than laboratory animals (Table C-5). Among the various animal species, plasma ENA 713 levels were lowest in the rat, with a dose-normalized C_{max} or AUC of ca. 3% of that observed in human, and highest in the dog, where the dose-normalized AUC at the higher doses was almost equal to that observed in the human. In mouse after a low or high dose, the dose-normalized AUC was ca. 20-30% of that observed in human. No quantitative comparison can be made with rabbit, because the blood levels of parent drug were below the limit of quantification. Repeated dosing of rats and dogs up to 104 and 52 weeks, respectively, revealed no change in the pharmacokinetics of ENA 713 (see Section 5.B. Toxicology Summary).

Following oral dosing, the decline of total blood radioactivity in all the animal species was biexponential, except in rat, where, after the high dose, the decline was triexponential. In humans, the decline of total plasma radioactivity was monophasic. As shown in Table C-6, the terminal half-life of total radioactivity, which represented the most slowly eliminated metabolite or group of metabolites, ranged from ca. 4 h (human) to ca. 156 h (rat). The terminal half-life of parent drug could be determined only in rat (0.4 h, multiple high oral dose), dog (2.5 h, high dose), and human (0.62-0.69 h), due

TABLE C-3: TOTAL RADIOACTIVITY CONCENTRATIONS (C_{max} AND AUC) IN BLOOD/PLASMA* FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED ENA 713

Species	Dose (mg/kg)	C_{max} (ngEq/mL)	AUC (ngEq·h/mL)	Dose-Normalized		Reference
				C_{max}	AUC	
Mouse	0.25	97.6	177	390	708	4
	1.56	493	992	316	636	4
Rat	0.38	59.9	316	158	832	5
	3.75	682	3995	182	1065	5
	3.75/day ^b	580	2873	155	766	5
Rabbit	1.12	601	1910	537	1705	7
Dog	0.038	29.7	333	782	8763	10
	1.88	878	11000	467	5851	10
Human	0.0132	[16.0]	[91.1]	[1212]	[6902]	16
	0.0310	[33.0]	[223]	[1064]	[7194]	16

* Results in plasma are given in brackets, []

^b Denotes multiple dose study; values of C_{max} and AUC were determined for one dose interval at steady state

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TABLE C-4: ENA 713 CONCENTRATIONS (C_{max} AND AUC) IN BLOOD/PLASMA* FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED ENA 713

Species	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng·h/mL)	Dose-Normalized		Reference
				C_{max}	AUC	
Mouse	0.25	4.16	4.64	16.6	18.6	4
	1.56	63.5	40.3	40.7	25.8	4
Rat	0.38	^b	^b	^b	^b	5
	3.75	8.19	9.49	2.18	2.53	5
	3.75/day ^d	7.24	5.56	1.93	1.48	5
Rabbit	1.12	^b	^b	^b	^b	7
Dog	0.038	^b	^b	^b	^b	10
	1.88	72.1	165	38.4	87.8	10
	0.11 ^c	2.20	2.10	20.0	19.1	11
	0.11/day ^{c,d}	2.30	2.15	20.9	19.5	11
	0.19 ^c	5.55	7.10	29.2	37.4	11
	0.19/day ^{c,d}	5.00	6.20	26.3	32.6	11
	0.29 ^c	6.30	7.45	21.7	25.7	11
	0.29/day ^{c,d}	4.50	5.85	15.5	20.2	11
	0.038 ^c	0.70	0.60	18.4	15.8	12
	0.038/day ^{c,d}	0.95	1.45	25.0	38.2	12
	0.38 ^c	9.40	13.0	24.7	34.2	12
	0.38/day ^{c,d}	14.5	19.4	38.2	51.0	12
2.25 ^c	146	301	64.9	134	12	
1.88/day ^{c,d}	92.0	166	48.9	88.3	12	

* Results in plasma are given in brackets, []

^b Blood levels were below the limit of detection

^c Nonradiolabeled dose; for Dog the value is the average of the male and female data

^d Denotes multiple dose study; values of C_{max} and AUC were determined for one dose interval at steady state

TABLE C-4: ENA 713 CONCENTRATIONS (C_{max} AND AUC) IN BLOOD/PLASMA* FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED ENA 713 - continued

Species	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng·h/mL)	Dose-Normalized		Reference
				C_{max}	AUC	
Dog - cont'd	0.11 ^c	4.02	4.61	36.5	41.9	14
	0.11/day ^{c,d}	2.48	3.72	22.5	33.8	14
	0.45 ^c	11.4	17.5	25.3	38.9	14
	0.45/day ^{c,d}	13.4	19.2	29.8	42.7	14
	1.58 ^c	89.7	113	56.8	71.5	14
	1.58/day ^{c,d}	46.1	83.6	29.2	52.9	14
	0.19 ^c	5.51	8.13	29.0	42.8	13
	0.19/day ^{c,d}	6.83	13.8	35.9	72.6	13
	0.38 ^c	9.69	10.3	25.5	27.1	13
	0.38/day ^{c,d}	12.3	25.7	32.4	67.6	13
	1.56 ^c	60.5	114	38.8	73.1	13
	1.31/day ^{c,d,e}	38.0	127	29.0	96.9	13
Human	0.0132	[0.227]	[0.305]	[17.2]	[23.1]	16
	0.0310	[1.92]	[2.91]	[61.9]	[93.9]	16

- * Results in plasma are given in brackets, []
- ^a Blood levels were below the limit of detection
- ^b Nonradiolabeled dose; for Dog the value is the average of the male and female data
- ^c Denotes multiple dose study; values of C_{max} and AUC were determined for one dose interval at steady state
- ^d Initial dose was 1.56 mg/kg; on Day 4 it was reduced to 1.31 mg/kg

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TABLE C-5: ANIMAL:HUMAN RATIOS OF DOSE-NORMALIZED PLASMA C_{max} AND AUC VALUES FOLLOWING A SINGLE ORAL DOSE OF ENA 713^a

	Species	Dose (mg/kg)	C _{max} Ratio	AUC Ratio
Radioactivity ^b	Mouse	0.25	0.366	0.0984
		1.56	0.297	0.0884
	Rat	0.38	0.148	0.116
		3.75	0.171	0.148
	Rabbit	1.12	0.505	0.237
	Dog	0.038	0.735	1.22
1.88		0.439	0.813	
0.038		0.297	0.168	
ENA 713 ^b	Mouse	0.25	0.268	0.198
		1.56	0.658	0.275
	Rat	0.38	- ^c	- ^c
		3.75	0.0352	0.0269
	Rabbit	1.12	- ^c	- ^c
	Dog	0.038	- ^c	- ^c
		0.038	0.297	0.168

^a Radioactivity data are extracted from Table C-3, ENA 713 data from Table C-4. The human ENA 713 C_{max} and AUC values for comparison were based on the higher of the two doses, 2.5 mg (0.031 mg/kg), estimated to be the minimum therapeutic dose.

^b Ratios were calculated using blood data for the animals, since plasma concentrations were unavailable. The blood to plasma distribution ratio for ENA 713 in rat, dog, and human was ca. 1 (see reference 17,18)

^c Blood levels of ENA 713 for the animals were below the limit of detection

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TABLE C-5: ANIMAL:HUMAN RATIOS OF DOSE-NORMALIZED PLASMA C_{max} AND AUC VALUES FOLLOWING A SINGLE ORAL DOSE OF ENA 713^a - continued

	Species	Dose (mg/kg)	C_{max} Ratio	AUC Ratio
ENA 713 ^b	Dog - cont'd	0.11	0.323	0.203
		0.11	0.590	0.446
		0.19	0.472	0.398
		0.19	0.468	0.456
		0.29	0.350	0.274
		0.38	0.399	0.364
		0.38	0.412	0.289
		0.45	0.409	0.414
		1.56	0.627	0.778
		1.58	0.918	0.761
		1.88	0.620	0.935
		2.25	1.05	1.43

^a Radioactivity data are extracted from Table C-3, ENA 713 data from Table C-4. The human ENA 713 C_{max} and AUC values for comparison were based on the higher of the two doses, 2.5 mg (0.031 mg/kg), estimated to be the minimum therapeutic dose.

^b Ratios were calculated using blood data for the animals, since plasma concentrations were unavailable. The blood to plasma distribution ratio for ENA 713 in rat, dog, and human was ca. 1 (see reference 17,18)

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TABLE C-6: HALF-LIVES OF RADIOACTIVITY AND ENA 713 AFTER ORAL ADMINISTRATION OF RADIOLABELED ENA 713

Species	Terminal Half-life (h) ^a		References
	ENA 713	Radioactivity	
Mouse	- ^b	12.4 ^c , 5.25 ^d	4
Rat	0.4 ^e	37.3 ^c , 155.7 ^d	5
Rabbit	- ^b	49 ^f	7
Dog	2.5 ^{d,g}	9.9 ^c , 11 ^d	10
Man	0.62 ^c , 0.69 ^d	3.5 ^c , 3.9 ^d	16

^a Single dose data

^b Data unavailable due to the insufficient number of concentration-time points of ENA 713 above the limit of quantification.

^c Determined from low dose data

^d Determined from high dose data

^e Determined based on data from multiple oral dosing

^f Only one oral dose administered

^g Blood levels of ENA 713 were below the detection limit at the low dose

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to the insufficient number of concentration-time data points of ENA 713 above the limit of quantification.

The total body clearance (CL) and steady-state volume of distribution ($V_{d,ss}$) of ENA 713 were calculated using blood level data after intravenous dosing as follows:

$$CL = \frac{\text{Dose}}{\text{AUC}} \quad V_{d,ss} = \frac{\text{Dose (AUMC)}}{(\text{AUC})^2}$$

where AUC is calculated from zero to the time point at which the concentration fell below the detection limit and AUMC is the area under the first moment of the concentration-time curve and was obtained by the trapezoidal rule. The results (Table C-7) showed that total clearance was lowest in man (1.4 L/h/kg) and highest in the rodents (ca. 12 L/h/kg). With the possible exception of the mouse, the difference in clearance between species appeared to be attributable to differences in the volume of distribution of ENA 713. The relatively small $V_{d,ss}$ in human was consistent with the higher circulating levels and greater systemic exposure to the drug than that observed in the animals.

3.3 Distribution

Blood

The *in vitro* binding of [³H]ENA 713 to plasma proteins and red blood cells of man, dog, and rat was determined using an ultrafiltration method (17). The percentage of radioactivity bound to plasma proteins was low (ca. 15%, 19%, and 43% for rat, dog, and human, respectively) without any major change of binding in the concentration range between 1 ng/mL and 400 ng/mL. The blood:plasma ratios of [³H]ENA 713 were ca. 1, while the fraction of drug associated with red blood cells was ca. 0.67, 0.53, and 0.43 for rat, dog, and human, respectively. Irrespective of the species tested, there was no evidence of concentration dependency of drug distribution in blood over the concentration range tested.

A subsequent study, using only human blood cells and plasma proteins, was performed to study the distribution and binding in the presence and absence of 1 mM physostigmine (18). This study confirmed that ENA 713 was stable for the duration of the study and that the binding of total radioactivity (see above) was representative of the distribution and binding of ENA 713. However, it was noted in this study that physostigmine competed with ENA 713 for the protein binding sites, resulting in a ca. 2-fold decrease in the binding of ENA 713.

Tissue and Organ Distribution

Tissue and organ distribution studies were conducted in the mouse (4) and rat (5). In Figure C-2, tissue and organ radioactivity levels at 2 h after a single dose are used to illustrate the distribution of ENA 713 and its metabolites in these species. In general, the dose-normalized concentrations were considerably higher in the rat than in the mouse. In both species, the highest radioactivity concentrations at 2 h were observed in the liver, kidneys, and salivary glands and the radioactivity in all tissues decreased rapidly such that at 96 h little or no residual radioactivity was detected. In the mouse,

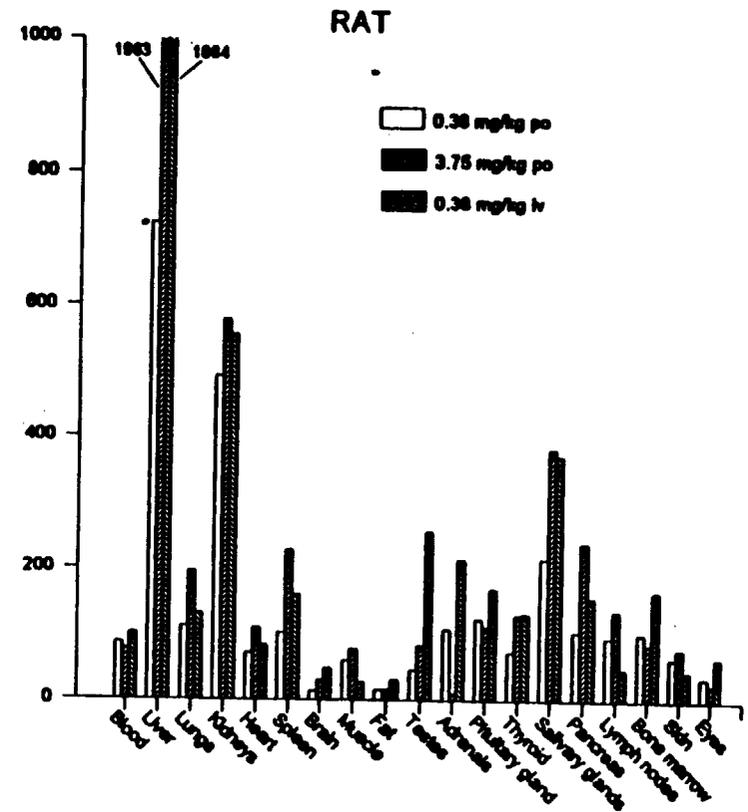
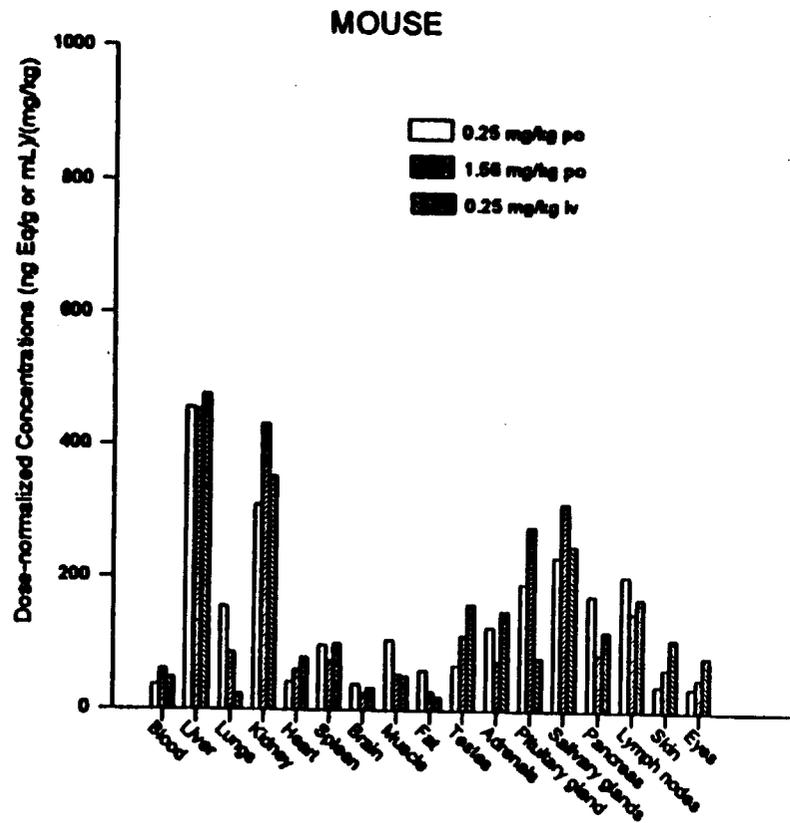
**TABLE C-7: TOTAL BODY CLEARANCE AND STEADY STATE VOLUME OF DISTRIBUTION
OF ENA 713**

Species	Dose (mg/kg)	Clearance (L/h/kg)	Vd _{ss} (L/kg)	Reference ^a
Mouse	0.25	12.3	2.96	4
Rat	0.38	12.1	8.24	5
Rabbit	1.12	3.45	3.34	7
Dog	0.038	4.92	2.33	10
Human	0.014	1.4	1.5	34

^a Clearance and Vd_{ss} were calculated using the correct doses as shown in the table. Blood (animals) and plasma (human) concentration data following intravenous administration are given in the referenced reports.

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FIGURE C-2: DOSE-NORMALIZED TISSUE AND ORGAN CONCENTRATIONS OF RADIOACTIVITY AT 2 H AFTER A SINGLE DOSE OF RADIOLABELED ENA 713 IN MICE AND RATS



the dose-normalized concentrations were similar between the low and high doses, thus reflecting similar absorption at both doses. In the rat, the dose-normalized concentrations were similar between the intravenous and high oral doses, but appeared to be lower in most tissues following the low oral dose. Following multiple oral dosing in the rat, no accumulation of drug-related material was apparent in the tissues.

Placental Transfer

The tissue distribution pattern during multiple dosing in pregnant rabbits (8) was similar to that observed in rodents following a single oral dose, i.e., only the liver and kidneys showing higher radioactivity concentrations than blood. The blood concentrations of radioactivity at 2 h post-dose in the maternal blood were relatively constant over days 10 to 19 p.c. (post-conception) and the tissue radioactivity appeared to have reached steady-state by day 10 p.c. (the fourth dose). The concentrations of radioactivity in the liver and kidneys were ca. 2.5- and 8-fold higher than the measured blood levels, respectively. The fetus:placentae ratio averaged 0.5 at 2 h and increased to 0.8-0.9 at 24 h and 48 h post-dose, indicating a moderate transfer of drug-related materials across the placenta. The concentrations of radioactivity in the amniotic fluid were similar to respective values in the fetuses. The blood concentrations of radioactivity in the pregnant rabbits at 2 h post-dose were similar to those previously reported in nonpregnant female rabbits after a single oral dose (7), suggesting little or no accumulation following multiple oral dosing.

Transfer Across the Blood-Brain Barrier (19)

Using an *in situ* rat brain perfusion/capillary depletion method, the blood-brain barrier permeability and extent of brain penetration of ENA 713 was determined. The data indicated a lack of sequestration of ENA 713 by the cerebral vasculature, that plasma protein binding of ENA 713 should not limit the quantity of drug available for uptake into the brain, and a high passage of ENA 713 into the brain. The brain extraction value was found to be 70%, a value that is higher than observed with amiridine (17%), another acetylcholinesterase inhibitor. The resulting permeability value of ENA 713 was 2.8×10^{-2} cm/min, which is similar to rimantadine, a drug actively transported through the blood-brain barrier.

3.4 Metabolism

The biotransformation of ENA 713 has been investigated in the mouse (28), rat (26, 31, 32), rabbit (29), dog (27,31) and human (30) following oral dosing. The main metabolite peaks found in plasma and urine (feces were not analyzed) are shown in Table C-8 and the structures (32) of the known metabolites are shown in Figure C-3.

ZNS 114-666 and 226-90 are both names referring to the phenol resulting from the hydrolysis of ENA 713. The compound 226-90 retains the stereochemistry of ENA 713, which is an optically pure enantiomer (see 2.1, this section), while ZNS 114-666 is the racemate. Since hydrolysis via the target enzymes does not involve metabolism at the chiral center, it can be assumed that chirality is retained. In the initial development, ZNS 114-666 was used as the reference compound, because it was readily available and, as a result, that name has been used to refer to the phenolic metabolite of ENA

TABLE C-8: MAIN^a METABOLITES OF ENA 713 AFTER SINGLE ORAL DOSING IN VARIOUS SPECIES

Peak Number	Identification	Metabolite Presence in Species and Sample Type ^b				
		Human	Mouse ^c	Rat	Rabbit ^c	Dog
2b	- ^d				U	
2c	- ^d		U			
3	- ^d	U			U	- ^d
4	- ^d	U	- ^d	- ^d		- ^d
5	M5 ^f	- ^d		U		U
6	- ^d			U		
7	M7 ^f	P,U		P,U		U
8	M8 ^f			U		U
9	- ^d		U			U
10	M10 ^f (ZNS 114-666)	P,U	U	P,U	U	P,U
11	- ^d			U		
15	- ^d		U			
20	ENA 713					U
23	- ^d					P

^a Metabolites representing 5% or greater sample content

^b P = plasma; U = urine

^c Plasma was not analyzed; blood was analyzed (ref. 4, 7) only for ENA 713 and ZNS 114-666

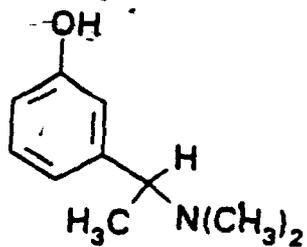
^d Not identified

^e Present between ca. 1-4%; not considered a "main" metabolite as defined in footnote "a"

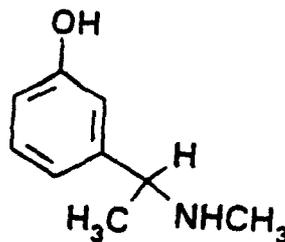
^f See Figure C-3 for structures

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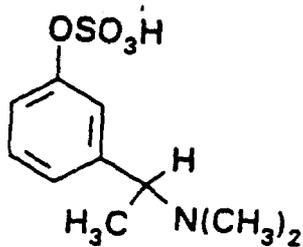
FIGURE C-3: STRUCTURES OF THE IDENTIFIED MAJOR METABOLITES OF ENA 713



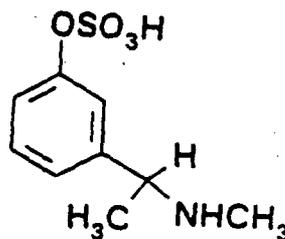
M 10
(ZNS 114-666)



M 8



M 7



M 5

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713 in both preclinical and clinical documents. The analytical methods used] cannot distinguish between ZNS 114-666 and 226-90. The usage of ZNS 114-666 as the name for the phenolic metabolite will continue in this section, recognizing that 226-90 is most likely the proper terminology for the phenolic metabolite.

In the human, following an oral dose of 1 or 2.5 mg, the major plasma and urinary metabolite was M7, the sulfate conjugate of ZNS 114-666. The phenol itself, ZNS 114-666 (M10), was also detected in the plasma and urine, but in a considerably lesser quantity. These two metabolites represented 47-50% of the plasma radioactivity (PRA) at C_{max} and 45-46% of the radioactivity recovered in the urine. No parent drug was detected in the urine following either dose. In both plasma and urine, the quantity of M7 remained approximately the same between the two doses, while the quantity of M10 increased by ca. 4 to 7 times greater at the higher dose, indicating probable saturation of the sulfation pathway. All major (greater than 5% of the dose) metabolites detected in the human were present in at least one of the animal species. Metabolite peak 4 (Table C-8), a major peak in human (but, detected only at the higher dose of 2.5 mg), was found only as a minor metabolite peak (ca. 1-4% of the dose) in the urine of mouse, rat, and dog. Together, all of the known metabolites (M7, M10, M5) detected in the human represented 51-54% of the PRA and 49-50% of the administered radioactivity.

All of the known metabolites detected in human (M7, M10, M5) were found in rat and dog, but not in the same proportions. The major circulating metabolites at C_{max} in the rat were M7 and M10, present in approximately equal quantities, while in the dog, M10 was the major circulating metabolite with M7 present in a minor quantity. At C_{max} in rat and dog plasma, these two metabolites, M7 and M10, represented ca. 32 % of the PRA in rat and ca. 44% of the PRA in dog. On a dose-normalized basis, the ng Eq/mL of M10 detected in the dog was 4-5 times that detected in human or rat.

The major urinary metabolite in rat was M10, present in ca. 2-fold greater quantity than its sulfate conjugate, M7 (which was the major human urinary metabolite). In dog, the major urinary metabolite was M8 (N-demethylated analog of ZNS 114-666) following a low dose and both M8 and M10, in approximately equal quantities, following a high dose. No parent drug was detected in rat urine, similar to the results in human; but, in dog, ENA 713 was detected in all of the urines (ca. 1% of the dose after a low dose and 16% \pm 14% of the dose after a high dose). In rat, the metabolism, both qualitatively and quantitatively, was similar between the low and high oral doses investigated, unlike in the human where the possible saturation of the sulfation pathway was noted. In dog, when the dose was increased ca. 50-fold, a saturation of metabolic pathways was indicated by the increased urinary excretion of ENA 713 and by an increase in the quantity of M10, with a concomitant decrease in the sulfate conjugates, M7 and M5.

The metabolism of ENA 713 in mouse and rabbit was similar to human only in that the major pathway, enzymatic hydrolysis to the phenol, M10, was detected. M10 was a major metabolite only in the mouse following a high dose, although neither its sulfate conjugate (M7), or its N-desmethyl analog (M8) were present as major metabolites. In the rabbit, none of these metabolites (M10, M7, M8) were present as major metabolites. These three known metabolites, together, represented only 3-9% of the

dose in mouse and ca. 12% of the dose in rabbit. In either species, no parent drug was detected in the urine.

Thus, the identified biotransformation pathways of ENA 713 (Figure C-4) in the species tested can be summarized as follows:

1. hydrolysis via the target enzymes, cholinesterases, to M10
2. N-desmethylation (M8)
3. conjugation with sulfuric or β -glucuronic acid (M7, M5, metabolite peaks 2b and 3)

The major metabolites and metabolic pathways identified *in vivo* were also identified *in vitro*, in addition to several minor metabolites not detected *in vivo* (33)

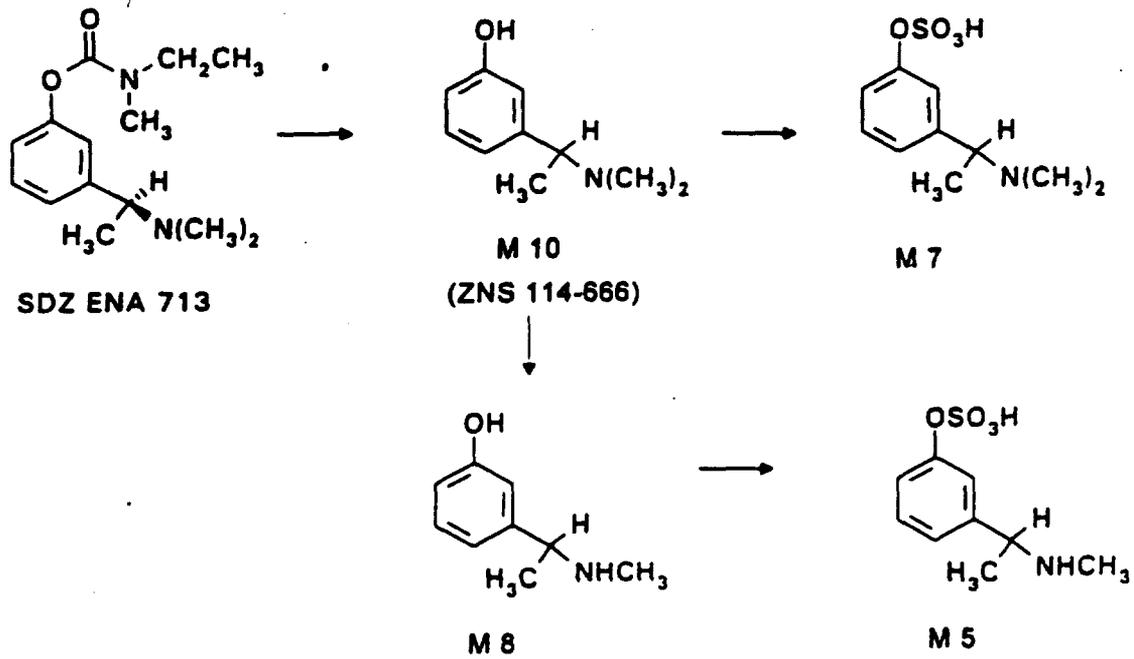
3.5 Excretion

The urinary and fecal excretion data following a single dose of radiolabeled ENA 713 are summarized in Table C-9. In all species, material balance was essentially achieved in the 96 h collection interval for the animals and the 120 h interval for human. The amount of total radioactivity recovered, including cage wash, was >88% except in the dog after a low oral dose (83%) and in the rat after an intravenous dose (83%). In all species, the renal route was the major elimination pathway, regardless of the dose or dose route, accounting for >76% of the administered radioactivity, except in the mouse following an oral dose (ca. 51-66%). The urinary excretion was rapid and nearly complete within 24 h of dosing. The remainder of the dose was eliminated in the feces and represented <11%, except for the mouse where it ranged between 19-24%. In bile-duct cannulated rats, 78.5% of the dose was recovered in the urine, compared to 5.6% in bile and 1.7% in feces (5). ENA 713 was not detected in the urine of any species, except dog, following an oral dose; however, after an intravenous dose, parent drug was detected in the urine of rat, rabbit, and dog. Because of the relatively small recovery of radioactivity in the feces, these samples were not analyzed for parent drug.

Following an oral dose of [³H]ENA 713 to lactating rabbits (9), transfer of drug-related materials (radioactivity) was rapid and extensive. Little or no parent drug was detected in the milk, although the milk:blood AUC ratios for total radioactivity and metabolite ZNS 114-666 were ca. 1.5 and 2.3, respectively.

Projecting the rabbit data to humans, it was estimated the maximum amount of ENA 713 and /or metabolite(s) that a breast fed infant could be exposed to by ingesting one liter of milk per day was 0.6% of the adult dose (9). With respect to the metabolite, ZNS 114-666, the exposure would be <0.1% of the adult dose.

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



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TABLE C-9: EXCRETION OF ENA 713 AND TOTAL RADIOACTIVITY FOLLOWING A SINGLE DOSE OF RADIOLABELED ENA 713

Species	Dose (mg/kg)	Dose Route	Amount Excreted (% of Dose)					Ref
			Urine			Feces		
			Radioactivity		ENA 713	Radioactivity		
			0-24 h	0-96 h	0-96 h	0-24 h	0-96 h	
Mouse	0.25	i.v. ^a	86.4	99.3	- ^a	5.8	7.5	4
	0.25	p.o.	34.7	51.2	- ^a	14.1	23.8	4
	1.56	p.o.	60.2	65.5	- ^a	16.8	19.2	4
Rat	0.38	i.v.	71.3	77.4	6.5	2.2	3.0	5
	0.38	p.o.	84.4	91.3	- ^a	3.4	4.3	5
	3.75	p.o.	86.4	95.0	- ^a	4.4	5.2	5
Rabbit	1.12	i.v.	83.3	85.6	7.2	0.87	1.1	7
	1.12	p.o.	82.5	86.7	- ^a	0.77	1.1	7
Dog	0.038	i.v.	74.3	84.2	2.2	2.7	4.7	10
	0.038	p.o.	64.1	76.0	1.2	2.2	6.0	10
	1.88	p.o.	71.0	79.5	16.5	8.2	10.6	10
Man	0.0132	p.o.	92.4	95.7 ^b	- ^a	0.04	0.40 ^b	16
	0.0310	p.o.	94.5	97.1 ^b	- ^a	0.02	0.32 ^b	16

^a Not detected

^b 0-120 h

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REFERENCES

- | | Vol. | Page No. |
|--|------|----------|
| 1) Susan A, Tang FYS, Jones L. [³ H]SDZ ENA 713 hta: Synthesis of SDZ ENA 713 hta with tritium in the phenyl-2-position. San-Joz Report CDH-84-69-7 (Document No. 303-159). | 58 | 5-11333 |
| 2) Tang FYS, Susan A, Jones L. [¹⁴ C] SDZ ENA 713 hta: Synthesis of Carbon-14 SDZ ENA 713 hta labeled at the benzylic carbon position. Sandoz Report CDH-84-69-8 (Document No. 303-158). | 58 | 5-11348 |
| 3) Pfefferkorn H, Tarapata R, Jones L, and Sunay U. [¹⁴ C] ENA 713 hta, Synthesis II. Sandoz Report CDH 84-34-97 (Document No. 303-303). | 58 | 5-11376 |
| 4) Habucky K. Absorption, Distribution, and Excretion of ³ H-ENA 713 in the Mouse Following a Single Oral and Intravenous Dose. Sandoz Report DM-1-3/16/94 (Document No. 303-238) and Amendment No. 1 (Document No. 303-254). | 58 | 5-11484 |
| 5) Ballard FH. SDZ 212-713- ³ H in the Male Rat: Absorption, Distribution, and Excretion Following Single and Multiple Doses. Sandoz Report DM-1-12/30/88 (Document No. 303-122). | 58 | 5-11490 |
| 6) Laplanche R, Perhaj Z. 2-Week Toxicity Study with SDZ ENA 713 in the Rat (Intravenous Application) Supplement No. 1: Toxicokinetic Results Part of Study RCC 341245. Sandoz Pharma Ltd., Basel, November 11, 1993 (Document No. 303-233). | 59 | 5-11579 |
| 7) Habucky K. Absorption, Blood Concentrations and Excretion of ³ H-ENA 713 in the Rabbit Following a Single Oral and Intravenous Dose. Sandoz Report DM-1-11/2/94 (Document No. 303-253). | 59 | 5-11619 |
| 8) Habucky K. Distribution of Radioactivity in Pregnant Rabbits During Multiple Oral Dosing of ³ H-ENA 713. Sandoz Report DM-1-3/8/95 (Document No. 303-259). | 59 | 5-11668 |
| 9) Gunn H, Habucky K. Passage of Radioactivity into Rabbit Milk Following an Oral Dose of ³ H-ENA 713. Sandoz Report DM-1-3/14/96 (Document No. 303-284). | 59 | 5-11696 |
| 10) Tse FLS. SDZ ENA 713- ³ H (212-713- ³ H) in the Dog: Absorption, Blood Levels and Excretion Following Single Oral and Intravenous Doses. Sandoz Report DM-1-12/20/88 (Document No. 303-120). | 59 | 5-11729 |
| 11) Jaffe JM. Blood Levels of SDZ ENA 713 (212-713) and its Phenolic Metabolite ZNS 114-666 (114-666) Following Single and Multiple Oral Doses of SDZ-ENA 713 in the Dog; PSA Project T-2604. Sandoz Report DM-1-10/5/89 (Document No. 303-119). | 59 | 5-11766 |
| 12) Tse FLS. Blood Levels of SDZ ENA 713 (212-713) and its Phenolic Metabolite ZNS 114-666 (114-666) Following Single and Multiple Oral Doses of SDZ ENA 713 in the Dog. Sandoz Report DM-1-6/28/89 (Document No. 303-118). | 59 | 5-11799 |
| 13) Cavanagh D. SDZ ENA 713: One year Oral Toxicity Study in the Dog on ENA 713 (Sandoz Project T-2723) August 6, 1992 (Document No. 203-203). | 46 | 5-8707 |

	Vol.	Page No.
14] Cavanagh DG. Twenty-Six Week Oral Toxicity Study in the Dog on 212-713 (ENA 713) (Sandoz Project T-2641). Sandoz Report T-2-8/1/90 (Document No. 203-144).	45	5-8264
15] Vit P. SDZ ENA 713: 2-Week Intravenous Toxicity Study in Beagle Dogs, Sandoz Pharma Ltd., Basel, February 19, 1996 (Document No. 203-275).	60	5-11834
16] Tse FLS, Anand R. Pharmacokinetic Characteristics of ENA 713 Following Single Oral Administration of Two Different Dose Levels of ¹⁴ C-ENA 713 in Healthy Male Volunteers. ENA 713 Study No. B151. Sandoz Report DM-1-5/29/92 (Document No. 303-208).	61	5-12035
17] Habucky K. ENA 713- ³ H]: <i>In Vitro</i> Binding Studies with Red Blood Cells and Plasma Proteins. Sandoz Report DM-1-8/17/92 (Document No. 303-206).	62	5-15491
18] Labbadia D. [¹⁴ C]SDZ ENA 713 (ENA 713): <i>In Vitro</i> Distribution and Binding Studies with Human Blood Cells and Plasma Proteins. Sandoz Report DM-1-7/11/96 (Document No. 303-297).	62	5-12514
19] Lemaire M. SDZ ENA 713: Blood-Brain Barrier Permeability of ³ H-SDZ ENA 713 and its Metabolite ³ H-ZNS 666. Sandoz Pharma Ltd., Basel, May 20, 1991 (Document No. 303-176).	62	5-12548
20] Perhaj Z, Laplanche R. SDZ ENA 713: Determination of the Unchanged Drug and Its Phenolic Metabolite ZNS 114-666 in Blood by Gas Chromatography-Mass Spectrometry. Sandoz Pharma Ltd., Basel, June 1, 1989 (Document No. 303-116).	62	5-12557
21] Hubert M, Frigola R, Lambert C. SDZ ENA 713: Determination of the Unchanged Drug and Its Phenolic Metabolite ZNS 114-666 in Blood by Gas Chromatography-Mass Spectrometry. Sandoz Laboratories, Rueil-Malmaison, February 24, 1992 (Document No. 303-199).	62	5-12574
22] Gurrieri P. SDZ ENA 713: Revalidation of a Method for the Determination of SDZ ENA 713 (212-713) and its Phenolic Metabolite ZNS 114-666 in Dog Blood Using Gas Chromatography-Mass Spectrometry. Sandoz Report DM-1-10/8/92 (Document No. 303-243).	62	5-12603
23] Perhaj Z, Laplanche R. SDZ ENA 713: Method of Determination of SDZ ENA 713 and ZNS 114-666 in Human Plasma by Gas Chromatography-Mass Spectrometry. Sandoz Pharma Ltd., Basel, July 7, 1995 (Document No. 303-263).	63	5-12711

	Vol.	Page No.
24] Laplanche R, Vogel D, Jean C. SDZ ENA 713: Evaluation of the Absorption, Distribution, and Excretion of Two Different Dosage Levels of ¹⁴ C-ENA 713 Following Single Dose, Oral Administration in Healthy Male Volunteers (Study No. B151). Determination of Unchanged SDZ-ENA 713 and of Its Metabolite ZNS 114-666 in Plasma Samples by Tandem Mass-Spectrometry. Sandoz Pharma Ltd., October 22, 1991 (page 238 of Document No. 303-208).	61	5-12035
25] Jean C, Laplanche R. Method of Determination of SDZ ENA 713 and ZNS 114-666 in Human Plasma and Cerebrospinal Fluid by Flow Injection-Tandem Mass Spectrometry. Sandoz Pharma Ltd., June 20, 1996 (Document No. 303-298).	63	5-12770
26] Orwig BA. 212-713- ³ H: Metabolite Patterns in the Urine of Rat. Sandoz Report DM-1-4/3/89 (Document No. 303-113).	63	5-12872
27] Orwig BA. 212-713- ³ H: Metabolite Patterns in the Urine of Dog. Sandoz Report DM-1-2/24/89 (Document No. 303-111).	63	5-12894
28] Orwig BA. Metabolism of SDZ ENA 713 in Mouse Following a Single Oral or Intravenous Dose of [³ H]ENA 713. Sandoz Report DM-1-6/15/94 (Document No. 303-239).	63	5-12913
29] Orwig BA. Metabolism in the Rabbit Following a Single Oral or Intravenous Dose of [³ H]SDZ ENA 713. Sandoz Report DM-1-12/7/94 (Document No. 303-252).	64	5-12936
30] Orwig BA. SDZ ENA 713 (212-713- ¹⁴ C): Biotransformation in Man Following a Single Oral Dose. Sandoz Report DM-1-2/26/92 (Document No. 303-201).	64	5-12959
31] Orwig B. SDZ ENA 713: Metabolite Profiles of SDZ ENA 713 in Rat and Dog Plasma Following a Single Oral Dose of SDZ ENA 713- ³ H and Comparison to Man. Sandoz Report DM-1-8/9/94 (Document No. 303-244).	64	5-13018
32] Orwig B. SDZ ENA 713 (212-713): Isolation and Identification of SDZ ENA 713- ³ H Metabolites from Rat. Sandoz Report DM-1-4/27/92 (Document No. 303-202).	64	5-13050
33] Fischer V, Vickers A, Zollinger M, and Enz A. SDZ ENA 713: Metabolism in human and rat liver slices, human intestine slices and human plasma. Sandoz Ltd., July 17, 1996 (Document No.303-302).	64	5-13080
34] Mancione L, Polinšky R, Hwang D, Garreffa S, and Habucky K. A partially double blind, randomized placebo controlled study to evaluate the pharmacokinetic and pharmacodynamic interaction of SDZ ENA 713 and digoxin and to determine the absolute bioavailability of SDZ ENA 713 in healthy subjects, Study No. W361-E-00/001. Sandoz Report CP-1-7/8/96 (Document No. 303-318).	65	5-13116

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Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Paul Andrews, Ph.D. HFD-150, Alternate Member
Barry Rosloff, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Barry Rosloff

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 20-823
Exelon (Rivastigmine tartrate)
Novartis

Mouse Carcinogenicity Study

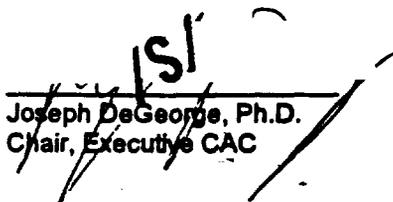
The two year gavage study was adequately conducted. Doses were adequate; MTD was reached based on toxic signs and decreased weight gain and food consumption. A slight statistically significant increase in incidence in mammary adenocarcinomas in HD F (4/70 vs 1/140 in controls) was not felt to be drug related since (1) the incidence of adenoma or hyperplasia was not increased, (2) the latency of tumor discovery was not decreased, (3) the incidence was within the sponsor's historical control range, and (4) there was no increase in mammary tumors in the rat study.

Rat Carcinogenicity Study

The two year (gavage) study was adequately conducted. Doses were adequate, MTD was reached based on toxic signs and decreased weight gain and food consumption. There were no drug-related increases in neoplasms.

Executive CAC Recommendations and Conclusions:

The mouse and rat carcinogenicity studies were adequate and do not indicate drug related-carcinogenesis.


Joseph DeGeorge, Ph.D.
Chair, Executive CAC

1/23/98

cc:1

- /Division File, HFD 120
- /BRosloff, HFD-120
- /WSchmidt, HFD-024

RECEIVED FEB 20 1998

Statistical Review and Evaluation

NDA #: 20,823

FEB 18 1998

Applicant: Sandoz Pharmaceuticals Corporation

Drug Name: Exelon (carbamoylatine hydrogen tartrate) Capsules

Document Reviewed:

"A two-year oral (gavage) carcinogenicity study in the rat on SDZ ENA 713" (T-2740),
"Lifetime oral (gavage) carcinogenicity study in the mouse on SDZ ENA 713" (T-2741),
Data diskettes.

Statistical Reviewer: Sue-Jane Wang, DB1/OEB, HFD-710

Pharmacologist: Berry Rosloff, ODEI, HFD-120

**APPEARS THIS WAY
ON ORIGINAL**

1 INTRODUCTION

In this submission, the sponsor reported the results of two 2-year carcinogenicity studies in rats and mice. The titles of the studies are "A two-year oral (gavage) carcinogenicity study in the rat on SDZ ENA 713" (Study T-2740) and "Lifetime oral (gavage) carcinogenicity study in the mouse on SDZ ENA 713" (T-2741). This review pertains to these studies.

2 STUDY OVERVIEW

2.1 T-2740 - the Rat Study

The study reported the results of two experiments, one in male and one in female rats. The study was designed to determine the oncogenic potential of the test article (SDZ ENA 713) after chronic administration to ~~SDZ ENA 713~~ y rats for a period covering the largest part of its life-span. It is based on the statistical analyses of survival and neoplastic lesions from a two-year chronic oral (gavage) study on the male and female Sprague-Dawley rats. There were two control groups and three dose groups (low, median and high). The following table summarizes the dose levels of compound SDZ ENA 713 administered.

Table. Dose levels administered in compound SDZ ENA 713* (T2740)

	I (Control I)	II (Control II)	III (Low)	IV (Mid)	V (High)
Salt*	0	0			
Base equivalent	0	0			

* SDZ ENA 713 is a hydrogen tartrate salt

! Base equivalent dose levels calculated using a conversion factor of 1.60. The dose levels stated are the hydrogen tartrate salt dose levels

All surviving animals were sacrificed in Week 104 with the objective of having at least 10 surviving animals/sex/group at terminal sacrifice. Seven hundred and fifty rats were randomly assigned to five groups each of 75 males and 75 females.

Inspections of individual clinical observations were recorded once daily during pretest and twice daily during the treatment phase (once prior to dosing and again after dosing). Two room checks for mortality were performed daily. Routine mass palpation examinations were performed weekly on all animals through Week 13 and once monthly thereafter. Any masses detected during routine handling of the animals were entered at the time of detection.

The sponsor's Results

a) Mortality

Survival was analyzed by life table techniques consisting of Kaplan-Meier product limit

estimates, Cox-Tarone binary regression on life table techniques, and Gehan-Breslow nonparametric methods (Thomas, Breslow, and Gart, 1977).

Trend probabilities were evaluated when there was existence of any monotone response. Continuity corrected test statistics were used for evaluation of all the incidence tables where appropriate. In the absence of any valid monotone trend, control versus treated group comparisons were made using Bonferroni adjustment to significance levels. The sponsor stated that 'no such adjustment was deemed appropriate for this study for the multiplicity of control groups because it would reduce the power of test considerably'.

The sponsor reported that "there were no statistically significant differences or trend in the survival data. Both sexes showed similar life-time survival probabilities across all groups".

b) Tumor Incidence

All nonpalpable tumor incidences were analyzed by Cochran-Armitage method for trend and Fisher-Irwin exact test for control versus treatment comparisons (Thakur, Berry, and Mielke, 1985). In the event of any possible intercurrent mortality differences due to competing toxicity among the treated groups, survival adjusted tumor analyses were performed as follows: the occult ("incidental" or nonpalpable) tumors were analyzed by logistic regression of tumor prevalence (Dinse and Lagakos, 1983). The ordinal dose levels, i.e., 0, 1, 2, and 3, were used to obtain continuity-corrected one-sided probability levels for trend. The benign and malignant neoplastic incidences were evaluated separately as well as combined (where appropriate). The criteria for combination were based on the work of McConnell et al. (1986).

The sponsor made the following observations:

1. There were a few instances of sporadic statistically significant findings in tumor analyses, specifically, the mammary gland fibrosarcoma incidence rate was significantly increased compared to the combined control incidence rate in the median dose (0.6 mg/kg/day) group of female rats.
2. There was no trend in this incidence and this increase was not significant when compared to the individual control group.
3. Most of the other findings were actually in the negative direction.
4. The fluctuating numbers are indicative of background noise in common neoplastic lesions in Sprague-Dawley rats.

The sponsor concluded that "based on the statistical analyses of survival and neoplastic lesion incidence data, there were no treatment related adverse toxic or oncogenic effects in either sex in this study".

Reviewer's Analysis and Comments

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor, using the programs provided by the statisticians of the SARB of the old Division of Biometrics, CDER/FDA. Since the two control groups are identical in design, the combined control groups was used in this reviewer's analyses.

Mortality

Two statistical tests were used (Cox test statistic and generalized Kruskal-Wallis test statistic) to examine the significance of the differences in survival among the treatment groups (the homogeneity test), and (2) to determine the significance of positive or negative linear trend (dose-mortality trend test).

The time intervals (0-52wk, 53-78wk, 79-91wk, 92-104wk, 104wk+) for two-year animal study were used. The cumulative mortality rates and the survival at terminal sacrifice for each dose group can be found in Table 1.

The results of this reviewer's analysis were consistent with the sponsor's results. Neither Cox test nor generalized K-W test showed a statistically significant ($p > 0.05$) positive linear trend or an increase in mortality in any treated group compared with the control in either sex. There was no statistical evidence indicating a difference in mortality among the four dose groups ($p > 0.31$). The survival curves are displayed in Figure 1 for male rats and Figure 2 for female rats.

Tumor Incidence

Using the method of Peto et al. (1980), this reviewer applied the death-rate method to fatal tumors and prevalence method to incidental tumors using standard time intervals of two-year animal studies. Tables 2 and 3 summarize the results of this reviewer's analysis for male and female rats that follows the FDA draft document "Guidance for industry on statistical aspects of design, analysis and interpretation of animal carcinogenicity studies". In general, the exact p-value is used to assess statistical significance, but for those tumors that are fatal to some animals but nonfatal to others the asymptotic p-value may be used. The p-values presented in the tables have not been adjusted for multiple testings. A rule proposed in the FDA draft Guidance document was used to adjust for multiple testings, based on which a positive linear trend is considered not to occur by chance alone if the p-value is less than 0.005 for a common tumor (tumor with incidence rate in the control group greater than 1%) and 0.025 for a rare tumor (tumor with incidence rate less than or equal to 1%). By use of this rule, subcutaneous tissue fibrosarcoma showed a significant positive linear trend (tumor incidence: 0 (control), 0 (low dose), 1 (median dose), 3 (high dose)) in male rats (see Table 2), but it was not significant in female rats (Table 3).

The results of this reviewer's analysis using standard two-year time intervals were consistent with the sponsor's results except the above finding (not reported by the sponsor) in terms of statistical significance after multiple testing adjustments.

2.2 T-2741 - the Mouse Study

The study reported the results of two experiments, one in male and one in female mice. The study was designed to determine the oncogenic potential of test article (SDZ ENA 713) based on the statistical analyses of survival and neoplastic lesions from a two-year chronic oral (gavage) study on the male and female CD-1 mouse. There were two control groups and three dose groups (low, median and high). The following table summarizes the dose levels of compound SDZ ENA 713 administered.

Table. Dose levels administered in compound SZD ENA 713* (T2741)

	I (Control I)	II (Control II)	III (Low)	IV (Mid)	V (High)
Salt*	0 (vehicle)#	0 (vehicle)#			
Base!	0	0			

* SDZ ENA 713 is a hydrogen tartrate salt

! Base equivalent dose levels calculated using a conversion factor of 1.60. The dose levels shown in this report are dose levels of the hydrogen tartrate salt.

Aqueous 1% carboxymethylcellulose (CMC)

The duration of the study (103 weeks) was based on exposing the animals to the test article for a majority of their adjust lifespan and having a minimum of 10 animals/sex/group at terminal sacrifice. Seven hundred mice were randomly assigned to five groups each of 70 males and 70 females.

Inspections for occurrence of clinical signs were made twice daily. Inspection of mortality was made twice daily except on weekends and holidays when a single check was done in the morning. Mass palpations were performed once during pretest, and monthly starting in Month 4, with the location, size and texture of each mass being recorded. A palpable mass was defined as having three measurable dimensions (length: measurement from head to tail; width: from left to right; and depth: from dorsal to ventral surfaces). The disappearance of a mass was noted. The initial onset of any mass was verified by the Study Director. Upon necropsy of the animals, all regions which had at any time exhibited a mass were examined in detail and observations of masses were recorded.

The sponsor's Results

a) Mortality

Survival was analyzed by life table techniques consisting of Kaplan-Meier product limit

estimates, Cox-Tarone binary regression on life table techniques, and Gehan-Breslow nonparametric methods (Thomas, Breslow, and Gart, 1977).

Trend probabilities were evaluated when there was existence of any monotone response. Continuity corrected test statistics were used for evaluation of all the incidence tables where appropriate. In the absence of any valid monotone trend, control versus treated group comparisons were made using Bonferroni adjustment to significance levels. The sponsor stated that 'no such adjustment was deemed appropriate for this study for the multiplicity of control groups because it would reduce the power of test considerably'.

In male mice, tests did not show any statistically significant change in mortality in any of the dosage groups. All control and treated groups had similar mortality.

In female mice, the high dose group showed significantly decreased mortality compared to control 2 and combined control. This group had the lowest mortality among all the groups studied. The significant negative trend in the female mortality was due to the decrease in this high dose group.

b) Tumor Incidence

All nonpalpable tumor incidences were analyzed by Cochran-Armitage method for trend and Fisher-Irwin exact test for control versus treatment comparisons (Thakur, Berry, and Mielke, 1985). In the event of any possible intercurrent mortality differences due to competing toxicity among the treated groups, survival adjusted tumor analyses were performed as follows: the occult ("incidental" or nonpalpable) tumors were analyzed by logistic regression of tumor prevalence (Dinse and Lagakos, 1983). The ordinal dose levels, i.e., 0, 1, 2, and 3, were used to obtain continuity-corrected one-sided probability levels for trend. The benign and malignant neoplastic incidences were evaluated separately as well as combined (where appropriate). The criteria for combination were based on the work of McConnell et al. (1986).

The sponsor made the following observations:

1. The combined skin mammary gland adenocarcinoma incidence rate in high dose group was marginally higher ($p=.0491$, adjusted) when compared to the combined controls in the females.
2. The rate was not statistically significant when compared against the individual control groups.
3. There was no other statistically significant neoplastic finding in the treated female groups.
4. There was no significant neoplastic finding in the males in this study.
5. All the incidences noted in both sexes were common backgrounds in this strain of mouse.

The sponsor concluded that "based on the statistical analyses of survival and neoplastic lesions, there was no adverse toxicological or oncogenic effect due to treatment in either sex in this study".

Reviewer's Analysis and Comments

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor, using the programs provided by the statisticians of the SARB of the old Division of Biometrics, CDER/FDA. Since the two control groups are identical in design, the combined control groups was used in this reviewer's analyses.

Mortality

The purposes of the survival data analysis are (1) to examine the significance of the differences in survival among the treatment groups (the homogeneity test), and (2) to determine the significance of positive or negative linear trend (dose-mortality trend test). Two statistical tests were used: Cox test statistic and generalized Kruskal-Wallis test statistic. The background for these tests can be found in Lin et al., 1994 and Thomas et al., 1976.

The standard time intervals (0-52wk, 52-78wk, 79-91wk, 92-103wk, 103wk+) for two-year animal studies were used. The cumulative mortality rates and the survival at terminal sacrifice for each dose group can be found in Table 4.

The results of this reviewer's analysis were consistent with the sponsor's results. Neither Cox test nor generalized K-W test showed a statistically significant ($p > 0.05$) positive linear trend or an increase in mortality in any treated group compared with the control in either sex. There was a significant decreased dose-mortality trend found in female mice. The decrease was primarily attributed to the high dose group which had the lowest mortality among all the groups studied. The survival curves are displayed in Figure 3 for male mice and Figure 4 for female mice.

Tumor Incidence

Using the method of Peto et al. (1980), this reviewer applied the death-rate method to fatal tumors and prevalence method to incidental tumors using standard time intervals of two-year animal studies. Tables 5 and 6 summarize the results of this reviewer's analysis for male and female mice that follows the FDA draft document "Guidance for industry on statistical aspects of design, analysis and interpretation of animal carcinogenicity studies". In general, the exact p-value is used to assess statistical significance, but for those tumors that are fatal to some animals but nonfatal to others the asymptotic p-value may be used. The p-values presented in the tables have not been adjusted for multiple testings. A rule proposed in the FDA draft Guidance document was used to adjust for multiple testings, based on which a positive linear trend is considered not to occur by chance alone if the p-value is less than 0.005 for a common tumor

(tumor with incidence rate in the control group greater than 1%) and 0.025 for a rare tumor (tumor with incidence rate less than or equal to 1%). By use of this rule, there were no significant positive linear trends in male mice (see Table 5) and female mice (see Table 6).

The results of this reviewer's analysis using standard two-year time intervals were consistent with the sponsor's results using different time intervals (0-52wk, 53-78wk, 79-91wk, 92-103wk, 103wk+) in terms of statistical significance after multiple testing adjustments.

3 VALIDITY OF THE DESIGN

To evaluate the validity of the experimental design of carcinogenicity studies, the following two issues are considered (1) Were enough animals exposed for a sufficient length of time to allow for late developing tumors? (2) Were the dose levels high enough to pose a reasonable tumor challenge in the animals? There has been no consensus among experts regarding the number of animals and length of time at risk, although most carcinogenicity studies are designed to run for two years with 50 animals per treatment group.

The following are some rules of thumb regarding these two issues as suggested by experts in the field. Haseman (1985) investigated the first issue. Based on the data from 21 studies using Fisher 344 rats and B6C3F1 mice conducted at the _____, he found that on the average, approximately 50% of the animals in the high dose group survived the 2-year study period. According to my personal communication with Dr. Karl Lin, Division of Biometrics II, CDER, FDA, Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals in the high dose group, after 80-90 weeks, would be considered as a sufficient number and adequate exposure. However, the percent could be lower or higher if the number of animals used in each treatment/sex group is larger or smaller than 50 so that there would be 20-30 animals still alive after the 80-90 weeks. Chu, Cueto and Ward (1981) proposed that "To be considered adequate, an experiment that has not shown a chemical to be carcinogenic would have groups of animals with greater than 50% survival at 1-year". From these sources, it appears that the proportions of survival at 1-year, week 80-90 and at 2-year are of interest in determining the adequacy of exposure and the number of animals at risk.

For the adequacy of the chosen dose levels, it is generally accepted that the high dose should be close to the MTD (maximum tolerated dose). Chu, Cueto and Ward (1981) suggested:

- (I) "A dose is considered adequate if there is a detectable weight loss of up to 10% in a dosed group relative to the controls."
- (II) "The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical".
- (III) "In addition, doses are considered adequate if the dosed animals show a slight increased mortality compared to the controls."

Bart, Chu, and Tarone (1979) stated that the mean body weight curves over the entire

study period should be taken into consideration with the survival curves, when adequacy of dose levels is to be examined. In particular, "usually, the comparison should be limited to the early weeks of a study when no or little mortality has yet occurred in any of the groups. Here a depression of the mean weight in the treated groups is an indication that the treatment has been tested on levels at or approaching the MTD."

Based on the above suggestions and recommendations, this reviewer examines the validity of the experimental design of the rat and the mouse studies.

3.1 T-2740 - Rat

The proportions of survival at the end of 1 year were 91% for male rats and 96% for female rats in the high dose group. Although these proportions were lower than 50% at the end of 2-year (17% for male rats and 31% for female rats), there were 30 or more animals still alive after 80-90 weeks corresponding to 47% for male rats and 60% for female rats. Using the above survival criteria, it is reasonable to conclude that there were enough number of rats exposed for sufficient amount of time to Exelon in both sexes.

Summary Table from Table 4A of the sponsor's report (T2740)

Sex	Group	Median Body weight in grams(n)		% weight gain	% reduction from Cntl
		Beginning of study	End of study		
Male rats	Cntl	133.5(150)	810(27)	507	
	Low	135.5(75)	785(16)	479	6%
	Med	136.5(76)	763(15)	459	9%
	High	137.5(75)	739(13)	437	14%
Female rats	Cntl	127.5(150)	631(42)	395	
	Low	130.5(75)	599(24)	359	9%
	Med	129.5(74)	555(20)	329	17%
	High	132.0(75)	544(23)	312	21%

The body weight gain information is summarized below (data are from Table 4A of the sponsor's report). Relative to the control, % reduction of body weight gain in the high dose group is 14% for male rats and 21% for female rats at the end of study. The mortality rates did not show any ordering with dose in either male or female rats. Thus, assessing whether the high dose was close to the MTD proved more difficult. There was weight reduction in the dosed animals. These reductions are higher than 10% for both male and female rats in the high dose group and for females receiving the medium dose. Mortality did not show any association with dose. To draw

conclusion in this regard, all clinical signs and histopathological effects in the treated rats should be taken into consideration.

3.2 T-2741 - Mouse

The proportions of survival at the end of 1 year were 93% for male mice and 93% for female mice in the high dose group. These proportions were 21% for male mice and 34% for female mice at the end of 2-year study. There were 30 or more animals still alive after 80-90 weeks corresponding to 30% for male rats and 44% for female rats. Using the above survival criteria, it is reasonable to conclude that there were enough number of mice exposed for sufficient amount of time to Exelon in both sexes.

The body weight gain information is summarized below (data are from Table 2A of the sponsor's report). Relative to the control, % reduction of body weight gain in the high dose group is 7% for male mice and 21% for female mice at the end of the study. The mortality rates did not show increased trend with dose in male mice. In female mice, the mortality decreased in the high dose group. It is of concern that their lack of tumor incidences may be due, at least in part, to the fact that leaner animals develop less tumors, and that the carcinogenic potential of this compound cannot be assessed in this sex and species. To draw a conclusion in assessing whether the high dose was close to the MTD, all clinical signs and histopathological effects in the treated mice should be taken into consideration.

Summary Table from Table 2A of the sponsor's report (T2741)

Sex	Group	Median Body weight in grams(n)		% weight gain	% reduction from Cntl
		Beginning of study	End of study		
Male mice	Cntl	29(140)	43(31)	48.3	
	Low	29(69)	44(19)	51.7	-7%
	Med	29(70)	43(17)	48.3	0%
	High	29(70)	42(15)	44.8	7%
Female mice	Cntl	24(140)	38(28)	58.3	
	Low	24(71)	39(14)	62.5	-5%
	Med	24(70)	37(13)	54.2	7%
	High	24(70)	35(24)	45.8	21%

4 CONCLUSION

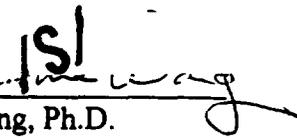
In the 2-year rat study, no statistically significant positive linear trend or heterogeneity in mortality between combined control group and three dose groups was detected in either sex. In male rats, tumor incidence of subcutaneous tissue - fibrosarcoma was shown to have a positive

linear trend with p-value of 0.0025 (asymptotic test). None of the other tested tumor types showed a statistically significant positive linear trend in either sex. The evaluation of the design validity suggested that it is not clear whether the high dose used in rat study reached the MTD. To draw any final conclusion for this issue, all clinical signs and histopathological effects in the treated rats should be taken into consideration.

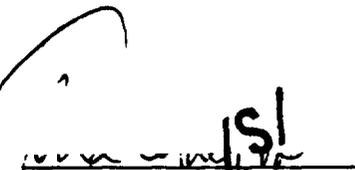
In the 2-year mouse study, no statistically significant positive linear trend or heterogeneity in mortality between the combined control group and three dose groups was detected in either sex. None of the tested tumor types showed a statistically significant positive linear trend in either sex. The evaluation of the design validity suggested that the high dose used in female mice may or may not be the MTD. To draw any final conclusion for this issue, all clinical signs and histopathological effects in the treated mice should be taken into consideration.

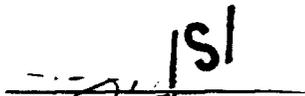
References:

1. Bart, Chi, and Tarone (1979). "Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity." Journal of the National Cancer Institute Vol.62, pp.957-974.
2. Chu, Cueto and Ward (1981). "Factors in the evaluation of 200 national cancer institute carcinogen bioassay." Journal of Toxicology and Environmental Health. Vol.8, pp.251-280.
3. Gart, J.J, Krewski, D., Lee, D.N., Tarone, R.E., and Wahrendorf, J. (1986). "Statistical methods in Cancer Research. Volume III - The design and analysis of long-term animal experiments", pp.1-219, International Agency for Research on Cancer, Lyon.
4. Haseman, J.K. (1983). "A re-examination of false-positive rates for carcinogenesis studies." Fundamental and Applied Toxicology 3, pp. 334-9.
5. Haseman, J.K. (1985). "Issues in carcinogenicity testing: Dose selection." Fundamental and Applied Toxicology. Vol. 5, pp. 66-78.
6. Lin et al. (1994). "Statistical Review and Evaluation of Animal Tumorigenicity Studies." Statistics in the Pharmaceutical Industry. Marcel Dekker, Inc. pp. 19-57.
7. Peto et al (1980). "Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments. In Long-Term and Short-Term Screening Assays for Carcinogens: A critical Appraisal, International Agency for Research on Cancer, Lyon, France. IARC Monographs Supplement 2. pp. 311-426.
8. Thomas et al. (1976). "Trend and Homogeneity Analyses of Proportions and Life Table Data." Computers and Biomedical Research. pp. 373-81.


Sue-Jane Wang, Ph.D.
Mathematical Statistician

Concur:


Todd Sahlroot, Ph.D.
Team Leader


George Chi, Ph.D.
Director, DB-I

cc: NDA # 20-823
HFD-120/Dr. Fitzgerald
HFD-120/Dr. Rosloff
HFD-120/Mr Nighswander, CSO
HFD-710/Dr. Chi
HFD-710/Dr. Sahlroot
HFD-710/Dr. Wang
HFD-700/Dr. Fairweather
HFD-710/chron

This review consists of 12 pages of text followed by 6 reviewer tables and 4 reviewer figures with a total of 24 pages.

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

DATE: March 4, 1998

FROM: Glenna G. Fitzgerald, Ph.D.
Pharmacology Team Leader
Division of Neuropharmacological Drug Products

TO: NDA 20-823
Exelon™, rivastigmine tartrate
1.5, 3.0, 4.5, 6.0 mg capsules
Sponsor: Novartis

SUBJECT: Approvability for Pharmacology and Toxicology

The pharmacology and toxicology studies submitted to this NDA for Exelon, an acetylcholine esterase inhibitor indicated for the treatment of mild to moderately severe dementia of the Alzheimer's type, have been reviewed by Dr. Barry Rosloff and are adequate to support its approval. I have incorporated Dr. Rosloff's recommendations for labeling changes and requests to the sponsor for information (see pages 47 - 49 of his review) into the sponsor's draft labeling, and a copy of the revised sections is attached [see Description, Clinical Pharmacology, Clinical Pharmacokinetics (Metabolism), Carcinogenesis, Mutagenesis, Impairment of Fertility, Pregnancy, and Overdosage].

Exelon was clastogenic in two *in vitro* assays, producing structural chromosomal aberrations in Chinese hamster lung cells and both structural and numerical aberrations in human lymphocytes. To assess the carcinogenic potential of Exelon, lifetime oral (gavage) studies were conducted in mice and rats. Both studies were adequate with respect to attaining a maximum tolerated dose, and neither showed an increase in tumors, as confirmed by the CAC-EC (report attached). However, the maximum doses tested represent less than the human exposure at a dose of 12 mg on a body surface area basis. Humans apparently tolerate much higher relative doses than rodents, and the relevance of this species difference in sensitivity with respect to evaluating carcinogenic risk is unknown.

The sponsor labeled Exelon Pregnancy Category C. They have cited no drug related effects, so it is not clear why they chose category C. Dr. Rosloff has recommended Category B. However, he notes in his labeling review that several studies showed decreased fetal and/or pup weights and recommends that the information be included in the pregnancy section. The doses at which this effect was noted varied across studies, there was not a clear dose relationship, the effects were slight, and they were undoubtedly due to developmental retardation rather than a direct effect on the fetus since they were usually associated with maternal toxicity. There were no effects on fetal deaths or pup survival. It becomes problematic to recommend Category B and also to include effects on the fetus in labeling. Section §201.57 CFR states "If animal reproduction studies have shown an adverse effect on the fetus, — the labeling shall state Pregnancy Category C." I therefore asked Dr. J.E. Fisher, our expert for reproductive toxicology, to look at the data to determine if he thought the effects on body weights would be considered a drug-related adverse effect on the fetus. His conclusion, and I agree, is that because of the minimal nature of the effects, lack of dose relationship and absence of more serious effects on fetuses/pups such as stillbirth/deaths, possible drug effects on fetal and pup weights should not be included in labeling and Exelon should be labeled Pregnancy Category B.

Recommendations:

This NDA is approvable for pharmacology/toxicology with the attached recommended labeling, and there are no outstanding issues.


Glenna G. Fitzgerald, Ph.D.

Attachments: 2

NDA 20-823

cc: / Div. File HFD-120

/ Leber/Levin/Rosloff/Fitzgerald/Nighswander

Executive CAC
12/23/97

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Paul Andrews, Ph.D. HFD-150, Alternate Member
Barry Rosloff, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Barry Rosloff

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 20-823
Exelon (Rivastigmine tartrate)
Novartis

Mouse Carcinogenicity Study

The two year gavage study was adequately conducted. Doses were adequate; MTD was reached based on toxic signs and decreased weight gain and food consumption. A slight statistically significant increase in incidence in mammary adenocarcinomas in HD F (4/70 vs 1/140 in controls) was not felt to be drug related since (1) the incidence of adenoma or hyperplasia was not increased, (2) the latency of tumor discovery was not decreased, (3) the incidence was within the sponsor's historical control range, and (4) there was no increase in mammary tumors in the rat study.

Rat Carcinogenicity Study

The two year (gavage) study was adequately conducted. Doses were adequate, MTD was reached based on toxic signs and decreased weight gain and food consumption. There were no drug-related increases in neoplasms.

Executive CAC Recommendations and Conclusions:

The mouse and rat carcinogenicity studies were adequate and do not indicate drug related-carcinogenesis.

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11/198

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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/BRosloff, HFD-120
/WSchmidt, HFD-024

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RELEASABLE

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5/4/00
9:03 AM

ExelonTM
(Rivastigmine Tartrate)
Capsules

1.5 mg, 3.0 mg, 4.5 mg, & 6.0 mg

NDA 20-823

Clinical/Statistical