2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were included in this submission.

2.6.6.3 Repeat-dose toxicity

No repeat-dose toxicity studies were included in this submission.

2.6.6.4 Genetic toxicology

The following genetic toxicology studies were submitted and are reviewed in this section:

- 1:1 combination of Naproxen sodium and Sumatriptan succinate: In vitro Chromosome Aberration Assay with Cultured Chinese Hamster Ovary (CHO) Cells
- 1:1 combination of Naproxen sodium and Sumatriptan succinate: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

1:1 combination of Naproxen sodium and Sumatriptan succinate: In vitro Chromosome Aberration Assay with Cultured Chinese Hamster Ovary (CHO) Cells

Study no.: [redacted] Study 2990/7; GSK Document WD2006/03218/00

Volume # , and page #: eNDA 21-926 #016, Module 4, Section 4.2.3.3.1.1, pages 1-76
Conducting laboratory and location: [redacted] North Yorkshire, UK
Date of study initiation: 04 AUG 2006
GLP compliance: Yes, UK 1999/2004, signed 31 OCT 2006 by Study Director
QA reports: yes, statement signed 31 OCT 2006 by the QA Representative
Drug, lot #, and % purity: Naproxen Sodium (NAP) Lot #NPXNAM-631 and #NPXNAM-635, Purity 100%; NAP doses were calculated as the free acid, using a correction factor of 1.1. Sumatriptan Succinate (SS) Lot #K058945 and #K026882, Purity 98.7% and 99.2%, respectively. SS doses were calculated as the free base, using a conversion factor of 1.4.

Methods

Strains/species/cell line:
CHO cells, originally supplied by [redacted] were maintained at [redacted] in tissue culture flasks containing [redacted] 5A medium with 10% (v/v) heat inactivated fetal calf serum, and 100 ug/mL gentamycin. The doubling time of this cell line is ~13 hrs, and its modal chromosome number is 21.

Doses used in definitive studies:
-S9: NAP alone at 1920 and 2500 ug/mL
SS alone at 1920 ug/mL
SS/NAP together at 1710/1710, 1815/1815, 1850/1850, and 1920/1920 ug/mL
+S9: NAP alone at 1780 and 2500 ug/mL
SS alone at 1780 ug/mL
SS/NAP together at 1640/1640, 1710/1710, 1745/1745, and 1780/1780 ug/mL

Basis of dose selection:
The primary measure of cytotoxicity in this study was mitotic index (MI), the percentage of cells in mitosis, based on scoring of at least 1000 cells per culture. Slides showing > 61% reduction in MI were not scored for chromosomal aberrations. The concentrations selected for analysis of chromosomal aberrations was agreed with the sponsor before scoring. Cytotoxicity was also measured based on cell counts (compared to mean vehicle control) and population doublings (PD = [log (N/X_0)]/log 2, where N = mean final cell count/culture at harvest, and X_0 = starting count at beginning of treatment).

Negative controls:
Purified water was used as the solvent control.

Positive controls:
4-Nitroquinolone 1-oxide (NQO, 0.25 and 0.30 ug/mL final concentration, from stock in DMSO, [redacted], was used as the positive controls in the absence of metabolic activation.

Cyclophosphamide (CPA, 6.25 and 12.5 ug/mL final concentration, from stock in DMSO, [redacted], was used as the positive control in the presence of metabolic activation.

Incubation and sampling times:
Duplicate cultures were prepared and treated with test article or positive control ± S9 metabolic activation (rat liver, [redacted], 2% final concentration) for 3 hrs as described above (vehicle controls were tested in quadruplicate cultures). Cells were harvested 17 hrs after the beginning of treatment, and 1.5 hrs after the addition of colchicine (1 ug/mL final concentration) to arrest dividing cells in metaphase. A cell count was determined from an aliquot of each cell suspension prior to centrifugation and resuspension of the cells [redacted], followed by centrifugation and resuspension several times to clean the cells. Slides were prepared after several drops of 45% (v/v) aqueous acetic acid were added to enhance chromosome spreading. Dried slides were **stained for 5 minutes in filtered 4% (v/v) Giemsia stain in Gurr's buffer (pH 6.8), rinsed,** dried, and mounted in DPX under coverslips.

The top four or five concentrations without excessive toxicity were scored for chromosome aberrations (100 metaphases from each of the duplicate flasks, providing 200 per concentration level, and 400 from the four vehicle-treated cultures). Only cells with 19-23 chromosomes were considered acceptable for analysis of chromosomal aberrations. The frequency of hyperdiploid, polyploid and endoreduplicated cells was also scored for each culture.

A 20-hr treatment in the absence of S9 was also performed, but was not analyzed for chromosomal aberrations because the 3-hr incubations without S9 were clearly positive.

Results

Study validity
Criteria for a valid assay were met for the 1:1 combination:

- **The highest concentrations analyzed (1920/1920 ug/mL –S9, 61% MI; 1780/1780** ug/mL +S9, 57% MI) both showed greater than the minimum 50% requirement for mitotic inhibition.
- At least 80% of the intended total cells per treatment (200 for test article and positive **controls; 400 for vehicle controls) were scored, except at the highest dose –S9** (1920/1920), where only 151 of the intended 200 cells were analyzed. This was not important since that concentration was clearly positive.
- The percentage of cells with aberrations in the solvent controls were within or close to laboratory historical control ranges.
- Positive control cultures showed clear, unequivocal positive responses as expected.

Study outcome:
Criteria for a positive response (chromosomal aberration (CA) frequency (excluding gaps) falling outside the historical vehicle control range, and statistically increased over vehicle controls) were met for the combination at ≥ 1815/1815 ug/mL without S9, and at ≥ 1745/1745 ug/mL with S9. No increase in the frequency of chromosomal aberrations was observed with NAP alone at up to 2500 ug/mL ± S9 or with SS alone at 1920 ug/mL (-S9) or 1780 (+S9). Both cytotoxicity (as measured by % Reduction in Cell Count) and frequency of chromosomal aberrations increased with increasing concentrations of NAP/SS. The frequency of numerical aberrations was also significantly increased at ≥ 1815/1815 ug/mL (-S9), primarily due to increased endoreduplication, but not in a clearly dose-related manner. Similarly, numerical aberrations were significantly increased at 1745/1745 ug/mL (+S9), but not at 1780/1780 ug/mL.

Treatment (3 hr Incubation Without S9)	Dose Level (ug/mL)	% Mitotic Inhibition	% Reduction In Cell Count	% Population Doubling Inhibition	% of Cells w/ Structural Aberrations (excluding gaps)
Purified Water	0	-	0	0	0.75
NAP/SS	1710/1710	0	44	54	1.50
NAP/SS	1815/1815	11	50	65	7.50*
NAP/SS	1850/1850	0	56	77	11.00*
NAP/SS	1885/1885	0	59	83	18.50*
NAP/SS	1920/1920	61	68	100	37.09*
NAP	1920	0	30	33	1.50
NAP	2500	0	33	37	2.50
SS	1920	0	24	25	1.00
4-NQO	0.3	ND	ND	ND	24.00*

* Statistically significant: p<0.001

Treatment (3 hr Incubation With S9)	Dose Level (ug/mL)	% Mitotic Inhibition	% Reduction In Cell Count	% Population Doubling Inhibition	% of Cells w/ Aberrations (excluding gaps)
Purified Water	0	-	0	0	1.19
NAP/SS	1640/1640	0	4	5	2.00
NAP/SS	1710/1710	2	32	52	3.50#
NAP/SS	1745/1745	26	42	73	9.50*
NAP/SS	1780/1780	57	52	96	22.50*
NAP	1780	0	31	49	1.00
NAP	2500	0	42	73	3.50
SS	1780	0	0	0	1.00
CPA	12.5	ND	ND	ND	70.50*

* Statistically significant: p<0.001 #Statistically significant: p< 0.05, but within historical control range.

Sponsor's Conclusions:
The sponsor concluded that the 1:1 combination of Naproxen Sodium and Sumatriptan Succinate was clastogenic when incubated with CHO cells for 3 hours in the presence and absence of metabolic activation. However, the sponsor also noted that no induction of chromosomal aberrations was observed in cultures with cytotoxicity ≤ 54% as **measured by inhibition of population doublings (PD, "a more reliable and robust measure of cytotoxicity,"** *page 29*, and that this assay would be considered negative if > 50% inhibition of PD were the cytotoxicity target used in dose selection instead of mitotic inhibition. The sponsor believes that this indicates that the chromosomal aberrations observed at higher concentrations of NAP/SS were caused by a non-genotoxic mechanism dependent on cytotoxicity, as described in Greenwood et al (*Environmental and Molecular Mutagenesis* 43:36-44, 2004).

Reviewer's Conclusions:
FDA has not yet adopted the cytotoxicity target of > 50% PD inhibition for the CHO chromosomal aberrations assay. Our current guidance states:

> **"The desired level of toxicity for in vitro cytogenetic tests using cell lines should be greater than** 50% reduction in cell number or culture confluency. For lymphocyte cultures, an inhibition of **mitotic index by greater than 50% is considered sufficient."**
> *(Guideline for Industry, Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, ICH S2A, April, 1996, page 3)*

Therefore, in the present study, the most appropriate measure of cytotoxicity is reduction in cell number. The combination of Naproxen Sodium and Sumatriptan Succinate should be considered positive for clastogenicity in CHO cells, since statistically significant dose-related increases in the frequency of cells with structural aberrations were observed without S9 at NAP/SS 1815/1815 ug/mL and 1850/1850 ug/mL associated with reductions in cell number of 50% and 56%, respectively; and with S9 at NAP/SS 1745/1745 ug/mL and 1780/1780 ug/mL associated with reductions in cell number of 42% and 52%, respectively.

The data also confirm suggestions from previous studies that the combination of NAP and SS produces a synergistic effect on both toxicity (a cytotoxic and/or cytostatic effect as measured by the reduction in cell number) and clastogenicity. Without S9, 1920 ug/mL NAP + 1920 ug/mL SS reduced cell number by 68%, more than the sum of NAP and SS alone at the same dose (30% and 24%, respectively; sum = 54%). Similarly, with S9, the combination at 1780/1780 ug/mL reduced cell number by 52%, more than the sum of NAP and SS alone (31% + 0% = 31%). The frequency of cells with chromosomal aberrations was increased 20-40-fold with the highest dose of the combination, but not at all with the same doses of NAP and SS alone.

1:1 combination of Naproxen sodium and Sumatriptan succinate: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

Study no.: [redacted] Study 2990/25; GSK Document WD2006/03038/00

Volume #, and page #: eNDA 21-926 #016, Module 4, Section 4.2.3.3.1.2, pages 1-75
Conducting laboratory and location: [redacted]
Date of study initiation: 04 AUG 2006
GLP compliance: Yes, statement signed 30 OCT 2006
QA reports: yes, statement signed 30 OCT 2006
Drug, lot #, and % purity: Naproxen Sodium (NAP) Lot #NPXNAM-631, Purity 100%; NAP doses were calculated as the free acid, using a correction factor of 1.1. Sumatriptan Succinate (SS) Lot #K026882, Purity 99.2%. SS doses were calculated as the free base, using a conversion factor of 1.4.

Methods

Strains/species/cell line:
The original cultures of mouse lymphoma L5178Y ($TK^{+/-}$) cells were obtained from [redacted] and stocks were stored frozen in liquid nitrogen. Each batch of frozen cells was purged of L5178Y ($TK^{-/-}$) mutants, checked for spontaneous mutant frequency and that they were mycoplasma free.

Doses used in definitive studies (combinations were tested in a separate study):
±S9, 3 hr: SS/NAP together at 400/400, 800/800, 1200/1200, 1350/1350, 1400/1400, 1500/1500, 1550/1550, and 1700/1700 ug/mL; NAP alone at 400, 800, 1200, 1350, 1400, 1500, 1550, and 1700 ug/mL; SS alone at 400, 800, 1200, 1350, 1400, 1500, 1550, and 1700 ug/mL
-S9, 24 hr: SS/NAP together at 75/75, 150/150, 200/200, 250/250, 300/300, 350/350, and 400/400 ug/mL; NAP alone at 75, 150, 200, 250, and 300 ug/mL; SS alone at 75, 150, 200, 250, 300, 350, 400, 450, 500, 600 ug/mL.

Basis of dose selection:
A preliminary cytotoxicity test assayed concentrations of NAP and SS alone and together from 78.13 to 2500 ug/mL (~10 mM NAP; ~ 6 mM SS). Cytotoxicity (reduction in Relative Total Growth, RTG) was observed at ≥ 1250/1250 ug/mL in the 3 hr treatment ±S9, and at ≥ 312.5 ug/mL in the 24 hr treatment –S9. No precipitates were observed. Based on these results, maximum doses of 1800/1800 ug/mL for the 3 hr assays and 600/600 ug/mL for the 24 hr assay were chosen.

Negative controls:
Purified water was used as the vehicle control.

Positive controls:
Methyl methanesulphonate (MMS, [redacted] in DMSO) was used as the positive control in the absence of metabolic activation, diluted with water to final concentrations of 5 or 15 ug/mL.

Benzo[a]pyrene (BaP, [redacted] in DMSO) was used as the positive control in the presence of metabolic activation, diluted with water to a final concentration of 3 ug/mL.

Incubation and sampling times:
Cultures were treated with test articles or controls for 3 hrs ± S9 (rat liver, 2% final concentration) or 24 hrs –S9, then replated and cultured for 48 hrs, then replated into 96-well plates to determine viability (for 3 days) and mutation frequency (for 1-2 weeks).

Results

Study validity
Criteria for a valid assay were met for the combination doses:

- The highest combination doses tested in each of the three assays resulted in toxicity within or near the desired range of 10-20% RTG (NAP/SS RTG = 23%, 15%, and 16% in the 3-hr –S9, 24-hr –S9, and 3-hr +S9 assays, respectively).
- Results with vehicle and positive controls were within the expected ranges.
- No confounding factors or excessive heterogeneity were observed.

However, the highest doses of NAP alone evaluated did not induce toxicity close to the desired range of toxicity in two of the three assays (NAP RTG = 68% at 1500 ug/mL after 3 hrs –S9; 17% at 300 ug/mL after 24 hrs –S9; and 59% at 1700 ug/mL after 3 hrs +S9). Similarly, the highest doses of SS alone evaluated did not induce toxicity close to the desired range of 10-20% (SS RTG = 82% after 3 hrs –S9; 73% after 24 hrs –S9; and 64% after 3 hrs +S9). For definitive results, NAP and SS alone should be tested in each assay up to concentrations associated with a RTG of ≤ 20% or 5000 ug/mL or 10 mM, whichever is lowest (10 mM = ~2300 ug/mL NAP and ~2940 ug/mL SS).

Study outcome:
In the 3-hr study without S9, the mutation frequency was not significantly increased with NAP/SS up to the limit of toxicity, 1500/1500 ug/mL, at which RTG was 23% of control. RTG at 1600/1600 ug/mL was 4%, below the acceptable limit of 10%. In the 24-hr study without S9, the mutation frequency was not significantly increased up to the limit of toxicity, 400/400 ug/mL, at which RTG was 15% of control. In the 3-hr study with S9, the mutation frequency was not significantly increased up to the limit of toxicity, 1700/1700 ug/mL, at which RTG was 16% of control.

NAP alone did not significantly increase the mutation frequency after 3 hrs –S9 (RTG 68% at 1500 ug/mL), after 24 hrs -S9 (RTG 17% at 300 ug/mL), or after 3 hrs +S9 (RTG 59% at 1700 ug/mL).

SS alone did not significantly increase the mutation frequency after 3 hrs –S9 (RTG 82% at 1500 ug/mL), after 24 hrs -S9 (RTG 73% at 600 ug/mL), or after 3 hrs +S9 (RTG 64% at 1700 ug/mL).

Treatment	Dose Level (ug/mL)	3 hr Incubation Without S9		3 hr Incubation With S9	
		% Relative Total Growth	Mutation Frequency (x 10^{-6})	% Relative Total Growth	Mutation Frequency (x 10^{-6})
Purified Water	0	100	58.65	100	54.51
NAP/SS	400/400	76	54.31	98	51.10
NAP/SS	800/800	71	67.34	80	65.41
NAP/SS	1200/1200	61	59.37	NE	NE
NAP/SS	1350/1350	NT	NT	66	70.74
NAP/SS	1400/1400	55	53.75	NE	NE
NAP/SS	1450/1450	23	100.93	NE	NE
NAP/SS	1500/1500	NT	NT	35	69.22
NAP/SS	1700/1700	NP	NP	16	114.83
MMS	15	35	769.25	NT	NT
BaP	3	NT	NT	27	880.29

NT=Not Treated; NP=Not Plated; NE=Not Evaluated

Treatment	Dose Level (ug/mL)	24 hr Incubation Without S9	
		% Relative Total Growth	Mutation Frequency (x 10^{-6})
Purified Water	0	100	58.98
NAP/SS	75/75	97	64.83
NAP/SS	150/150	76	73.37
NAP/SS	200/200	68	66.47
NAP/SS	250/250	44	62.56
NAP/SS	300/300	32	64.83
NAP/SS	350/350	21	74.20
NAP/SS	400/400	15	76.33
MMS	5	30	1070.51

NT=Not Treated; NP=Not Plated; NE=Not Evaluated

Test Article	Dose Level[1] µg/mL	3 hr Treatment –S9-mix Mean Relative Total Growth (%)	3 hr Treatment –S9-mix Mean Mutant Frequency ($x10^{-6}$)	3 hr Treatment +S9-mix Mean Relative Total Growth (%)	3 hr Treatment +S9-mix Mean Mutant Frequency ($x10^{-6}$)	Dose Level[1] µg/mL	24 hr Treatment –S9-mix Mean Relative Total Growth (%)	24 hr Treatment –S9-mix Mean Mutant Frequency ($x10^{-6}$)
Purified water	0	100	70.57	100	58.64	0	100	60.88
Naproxen	400	111	55.13	82	48.85	75	43	57.16
Naproxen	800	116	62.86	62	65.53	150	61	53.40
Naproxen	1200	133	70.98	NE	NE	200	58	52.28
Naproxen	1350	NT	NT	87	72.26	250	NE	NE
Naproxen	1400	73	59.34	NE	NE	300	17	70.95
Naproxen	1500	68	78.58	NE	NE	NT	NT	NT
Naproxen	1550	NT	NT	77	61.56	NT	NT	NT
Naproxen	1700	NE	NE	59	52.62	NT	NT	NT
Methyl methane sulphonate	15	44	448.96	NT	NT	NT	NT	NT
Methyl methane sulphonate	NT	NT	NT	NT	NT	5	21	679.11
Benzo[a]pyrene	3	NT	NT	36	648.98	NT	NT	NT

1. All concentrations of Naproxen sodium are expressed in terms of parent compound
NT Not treated
NE Not evaluated

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Test Article	Dose Level[1] µg/mL	3 hr Treatment -S9-mix Mean Relative Total Growth (%)	3 hr Treatment -S9-mix Mean Mutant Frequency ($x10^{-6}$)	3 hr Treatment +S9-mix Mean Relative Total Growth (%)	3 hr Treatment +S9-mix Mean Mutant Frequency ($x10^{-6}$)	Dose Level[1] µg/mL	24 hr Treatment -S9-mix Mean Relative Total Growth (%)	24 hr Treatment -S9-mix Mean Mutant Frequency ($x10^{-6}$)
Purified water	0	100	60.26	100	41.23	0	100	66.36
Sumatriptan	400	95	56.39	74	52.30	75	94	43.83
Sumatriptan	800	107	57.13	75	42.62	150	108	44.67
Sumatriptan	1200	120	50.68	NE	NE	200	94	65.11
Sumatriptan	1350	NT	NT	88	68.09	250	77	57.46
Sumatriptan	1400	88	44.34	NE	NE	300	103	57.89
Sumatriptan	1500	82	70.08	NE	NE	350	100	53.74
Sumatriptan	1550	NT	NT	89	58.74	400	112	67.57
Sumatriptan	1700	NE	NE	64	68.34	450	94	70.32
Sumatriptan	NT	NT	NT	NT	NT	500	71	68.13
Sumatriptan	NT	NT	NT	NT	NT	600	73	60.40
Methyl methane sulphonate	15	28	410.78	NT	NT	NT	NT	NT
Methyl methane sulphonate	NT	NT	NT	NT	NT	5	27	1087.49
Benzo[a]pyrene	3	NT	NT	24	877.66	NT	NT	NT

1. All concentrations of Sumatriptan succinate are expressed in terms of parent compound
NT Not treated
NE Not evaluated

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Sponsor's Conclusions:

The 1:1 combination of NAP and SS was not genotoxic in the mouse lymphoma L5178Y TK +/- assay in the presence or absence of S9 metabolic activation, when tested up to the limits of cytotoxicity.

Reviewer's Conclusions:

This study presented valid negative results for the 1:1 combination of NAP and SS up to appropriate levels of toxicity. However, the study did not evaluate sufficiently high doses of NAP and SS alone to conclude that they are not genotoxic in this assay, except in the 20-hr assay without S9 for NAP. It is not clear why NAP was not genotoxic in this assay at concentrations much higher than those that were shown to be genotoxic in a previous mouse lymphoma assay [redacted] #AA33KS, JL, KC, .7040002.BTL, Pozen #MT100-T26) submitted to support [redacted] (dose-dependent increases in mutation frequency were observed at 50, 150, and 300 ug/mL NAP alone in a 4-hr assay with S9).

2.6.6.5 Carcinogenicity

No carcinogenicity studies were included in this submission.

2.6.6.6 Reproductive and developmental toxicology

No reproductive and developmental toxicology studies were included in this submission.

2.6.6.7 Local tolerance

No local tolerance studies were included in this submission.

2.6.6.8 Special toxicology studies

No special toxicology studies were included in this submission.

2.6.6.9 Discussion and Conclusions

See Overall Conclusions and Recommendations.

2.6.6.10 Tables and Figures

Tables and Figures were included within the text.

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS:

In the original NDA submission, an in vitro chromosomal aberration assay in CHO cells (Study MT400/T07, #0735/0736-3110) demonstrated greater clastogenic effects with the combination of naproxen and sumatriptan compared with naproxen alone, raising the concern that the two compounds together may have carcinogenic effects not observed with either drug alone. The clastogenic effects were observed only at concentrations producing substantial cytotoxicity, making the biological significance of the effects unclear.

In the approvable letter dated 08 JUN 2006, the sponsor was asked to attempt to clarify this issue by repeating the chromosomal aberrations assay testing concentrations of the 1:1 NAP/SS combination between those exhibiting minimal toxicity (1250/1250 ug/mL) and those inducing substantial toxicity (2500/2500 ug/mL without S9 activation, and 2000/2000 ug/mL with S9 activation), and by conducting an in vitro mouse lymphoma tk assay testing naproxen and sumatriptan alone and in combination.

The current submission contains final reports from the requested genotoxicity studies with NAP and SS alone and in combination at 1:1.

In the new assay for chromosomal aberrations in CHO cells, the frequency of cells with structural aberrations was increased dose-dependently by NAP/SS at concentrations that reduced the total cell count by 50-68% in the absence of S9 metabolic activation (1815/1815 to 1920/1920 ug/mL), and by 42-52% in the presence of S9 (1745/1745 to 1780/1780 ug/mL). Neither NAP nor SS alone at the same concentrations resulted in significant clastogenicity. NAP alone was also negative at the highest concentration tested, 2500 ug/mL, which is equivalent to ~10.9 mM, exceeding the recommended maximum concentration of 10 mM (Note: these calculations are based on MW = 230.266 for naproxen free acid, and 295.406 for sumatriptan free base). These data demonstrate a synergistic clastogenic effect of the combination of the two drugs at concentrations greater than or equal to ~7.6 mM NAP and ~5.9 mM SS, associated with reductions in cell number of $\geq$ 42%. NAP and SS also had synergistic effects on cytotoxicity at these concentrations. The sponsor argues that using reduction in cell count underestimates the cytotoxicity, and that this assay would be considered negative if inhibition of population doublings (PD) were used instead. However, the current ICH guidance recommends **testing test article concentrations that cause "greater than 50% reduction in cell number or culture confluency."** *(Guideline for Industry, Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, ICH S2A, April, 1996, page 3)*

In the new in vitro mouse lymphoma assay, the 1:1 NAP/SS combination was not genotoxic at concentrations up to those inducing cytotoxicity within (or close to) the **desired range of 10-20% Relative Total Growth (1450/1450 ug/mL after 3 hrs –S9; 1700/1700 ug/mL after 3 hrs +S9; and 400/400 ug/mL after 24 hrs –S9). It is not clear** why NAP was not genotoxic in this assay at up to 1700 ug/mL +S9 when a previous mouse lymphoma assay showed dose-dependent increases in mutation frequency at 50, 150, and 300 ug/mL NAP alone after 4-hr treatments with S9 [redacted]

The original NDA submission for Trexima included negative results for a valid bacterial reverse mutation assay (up to 2500 ug/plate NAP/SS) and for a valid in vivo mouse micronucleus assay (up to an MTD of 500/1500 mg/kg NAP/SS (M) or 375/1625 mg/kg (F)). Negative carcinogenicity studies are described in the current labeling for SS (Imitrex; rat and mouse) and NAP (Anaprox; rat). In addition, the current sponsor conducted a two-year rat study to support [redacted] that demonstrated no increases in neoplasms in rats receiving NAP alone at the MTD of 8 mg/kg/day. Finally, a 26-week carcinogenicity study in $p53^{+/-}$ mice was negative in mice given 50 mg/kg/day NAP in combination with 50 or 1.6 mg/kg/day metoclopramide (see [redacted]

The approval letter of 08 JUN 2007 also included the following statement:

> You need to include the results of the in vitro mouse lymphoma tk assays (Studies MT 100 T25, MT100 T26) and the carcinogenicity study in $p53^{+/-}$ heterozygous mice (Study MT 100 T35) for naproxen in product labeling.

The sponsor has agreed to include the following statement in labeling to address the first point:

[redacted]

Reviewer's Conclusion:
The data demonstrate that the combination of NAP and SS induce genotoxic effects in CHO cells in the presence and absence of S9 that are not observed at similar concentrations of either drug alone. These effects occur at moderate levels of cytotoxicity (≥ 42% reduction in cell number) and at relatively high concentrations (≥ 7.6 mM NAP; ≥ 5.9 mM SS). This reviewer believes it might be reasonable to argue that these concentrations exceed the recommended maximum concentration of 10 mM because 7.6 + 5.9 = 14.5 mM. Considering the negative findings in the mouse lymphoma assay, the bacterial reverse mutation assay, and the in vivo mouse micronucleus assay with NAP/SS, and the negative carcinogenicity findings for each of the components of the combination, this reviewer believes that including the positive genotoxicity findings in the labeling for Trexima is sufficient to address this issue. No additional nonclinical studies are needed.

Unresolved toxicology issues:
The discrepancy between the dose-dependent positive findings in a previous mouse lymphoma tk assay with NAP alone in the presence of S9 metabolic activation and the negative findings in the present mouse lymphoma tk assay with NAP alone (+S9) at much higher concentrations remains unexplained.

Recommendations:

The nonclinical package is adequate to support an approval action for NDA 21-926 TREXIMA (sumatriptan succinate/naproxen sodium) Tablets for the acute treatment of migraine.

9 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

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/s/

David Hawver
8/1/2007 06:18:33 PM
PHARMACOLOGIST

Lois Freed
8/1/2007 06:21:29 PM
PHARMACOLOGIST
Please see separate memo for comments.

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: June 9, 2006

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 21-926 (TREXIMA; sumatriptan/naproxen)

The nonclinical data submitted in support of the NDA for TREXIMA were reviewed by David B. Hawver, Ph.D. (Pharmacology/Toxicology Review and Evaluation NDA 21-926, June 9, 2006). Based on his review, Dr. Hawver has concluded that the nonclinical data support an approvable action; however, he identifies several unresolved toxicology issues:

(a) whether or not naproxen exacerbates the cardiovascular effects (i.e., vasoconstriction, coronary artery vasospasm) of sumatriptan.

Dr. Hawver notes that the special toxicology study to assess the effects of naproxen on sumatriptan-induced vasoconstriction/vasospasm was methodologically flawed and, thus, provided no reliable data to address the issue. However, he concludes that issues of technical feasibility and interpretability argue against requiring a new cardiovascular safety study as a condition for approval.

Comment: although it is not clear that the technical difficulties encountered during the conduct of the study necessarily means that an interpretable combination cardiovascular study could not be conducted, it seems unlikely that a new or repeat study would provide data that would cause greater concern of cardiovascular risk than that already acknowledged based on data in humans. Therefore, I agree that no additional nonclinical study is needed.

(b) the potential for additive/synergistic or novel toxicities with the combination of naproxen and sumatriptan compared to each drug alone.

Two 90-day combination toxicity studies were conducted in mice (Study #907-009 and Study 04-293/MT400-T19). Study #907-009 tested sumatriptan (S) and naproxen (N) in combination (S/N) and each alone at oral (gavage) doses of 0/0, 25/12, 105/50, 210/100, 320/150, 320/0, and 0/150 mg/kg/day in males and 0/0, 50/12, 110/25, 210/50, 320/75, 210/0, and 0/50 mg/kg/day in females. In males, the dose of naproxen (alone and in combination with sumatriptan) was lowered from 150 to 100 mg/kg/day on Day 62 (after a drug holiday from Days 57-61) due to increased mortality. In females, the 320/75 mg/kg/day group was terminated on Day 65 due to increased mortality; mortality was also increased in the 0/50 mg/kg/day group. Dr. Hawver noted that no sumatriptan-related toxicity was observed in either males or females. Dr. Hawver also noted that "concerns regarding the accuracy and homogeneity of the dosing solutions" made interpretation of the study difficult. It was also the sponsor's conclusion that Study #907-009 was not definitive due to methodological problems.

Study 04-293/MT400-T19 tested sumatriptan (S) and naproxen (N) in combination (S/N) and each alone at oral doses of 0/0, 30/10, 100/30, 320/100, 320/0, and 0/100 mg/kg/day in males and 0/0, 30/10, 100/30, 320(210)/50, 320(210)/0, and 0/50 mg/kg/day in females. In females, the high dose of sumatriptan was decreased from 320 to 210 mg/kg/day at the start of dosing Week 4 due to death in 3/18 and 2/18 females in the 320/50 and 320/0 mg/kg/day groups, respectively. Other than these deaths, no sumatriptan-related toxicity was observed. Toxicity induced by naproxen was characterized by Dr. Hawver as a "low level of toxicity" and by the sponsor as "subtle". The sponsor also concluded that "There were no deaths or clinical signs that were considered to be test article-related". There were, however, a number of unscheduled deaths which were attributed to gavage error or lower urinary tract obstruction.

Based on the lack of clear dose-related toxicities for either sumatriptan or naproxen (or the combination) in the definitive 90-day study, Dr. Hawver recommends that another 90-day study be conducted in mice testing up to a maximum tolerated or maximum feasible dose of each drug alone and in combination.

Comment: based on Dr. Hawver's review of the data from the two 90-day mouse studies, it would appear that sumatriptan could have been tested at higher doses. However, the high doses of naproxen tested in Study 04-293/MT400-T19 would appear to have been maximum tolerated doses (MTDs). Although 100 and 50 mg/kg were not associated with notable toxicity in males and females, respectively, in that study, the high doses of naproxen used in Study #907-009 (150 and 75 mg/kg in males and females, respectively) exceeded the MTD based on increased mortality. Comparing the data from the two studies, it would appear that the plasma exposures (AUC) for naproxen were fairly similar between studies.

The high dose of sumatriptan used in both studies (320 mg/kg in males and females) does not appear to have been associated with any toxicity. Although the sponsor lowered the dose from 320 to 210 mg/kg/day in females in Study 04-293/MT400-T19 due to two deaths, the sponsor concluded that no drug-related deaths occurred in that study. In the 28-day dose-range finding study in mice, a dose of 500 mg/kg was well-tolerated (i.e., no

sumatriptan-related toxicity was detected). Therefore, there are no data that establish an MTD for sumatriptan in mice. However, there is a large safety margin between the plasma exposure (AUC) at the high dose in males (≈100 fold at 320 mg/kg/day) and females (≈80 fold at 210 mg/kg/day) and the plasma AUC associated with the recommended daily dose of 85 mg/day in humans.

Although I agree with Dr. Hawver that higher doses of sumatriptan should have been used in the combination study, I don't believe that a repeat study is necessary since (a) naproxen was tested at an MTD, (b) there is a large safety margin between the plasma exposures at the high doses of sumatriptan used in the 90-day mouse study and those anticipated in humans at the recommended daily dose of sumatriptan, (c) no novel toxicities were detected with naproxen and sumatriptan in combination, and (d) according to the clinical team there is a robust safety database for the combination in humans.

(c) the effects of sumatriptan on the clastogenicity of naproxen.

Dr. Hawver concludes that the possibility that sumatriptan may potentiate the clastogenic effects of naproxen "remains unresolved". However, it is Dr. Hawver's opinion that experiments to further investigate this possibility are not necessary since naproxen was not tumorigenic in carcinogenicity studies in rats at maximum tolerated doses.

Comment: naproxen and sumatriptan were negative in the Ames assay and the in vivo micronucleus assay in mice when tested alone and in combination. However, naproxen, alone and in combination with sumatriptan, was clastogenic in an in vitro chromosomal aberration assay in Chinese Hamster Ovary cells, without and with metabolic activation. In addition, the magnitude of the effect (both in the absence and presence of metabolic activation) was greater with the combination than with naproxen alone. Sumatriptan was negative in this assay when tested alone.

Naproxen and sumatriptan were negative for tumorigenicity when tested alone in carcinogenicity studies. The possibility that the tumorigenic potential could be increased when administered in combination could have serious implications for approvability. Therefore, it is my opinion that the potential for a synergistic clastogenic effect needs to be further investigated.

The data from Study #MT400-T07 are summarized in the sponsor's Tables 1 and 2 (page 4 of this memo). Although naproxen and the combination of naproxen and sumatriptan were clastogenic only at concentrations associated with clear cytotoxicity (relative MI = 20-37%), the next lowest concentrations tested were associated with little or no cytotoxicity (relative MI = 84-153%). The sponsor needs to carefully investigate the interval between the highest and next highest concentrations used (without and with metabolic activation) in order to more fully evaluate the clastogenic effects at concentrations associated with intermediate cytotoxicity. In addition, the sponsor should also evaluate naproxen and sumatriptan, alone and in combination, in an in vitro mouse lymphoma tk assay. The data from the in vitro mouse lymphoma assay will help in

interpreting the results of the in vitro chromosomal aberration assay, particularly if the original results are replicated.

In the in vitro chromosomal aberration assay, the biological significance of clastogenic effects associated only with substantial cytotoxicity is somewhat controversial. However, if synergestic clastogenic effects are also observed in the in vitro mouse lymphoma assay, similar findings in the in vitro chromosomal aberration assay would be difficult to dismiss even if only observed at concentrations associated with substantial cytotoxicity.

Table (1) Relative Mitotic Indices and % Cells with Aberrations (- S9): 3 Hour Treatment with 18 Hour Harvest

NAP/SS[a] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	NAP[c] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	SS[d] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]
Solvent	100	0.0	Solvent	100	0.0	Solvent	100	0.0
250/250	160		500	97		500	119	
500/500	151	0.0	1000	91	0.0	1000	134	0.0
1250/1250	153	0.0	2500	108	0.5	2500	94	0.0
2500/2500	29	10.0**	5000	37	4.5*	5000	110	0.5
MMC 0.4[e]	81	27.0**	MMC 0.4[e]	69		MMC 0.4[e]	69	
MMC 0.8[e]	39		MMC 0.8[e]	66	29.0**	MMC 0.8[e]	66	29.0**

a. Naproxen sodium / Sumatriptan (dose calculated as the base)
b. % of cells with chromosome aberrations
c. Naproxen sodium
d. Sumatriptan (dose calculated as the base)
e. MMC - positive control Mitomycin C

** $p \leq 0.0001$
* $p = 0.028$

Table (2). Relative Mitotic Indices and % Cells with Aberrations (+ S9): 3 Hour Treatment with 18 Hour Harvest

NAP/SS[a] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	NAP[c] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	SS[d] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]
Solvent	100	0.0	Solvent	100	1.5	Solvent	100	1.5
250/250	85		5	116		5	91	
500/500	91	0.0	50	88	0.0	50	130	0.0
1250/1250	84	0.0	500	99	0.5	500	119	0.0
2000/2000	20	11.0**	2500	29	4.0[f]	5000	100	0.0
CP 7.5[e]	10	30.0**	CP 7.5[e]	14	31.0**	CP 7.5[e]	14	31.0**
CP 12.5[e]	13		CP 12.5[e]	11		CP 12.5[e]	11	

a. Naproxen sodium/Sumatriptan (dose calculated as the base)
b. % of cells with chromosome aberrations
c. Naproxen sodium
d. Sumatriptan (dose calculated as the base)
** $p \leq 0.0001$
e. CP – positive control Cyclophosphamide
f. Value was not statistically different from solvent control due to the high % of cells with aberrations in the solvent control group

Information to be relayed to the sponsor:

1. The results of the in vitro chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells (Study MT400/T07, #0735/0736-3110) demonstrated clastogenic effects of naproxen alone and in combination with sumatriptan. The magnitude of the clastogenic

effect was greater with the combination of naproxen and sumatriptan than with naproxen alone, both in the absence and presence of metabolic activation. (Sumatriptan was negative in this assay.) These results raise the concern that naproxen and sumatriptan in combination may have carcinogenic effects not observed with either drug alone. However, since the clastogenic effects were observed only at concentrations producing substantial cytotoxicity, the biological significance of these effects is unclear, but cannot be dismissed. Therefore, you need to conduct the following additional studies:

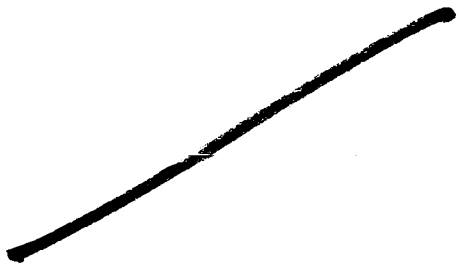
a. a repeat in vitro chromosomal aberration assay in CHO cells testing concentrations between those exhibiting minimal or no cytotoxicity (i.e., 1250/1250 µg/mL naproxen/sumatriptan) and those resulting in substantial cytotoxicity (i.e., 2500/2500 and 2000/2000 naproxen/sumatriptan in the absence and presence of metabolic activation, respectively).

b. an in vitro mouse lymphoma tk assay (with colony sizing) testing naproxen and sumatriptan alone and in combination.

The results of these studies will determine the need for additional nonclinical studies.

2. You need to include the results of the in vitro mouse lymphoma tk assays (Studies MT100 T25, MT100 T26) and the carcinogenicity study in $p53^{+/-}$ heterozygous mice (Study MT100 T35) of naproxen in product labeling.

Recommended labeling (based on recommendations by Dr. Hawver and, for the reproductive toxicology data, J. Edward Fisher, Ph.D.):



3 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox- 6

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Lois Freed
6/9/2006 06:33:15 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-926**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**08/05/2005**
PRODUCT:	**Trexima (Sumatriptan/Naproxen)**
INTENDED CLINICAL POPULATION:	**Migraine patients**
SPONSOR:	**POZEN Inc.**
DOCUMENTS REVIEWED:	**eNDA**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**David B. Hawver, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Lana Chen**

Date of review submission to Division File System (DFS): 09 JUN 2006

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The nonclinical package is adequate to support an approvable action for NDA 21-926 TREXIMA (sumatriptan succinate/naproxen sodium) Tablets for the acute treatment of migraine.

B. Recommendation for nonclinical studies

A new 90-day repeated dose general toxicity study should be conducted with SS and NAP, including Maximum Tolerated or Maximum Feasible Doses for each sex, of each drug, alone and in combination.

C. Recommendations on labeling

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Nonclinical studies submitted in support of the NDA for TREXIMA included a coronary artery vasoconstriction safety pharmacology study in dogs; general toxicology studies in mice, rats, and minipigs; a standard battery of genetic toxicology studies; and embryofetal toxicity studies in rabbits and rats.

The sponsor was asked to assess the effect of naproxen on the known capacity of sumatriptan to induce coronary artery vasoconstriction. A cardiovascular safety pharmacology study (MT400-T15) was conducted in which 6 female beagles were surgically implanted with tiny electronic devices and probes to collect information on coronary artery diameter, carotid artery diameter, blood pressure and several other parameters at baseline and after IV injection of sumatriptan succinate (SS) in the presence or absence of naproxen sodium (NAP), infused for 1 minute just prior to the SS injection. No statistically significant differences in changes from baseline were observed in any parameters measured between treatments with SS + vehicle and SS + NAP.

Unfortunately, no reliable conclusions can be drawn from this study due to wide inter-individual and intra-individual variation observed in most parameters measured and serious design flaws. The most serious design flaw was the omission of a vehicle control injection for SS. Without this control (or a low, ineffective dose of SS) it is not clear whether the minimal or maximal changes from baseline parameters recorded in the one hr after SS injection are due to the injection procedure, the vehicle, natural variation, or the SS. Hence, it is not clear that any SS-induced changes were measured here, so the meaning of any effects (or lack of effects) of NAP on these changes is questionable. Other design flaws included insufficient numbers of dogs per group (N=6) and insufficient numbers of repetitions of treatments to detect a significant NAP-induced change in the predicted SS-induced ~5% reduction in the coronary artery diameter (and similarly small changes in other parameters).

In general toxicology studies conducted in mice (two 90-day studies and one 28-day dose-ranging study), rats (two 28-day dose-ranging studies), and minipigs (one 28-day dose-ranging study), high dose NAP induced occasional slight renal toxicity (tubular dilatation and/or regeneration) and consistent gastrointestinal toxicity characteristic of non-steroidal anti-inflammatory drugs (NSAIDs; ulcer, erosion, inflammation of the glandular stomach, and secondary changes in hematology and clinical chemistry). Coadministration of SS did not consistently exacerbate these NAP-related toxicities. However, the pivotal 90-day mouse study was inadequate to support approval of this NDA due to the use of insufficiently high doses of both SS and NAP, and due to unexpected complications and unusual procedures that call into question the reliability of the results.

The results of the *in vitro* bacterial mutagenicity assay and *in vivo* mouse micronucleus assay with SS and NAP, alone and in combination, were clearly negative in valid studies. In contrast, high dose NAP ± high dose SS was positive in the initial CHO cell chromosomal aberrations assay, in the presence or absence of metabolic activation, in association with moderate, but not excessive, cytotoxicity. This positive result was confirmed in a non-GLP follow-up study testing only HD NAP + HD SS in the absence of metabolic activation. SS was not genotoxic alone, but there was some evidence to suggest that SS might exacerbate the genotoxicity of NAP: the frequency of chromosomal aberrations was increased more than 2-fold in HD NAP + HD SS cultures vs. HD NAP alone cultures with and without metabolic activation, despite 50% lower NAP concentrations in the NAP + SS cultures.

In the definitive embryo-fetal development study in rabbits, significant reductions in maternal and fetal weights were observed even at the lowest combination dose tested, but significant increases in resorption parameters and specific malformations and variations were only observed in HD NAP groups ± SS. The slight increases in specific malformations and variations attributed to HD NAP included interventricular septal defects, fused caudal vertebrae, absent intermediate lobe of the lung, irregular ossification of the skull, and incompletely ossified sternal centra. Effects of SS and NAP on maternal and fetal body weights appeared to be additive, but SS did not exacerbate increases in resorption parameters, malformations, or variations observed with high dose NAP.

B. Pharmacologic activity

No new pharmacologic activity studies were conducted.

C. Nonclinical safety issues relevant to clinical use

The potential for naproxen to exacerbate the known capacity of sumatriptan to induce coronary artery vasoconstriction remains unknown, due to the lack of a valid nonclinical study addressing this issue. However, questions concerning the technical feasibility, as well as the interpretability, of a new cardiovascular safety pharmacology study in dogs to address this issue argue against requiring such a study as a condition for approval of TREXIMA.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

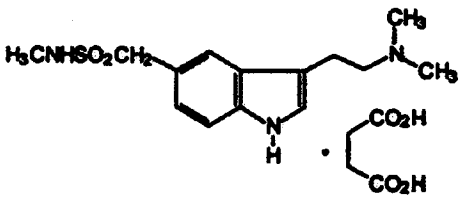
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-926
Review number: 1
Sequence number/date/type of submission:
000/05 AUG 2005/505 (b)(2) Original Application
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: POZEN Inc., Chapel Hill, NC
Manufacturer for drug substance:
Sumatriptan Succinate (SS): Glaxo Wellcome Manufacturing Pte Limited, Singapore
Naproxen Sodium (NAP): [redacted]

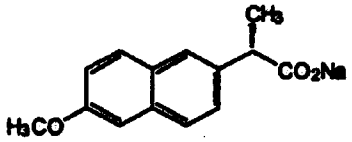
Reviewer name: David B. Hawver, Ph.D.
Division name: Division of Neurology Products
HFD #: 120
Review completion date: 05 JUN 2006

Drug:

Trade name: Trexima™
Generic name: sumatriptan succinate/naproxen sodium
Code name: MT400
Chemical name:
SS: 3-[2-(dimethylamino)ethyl]-N-methyl-indole-5-methanesulfonamide succinate (1:1)
NAP: (S)-6-methoxy-(alpha)-methyl-2-naphthaleneacetic acid, sodium salt
CAS registry number: 103628-48-4 (sumatriptan succinate)
26159-34-2 (naproxen sodium)
Molecular formula/molecular weight:
sumatriptan succinate: $C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$ MW 413.5
naproxen sodium: $C_{14}H_{13}NaO_3$ MW 252.25
Structure:



sumatriptan succinate



naproxen sodium

Relevant INDs/NDAs/DMFs:

IND 68,435 MT 400 for migraine, POZEN's current IND for sumatriptan/naproxen combined in one [redacted] tablet; submitted 18 DEC 2003

IND 60,669 MT 400 for migraine, POZEN's initial IND for sumatriptan/naproxen using marketed products in combination; submitted 26 JUL 2000

NDA 20-132 IMITREX® Tablets, sumatriptan succinate for migraine; Glaxo Inc.; approved 01 JUN 1995

NDA 17-581 NAPROSYN® Tablets, naproxen for rheumatoid arthritis, now also for acute pain, ankylosing spondylitis, tendonitis, bursitis, and acute gout; Roche (originally Syntex, Inc.); approved 11 MAR 1976

NDA 18-164 ANAPROX® Tablets, naproxen sodium for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis; Roche/Syntex; approved 04 SEP 1980

[redacted]

Drug class:

Sumatriptan succinate is a selective 5-HT1_D receptor agonist.

Naproxen sodium is a nonsteroidal anti-inflammatory drug (NSAID).

Intended clinical population:

The proposed indication for Trexima® Tablets is for the treatment of acute migraine headache with or without aura in adults.

Clinical formulation:

Each Trexima® Tablet contains 119 mg sumatriptan succinate (equivalent to 85 mg sumatriptan) and 500 mg naproxen sodium. Inactive ingredients (which are all GRAS for use in oral pharmaceuticals) include: [redacted] (microcrystalline cellulose), croscarmellose sodium, dibasic calcium phosphate, magnesium stearate, microcrystalline cellulose, [redacted] sodium bicarbonate and talc; the aqueous film coat contains sodium carboxymethyl-cellulose, maltodextrin, dextrose monohydrate, titanium dioxide, lecithin and FD&C Blue No. 2.

Route of administration: Oral tablet

Disclaimer:

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

Data reliance:
Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-926 are owned by POZEN Inc. or are data for which POZEN Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-926 that POZEN Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed **drug, as described in the drug's approved** labeling. Any data or information described or referenced below from a previously approved application that POZEN Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-926.

Studies reviewed within this submission:

Study to Determine Coronary and Carotid Arterial Blood Flow, Resistance, and Diameter following Intravenous Administration of Sumatriptan Succinate with and without Naproxen Sodium to Conscious Beagle Dogs

An Investigational Range-Finding Study to Determine Coronary Blood Flow and Resistance Following Intravenous Administration of Sumatriptan Succinate to Anesthetized Beagle Dogs

A 28-Day Oral Gavage Dose Range-Finding Toxicity Study in Female Gottingen Minipigs with Naproxen Sodium and Sumatriptan Succinate

A 28-Day Oral Range-Finding Toxicity Study in Rats with Sumatriptan Succinate Combined with Naproxen Sodium

A Second 28-Day Oral Range-Finding Toxicity Study in Rats with Sumatriptan Succinate Combined with Naproxen Sodium

A 28-Day Oral Range-Finding Toxicity Study in Mice with Sumatriptan Succinate Combined with Naproxen Sodium

A 90-Day Oral Toxicity Study in CD-1 Mice with Sumatriptan Succinate Combined with Naproxen Sodium

90-Day Oral Toxicity Study in CD-1 Mice with Sumatriptan Succinate and Naproxen Sodium as Single Entities and in Combination

Evaluation of a Test Article in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation/Preincubation Mutation Assay in the Presence and Absence of Induced Rat Liver S-9

In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells

Test for Chemical Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation

Non-GLP Test for Chemical Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO BWL) Cells With and Without Metabolic Activation

Oral (Stomach Tube) Developmental Toxicity Study of Sumatriptan Succinate Combined with Naproxen Sodium in Rabbits

Oral (Stomach Tube) Dosage-Range Develomental Toxicity Study of MT 400 in Rabbits

Oral (Gavage) Dosage-Range Developmental Toxicity Study of MT 400 in Rats

Studies not reviewed within this submission: None.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

(The section below is reprinted directly from the corresponding section in the sponsor's eNDA 21-926, Module 2, Section 2.6, Page 4)

Nonclinical primary and secondary pharmacology studies and pharmacodynamic drug interaction studies were not conducted for the sumatriptan succinate (SS)/naproxen sodium (NAP) combination, as the individual components have already been well characterized.

Two safety pharmacology studies were conducted to support the SS and NAP combination. Both studies were designed to determine whether combined administration of NAP with SS would alter the potential vasoconstrictive effects of SS on the coronary arterial vessel.

The first study was conducted using an anesthetized instrumented open-chest canine (beagle) model (Section 2.6.2.4.1; Section 2.6.3.4; MT400-T17). This study was conducted to evaluate the effects of SS (dose calculated as the base) on coronary and carotid vessel blood flow and resistance after IV administration of a wide range of escalating doses (0.7-1434 µg/kg SS as the base compound). Special measures were taken in some of the animals (establishment of a critical stenosis on the coronary vessel or pre-treatment of the animal with a β-blocker, propranolol) to increase the sensitivity of the coronary vessel to the potential effects of SS. Biologically meaningful test article-related changes in coronary arterial blood flow and calculated coronary arterial vascular resistance were not observed in this study.

A second study was conducted using a conscious chronically instrumented canine (beagle) model (Section 2.6.2.4.2; Section 2.6.3.4; MT400-T15). The primary purpose of this study was to evaluate whether combined administration of NAP with SS would alter the ability of the latter to reduce the diameter of the coronary artery. Changes in the diameter of the coronary artery were measured using piezoelectric crystals and coronary flow was measured using a magnetic flow probe. Similar measurements in diameter and blood flow were made for the carotid artery, as SS is well-documented to cause sustained reductions in both diameter and blood flow in the carotid artery. Co-administration of NAP with SS did not significantly alter the vasoconstrictive effect of SS alone on the coronary and carotid arteries of conscious chronically instrumented beagle dogs. There were also no statistically significant, biologically meaningful additive effects of NAP on SS for the other cardiovascular parameters evaluated in this study.

Reviewer's Note:
Study MT400-T15 should be considered invalid due to wide inter-individual and intra-individual variation observed in most parameters measured and serious design flaws.

2.6.2.2 Primary pharmacodynamics

"No primary pharmacodynamic studies were conducted with the combination of sumatriptan **succinate and naproxen sodium."** *(directly from the eNDA 21-926, Module 2, Section 2.6, Page 4)*

Mechanism of action of sumatriptan Succinate: *(directly from labeling of Imitrex®, PDR FEB 2004)*

Sumatriptan is an agonist for a vascular 5-hydroxytryptamine $_1$ receptor subtype (probably a member of the 5-HT $_{1D}$ family) having only a weak affinity for 5-HT $_{1A}$, 5-HT $_{5A}$, and 5-HT $_7$ receptors and no significant affinity (as measured using standard radioligand binding assays) or pharmacological activity at 5-HT $_2$, 5-HT $_3$, or 5-HT $_4$ receptor subtypes or at alpha$_1$-, alpha$_2$-, or beta-adrenergic; dopamine $_1$; dopamine $_2$; muscarinic; or benzodiazepine receptors.

The vascular 5-HT $_1$ receptor subtype that sumatriptan activates is present on cranial arteries in both dog and primate, on the human basilar artery, and in the vasculature of human dura mater and mediates vasoconstriction. This action in humans correlates with the relief of migraine headache. In addition to causing vasoconstriction, experimental data from animal studies show that sumatriptan also activates 5-HT $_1$ receptors on peripheral terminals of the trigeminal nerve innervating cranial blood vessels. Such an action may also contribute to the antimigrainous effect of sumatriptan in humans.

In the anesthetized dog, sumatriptan selectively reduces the carotid arterial blood flow with little or no effect on arterial blood pressure or total peripheral resistance. In the cat, sumatriptan selectively constricts the carotid arteriovenous anastomoses while having little effect on blood flow or resistance in cerebral or extracerebral tissues.

Mechanism of action of naproxen sodium: *(directly from labeling of Anaprox® DS, PDR MAR 2005)*

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. The sodium salt of naproxen has been developed as a more rapidly absorbed formulation of naproxen for use as an analgesic. The mechanism of action of the naproxen anion, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition.

2.6.2.3 Secondary pharmacodynamics

"No secondary pharmacodynamic studies were conducted with the combination of sumatriptan succinate and naproxen sodium." *(directly from the eNDA 21-926, Module 2, Section 2.6, Page 4)*

2.6.2.4 Safety pharmacology

Neurological effects:
No CNS Safety Pharmacology studies were submitted.

Cardiovascular effects:
Two cardiovascular safety pharmacology studies in dog (MT400-T15 and MT400-T17) were conducted to support this NDA in response to a request from the Division to "assess the effect of naproxen on the risk of sumatriptan-induced vasoconstriction of the coronary artery." *(see the Division's Pre-IND Meeting Minutes dated 02 JUL 2002)*

Study MT400-T17 was a pilot study in 5 anesthetized beagle dogs (1 F, 4 M) to explore the effects of IV sumatriptan succinate (SS) on coronary arterial blood flow and coronary arterial resistance in preparation for a subsequent study examining the potential for naproxen sodium (NAP) to exacerbate the known vasoconstrictive effects of SS. Unfortunately, the procedures used in this study failed to result in a reproducible dose-dependent reduction in coronary flow as a function of increasing IV doses of SS, up to 1434 ug/kg, even after sensitizing two dogs with coronary artery stenosis and one dog with beta blocker pre-treatment.

In response to the Division's request, the Sponsor submitted a draft protocol for Study MT400-T15, which was reviewed and accepted by the Division as appropriate to address this issue. The original protocol included only Phases I and II; Phase III was added later by amendment.

In Phases I and II of Study MT400-T15, the following cardiovascular parameters were analyzed in 6 female beagle dogs for one hour following 80 ug/kg IV SS on each of three successive days: left circumflex (LCX) coronary artery diameter, blood flow, and resistance; carotid artery diameter, blood flow, and resistance; mean arterial pressure (MAP), heart rate (HR), and left ventricular pressure (± dP/dt). In Phase 1, the SS injection was preceded by a 1-minute IV infusion of vehicle on Day 1, but not on Days 2 or 3. In Phase II, the SS injection was preceded by a 1-minute IV infusion of 20 mg/kg NAP on Day 1 only.

Unfortunately, no reliable conclusions can be drawn from this study due to wide inter-individual and intra-individual variation observed in most parameters measured and serious design flaws. The most serious design flaw was the omission of a vehicle control injection for SS. Without this control (or a low, ineffective dose of SS) it is not clear whether the minimal or maximal changes from baseline parameters recorded in the one hr after SS injection are due to the injection procedure, the vehicle, natural variation, or the SS. Hence, it is not clear that any SS-induced changes were measured here, so the meaning of any effects (or lack of effects) of NAP on these changes is questionable. Other design flaws included insufficient numbers of dogs per group and insufficient numbers of repetitions of treatments to detect a significant NAP-induced change in the predicted SS-induced ~5% reduction in the coronary artery diameter (and similarly small changes in other parameters).

In Phase III, two doses of 200 ug/kg IV SS were given ~1 hr apart, with a 1-minute infusion of 20 mg/kg IV NAP given just prior to the second SS dose. However, the half-life of SS is ~ 2 hrs in dog, so the second "baseline" was not a true baseline, and the SS plasma levels were 65% higher than those after the first dose of SS. Therefore, any differences (or lack of differences) seen between the first SS treatment without NAP and the second with NAP may be due to the higher SS plasma levels rather than to the presence of NAP, so no conclusions can be reached. This experiment also lacked appropriate vehicle controls for both SS and NAP.

While the Sponsor's conclusion that no statistically significant effects of NAP were observed is accurate, there appeared to be trends toward NAP-related enhancement of SS-induced effects on coronary artery diameter, carotid artery diameter, mean arterial blood pressure (MAP), and coronary artery resistance. The importance of such trends is questionable, though, in the context of the wide inter-individual and intra-individual variation observed and the design flaws noted above.

The Sponsor's overall conclusion that "co-administration of NAP with SS did not alter the vasoconstrictive effect of SS on the coronary arteries of conscious, chronically instrumented female beagle dogs, nor did it alter any of the other cardiovascular parameters measured in this study," is not justified.

This study (MT400-T15) should be considered invalid.

A Study to Determine Coronary and Carotid Arterial Blood Flow, Resistance, and Diameter following Intravenous Administration of Sumatriptan Succinate with and without Naproxen Sodium to Conscious Beagle Dogs

POZEN Study Number: MT400-T15.

[redacted] OCBW-0106.
QCBW-0106-163

Study Initiation Date: 05 JAN 2004
Dose Administration Initiation: 18 JAN 2004
In-life Phase Completion Date: 24 FEB 2004
Report Issue Date: 02 JUL 2004
Report Location: Module 4, Section 4.2, Page 1-7465
Report Length: 449 pages.
Study Location: [redacted]

Study Director: [redacted] signed 02 JUL 2004
Compliance Statement: Compliant with FDA GLP Regulations as in 21 CFR Part 58, **"except that the** [redacted] (use to collect all hemodynamic data) and the [redacted] (used to collect all vessel diameter data) were not Part 11 validated at the time of data acquisition. The bioanalytical and toxicokinetics reports will be reported separately and are the responsibility of the Sponsor. Deviations are listed in Appendix 7. There were no deviations from the aforementioned standards that affected the quality or integrity of the study or the interpretation of **the results in the report."**
Signed by the Study Director 02 JUL 2004.
Quality Assurance Statement: "The results as presented accurately reflect the raw data."
Signed by [redacted] QA Auditor, 02 JUL 2004.
Test Article: Naproxen Sodium (NAP), USP, Lot Number A03L263, 99.9% purity, from GSK. Sumatriptan Succinate (SS), Lot Number A03L58, 98.7% purity, from GSK.
Vehicle: Sterile Water for Injection, Lot # 05-254-DK, from [redacted]
Characterization and Stability: Responsibility of Sponsor.

Key Points:

- No reliable conclusions can be drawn from this study due to wide inter-individual and intra-individual variation observed in most parameters measured and serious design flaws. This study should be considered invalid.
- The most serious design flaw was the omission of a vehicle control injection for SS. Without this control (or a low, ineffective dose of SS) it is not clear whether the minimal or maximal changes from baseline parameters recorded in the one hr after SS injection are due to the injection procedure, the vehicle, natural variation, or the SS. Hence, it is not clear that any SS-induce changes were measured here, so the meaning of any effects (or lack of effects) of NAP on these changes is questionable.

- Other design flaws included: insufficient numbers of dogs per group and repetitions of treatments to detect a significant NAP-induced change in the predicted SS-induced ~5% reduction in the coronary artery diameter; lack of SS and NAP vehicle controls in Phase III; and the insufficient one hr washout period between the first SS dose and the SS/NAP dose invalidated Phase III, since it was impossible to determine whether any changes (or lack of changes) were due to the NAP or to the 65% higher SS levels after the second dose compared to the first.
- **While the Sponsor's conclusion that no statistically** significant effects of NAP were observed is accurate, there were apparent trends toward NAP-related enhancement of SS-induced effects on coronary artery diameter, carotid artery diameter, mean arterial blood pressure (MAP), and coronary artery resistance. The importance of such trends is questionable, though, in the context of the wide inter-individual and intra-individual variation observed and the design flaws noted above.
- **The Sponsor's overall conclusion that "co-administration** of NAP with SS did not alter the vasoconstrictive effect of SS on the coronary arteries of conscious, chronically instrumented female beagle dogs, nor did it alter any of the other **cardiovascular parameters measured in this study," is not warranted.**

Methods:

Surgical Procedures:

- A [redacted] flow probe (1.5-2.0 mm) and a pair of ultrasonic dimension crystals (5 MHz, 0.024 mm sensitivity, able to detect changes in distance between two crystals that are as little as 1 mm and up to 80 mm apart, manufactured by [redacted]) were placed around the isolated left common carotid artery just proximal to the bifurcation of the internal and external carotid arteries. The flow probe was positioned distal to the dimension crystals, and care was taken not to occlude the vessel in any way. Wires from the flow probe and dimension crystals were tunneled subcutaneously and externalized intrascapularly, and the wound was closed in layers with appropriate sutures. (*eNDA 21-926, Module 4, Section 4.2, Page 26)*
- "A [redacted] pressure catheter was inserted into the left ventricle through the apex of the ventricle (for recording of left ventricular pressure), and a second [redacted] pressure catheter was inserted into the descending thoracic aorta (for recording of systemic arterial pressure). A segment of the left circumflex coronary artery (LCX) proximal to the first branch was isolated (approximately 2–4 cm from the vessel origin). A [redacted] **flow probe (1.5–2.0 mm) and** a pair of ultrasonic dimension crystals (5 MHz, 0.024 mm sensitivity, detection levels between 1 and 80 mm, manufactured by [redacted]) were placed around the isolated section of the LCX, with care not to occlude the vessel in any way (flow probe was positioned distal **to the dimension crystals).**" (*eNDA 21-926, Module 4, Section 4.2, Page 27)*

Dose Administration:

- Phase I: On Day 1, 6 adult female beagle dogs each received a 1-minute IV infusion of vehicle followed by an IV bolus of 80 ug/kg SS. On Days 2 and 3, animals received only the IV bolus dose of 80 ug/kg SS.
- Phase II: On Day 1, 6 adult female beagle dogs each received a 1-minute IV infusion of 20 mg/kg NAP followed by an IV bolus of 80 ug/kg SS. On Days 2 and 3, animals received only the IV bolus dose of 80 ug/kg SS.
- Phase III: 6 adult female beagle dogs each received two IV bolus injections of 200 ug/kg SS, about 1 hr apart, with a 1-minute IV infusion of NAP given just prior to the second SS dose.

Measurements:

- Cardiovascular data were acquired for up to 6 hrs following treatment during Phases I and II; data collected during the first hour following dosing was used to evaluate SS-related effects.
- Cardiovascular data were acquired for up to 1 hr following each SS dose during Phase III.
- Clinical observations, physical exam results, and body weights were recorded.
- Blood plasma samples were collected and sent to a Sponsor-designated laboratory for bioanalytical evaluation.
- Necropsy was performed after euthanization to retrieve the catheters, flow probes, and dimension crystals, without further gross or microscopic pathology evaluation.

Study Design Diagrams:

Text Table 1
Study Design – Phase I

Gr. No.	No. of Females	Test Substance						Monitoring Period
		Vehicle (Sterile Water for Injection, USP)			Sumatriptan Succinate[a]			
		Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Regimen	Dose Level (µg/kg)	Dose Volume (mL/kg)	Dose Regimen	
1	6	0	0.1	Day 1: 1-minute IV infusion	80	0.1	Days 1, 2, and 3: IV bolus; once per day	Day 1: From 30 minutes prior to vehicle dose through up to 6 hours following sumatriptan succinate dose Days 2 and 3: From 30 minutes prior to sumatriptan succinate dose through up to 6 hours following sumatriptan succinate dose

[a] Sumatriptan succinate dose was calculated as the base (1.4 correction factor).
Gr. = Group; No. = Number; IV = Intravenous
Note: A 5- to 6-day wash-out period occurred prior to Phase II.

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 18)

Text Table 2
Study Design – Phase II

Gr. No.	No. of Females	Test Substance						Monitoring Period
		Naproxen Sodium			Sumatriptan Succinate[a]			
		Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Regimen	Dose Level (µg/kg)	Dose Volume (mL/kg)	Dose Regimen	
1	6	20	0.1	Day 1: 1-minute IV infusion	80	0.1	Days 1, 2, and 3: IV bolus, once per day	Day 1: From 30 minutes prior to naproxen sodium dose through up to 6 hours following sumatriptan succinate dose Days 2 and 3: From 30 minutes prior to sumatriptan succinate dose through up to 6 hours following sumatriptan succinate dose

[a] Sumatriptan succinate dose was calculated as the base (1.4 correction factor).
Gr. = Group; No. = Number; IV = Intravenous

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 19)

Text Table 3
Study Design – Phase III

Gr. No.	No. of Females	Test Substance						Monitoring Period
		Naproxen Sodium			Sumatriptan Succinate[a]			
		Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Regimen	Dose Level (µg/kg)	Dose Volume (mL/kg)	Dose Regimen	
1	6	20	0.1	Day 1: 1-minute IV infusion given just prior to the second sumatriptan succinate dose	200	0.1	Day 1: two IV bolus injections, administered about 1 hour apart	Day 1: From 30 minutes prior to first sumatriptan succinate dose through up to 1 hour following second sumatriptan succinate dose

[a] Sumatriptan succinate dose was calculated as the base (1.4 correction factor).
Gr. = Group; No. = Number; IV = Intravenous

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 19)

Justification for Species, Sex, Doses, and Procedures Used:

- Dogs are an established animal species for cardiovascular studies.
- FDA recommended us of an *in vivo* dog model for this evaluation.
- One sex was selected to limit the number of animals required to obtain the data.
- Females were selected because the test article is intended for us in the treatment of migraine, and most migraine patients are female.
- IV SS at 100 ug/kg was associated with a mean reduction in coronary arterial diameter of 137 ± 21 um in a study in conscious dogs by Carel et al. (2001, *Br J Pharmacol* 132:1071-1083).
- Six dogs were chosen to provide a power of more than 80% of detecting this change.
- Conscious, chronically instrumented dogs were us to measure drug-induced changes in coronary vessel diameter in accordance **with FDA's recommendation to assess the** vasoconstrictive potential of the combination of SS and NAP in an in vivo model using the instrumentation (dimensions crystals) successfully employed by Gupta et al., [(1995), *Br J Pharmacol* **116**(5):2385-2390; and (2000) *Eur J Pharmacol* **398**:73-81], and Carel et al., (2001).
- The conscious animal method of Carel et al. was used instead of the anesthetized method of Gupta et al. because anesthesia is known to alter baseline hemodynamic parameters (e.g., increased blood pressure and heart rate) compared to conscious animals, thus potentially altering the cardiovascular reserve of the animal.
- Carel et al. were able to show that a dose-dependent reduction in coronary and carotid artery diameters could be achieved using the doses of 0.1-100 ug/kg sumatriptan (free base). External coronary artery diameter was reduced 5.3% (-137 um) at 100 ug/kg sumatriptan (free base). Higher doses were not tested.
- Gupta and colleagues used an anesthetized dog model to show that sumatriptan-induced reductions in external coronary artery diameter appeared to reach a maximum at100 ug/kg, as doses above this level produced no greater response.
- The dose level of 80 ug/kg sumatriptan (free base) was selected for this study in order to evaluate the potential of NAP to exacerbate the sumatriptan-induced coronary artery vasoconstriction.
- Administration of NAP within one minute of SS was selected rather than a 15- to 30-minute NAP pretreatment since the former was considered more likely to mimic human exposure to NAP and SS after a single oral administration of MT 400 (combination of SS and NAP at a ratio of 85/500 mg), where both components are absorbed simultaneously.
- The dose level of NAP selected for the current study (20 mg/kg, sodium salt) was known to be within the range of pharmacologically effective doses: 10-30 mg/kg NAP blocked prostaglandin synthesis almost immediately after single IV administration in published dog studies.
- Since the half-life of NAP has been shown to be 35-40 hrs in dog, the plasma levels of NAP were expected to remain within the range of pharmacologically effective concentrations during all three days of SS administration after the single IV injection of 20 mg/kg NAP on Day 1 in Phase 2 of the current study.

- The period of 24 hrs between successive doses of SS in Phases 1 and 2 was considered adequate to allow washout of its effects, since the half-life of SS in dogs is only about 2 hrs.

Results:

Dose Formulation Analysis: *(reproduced directly from Module 4 Section 4.2 Page 34)*

> Stability of sumatriptan succinate (SS) at 0.8 or 2.0 mg/mL after 24 hours at room temperature and at eight days at -20°C was within the acceptable limit of ±10% of both theoretical concentrations. Formulation analysis of the samples collected prior to dosing during each of the three phases demonstrated that the formulations were within ±10% of the intended theoretical dose concentrations on all days with one exception. Duplicate samples of the Phase I Day 1 SS dose solution collected on January 21, 2004, demonstrated a concentration of SS of 0 mg/mL. The corresponding vehicle sample (and its back-up) collected on the same day contained 0.8146 mg/mL sumatriptan (+1.8% of the 0.8 mg/mL theoretical concentration of the test article). It is reasonable to believe that the vehicle and SS formulation samples collected on this date were inadvertently placed into the incorrect labeled sampling tubes. It is also reasonable to believe the animals (Animal Nos. 1101 and 1102) were correctly treated with vehicle followed by SS on this date because the vehicle solution was transported **to the treatment room in the vendor's** original plastic bottle, labeled by the vendor as sterile water for injection, while the formulated dose of SS was contained within an appropriately labeled sterile glass bottle. Stability of naproxen sodium (NAP) at 200 mg/mL after 24 hours at room temperature and at 7 days at -20°C was within the acceptable limit of ±10% of the theoretical concentration. Formulation analysis of the samples collected prior to dosing during Phases II and III demonstrated that the formulations were within ±10% of the intended theoretical dose concentration on all days except the Phase II Day 1 sample collected on January 29, 2004. This sample was measured to be 12.3% below the theoretical formulation concentration of 200 mg/mL (175.5 mg/mL).

Mortality:

Eight female dogs were instrumented for **this study, but one (#1103) "did not have a** detectable systemic arterial blood pressure signal on Day 7 following the surgical instrumentation and was subsequently euthanized after being observed in discomfort. During the necropsy evaluation on Animal No. 1103, the [redacted] pressure catheter inserted into the descending thoracic aorta was observed to have come out of the vascular insertion site.

Clinical Observations:

No test article related clinical observations were noted, though one or more animals showed signs related to the surgical procedures, instrumentation, and bandaging required to protect the exteriorized catheters (lethargy, anorexia, wound scabs, skin erythema, eye squint, swelling, alopecia, and discharge).

Body Weights:

Individual body weights ranged from 7.1 to 8.5 kg in Phase I, 7.1 to 8.2 kg in Phase II, and 6.8 to 8.5 kg in Phase III. Group mean body weight decreased 0.3 kg between Phases I and II, which may have been due to SS administration. Mean body weight increased 0.1 kg between Phases II and III.

Toxicokinetics:

(POZEN TK Report MT400-T15: Toxicokinetic Data from: A Study to Determine Coronary and Carotid Arterial Blood Flow, Resistance, and Diameter following Intravenous Administration of Sumatriptan Succinate with and without Naproxen Sodium to Conscious Beagle Dogs; *see eNDA 21-926, Module 4, Section 4.2, page 910*)

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, page 916)
Blood samples were collected during Phases I and II on Days 1, 2 and 3 at baseline (pre-dose) and at 0.5, 1, 2, 4, 8, and 12 hours post sumatriptan Succinate administration; also, 24 hours after the final (Day 3) dose of sumatriptan succinate. During Phase III, blood samples were collected predose and also at 0.5 and 1.0 hours after administration of the first and second bolus injections of sumatriptan succinate. Plasma samples were analyzed for sumatriptan and naproxen using validated LC/MS/MS assays.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, page 917)
Pharmacokinetic analysis of Phase III data was not conducted since the two doses of sumatriptan were administered just one hour apart. Because sumatriptan is not completely eliminated in one hour of time, the two doses of sumatriptan were cumulative. No conclusions can be drawn regarding the impact of naproxen on the pharmacokinetics of sumatriptan from the Phase III data.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, page 920-921)
The majority of the blood samples were collected within + 5% of the scheduled times. Exceptions included on Day 3 of Phase I (February 4, 2004), the 0.5-hour blood samples for Animal Nos. 1107 and 1108 were collected later than the allowable time range (18 and 14 minutes late, respectively), and on Day 2 of Phase II (February 10, 2004), the 0.5-hour blood sample for Animal No. 1107 was collected 2 minutes late. Based upon the appearance of the concentration-time profile, the samples collected from animals 1101 and 1102 at the same time on Day 2 (12 hour sampling time) were apparently inadvertently transposed upon collection or processing. These samples were included in the WinNonlin input file as reported and the analysis was also performed with the samples switched to reflect the anticipated pharmacokinetic profile. Since the apparent inadvertent transposition of the samples did not appear to have a major impact upon the pharmacokinetic profiles, the original values were maintained in the final data analysis.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, page 923)
Conclusions:

- Sumatriptan pharmacokinetic parameters were similar during Phase I (water vehicle/sumatriptan on Day 1 and sumatriptan only on Days 2 and 3) and Phase II (naproxen/sumatriptan on Day 1 and sumatriptan only on Days 2 and 3). Measurable plasma concentrations of naproxen were present over the entire 3-day Phase II study period.
- These results indicate that there was not a pharmacokinetic drug interaction by naproxen on sumatriptan.

Table 4. Sumatriptan Toxicokinetic Parameters in Beagle Dogs on Days 1, 2, and 3 of Phase I and Phase II

Treatment (Phase)		C_{max} (ng/mL)			T_{max} (hr)			Half-life (hr)			AUC_{inf} (hr*ng/mL)		
	Animal ID	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Vehicle + Sumatriptan (Phase I)	1101	18.5	22.8	21.5	0.50	0.50	0.50	1.1	0.9	0.9	34.3	32.7	31.0
	1102	20.3	15.7	20.3	0.50	0.50	0.50	1.2	1.0	1.0	37.0	25.8	29.6
	1104	20.0	22.4	19.9	0.50	0.50	0.50	1.3	1.0	1.1	38.6	37.5	36.3
	1105	26.1	17.9	19.6	0.50	1.00	0.50	0.9	1.4	1.3	39.8	33.4	39.7
	1107	22.0	17.9	15.6	0.50	0.50	1.00	0.9	1.0	1.4	34.6	31.2	33.4
	1108	21.2	20.9	13.5	0.50	0.50	0.73	1.0	1.1	1.4	33.5	33.0	29.8
	Mean	21.4	19.6	18.4	0.50	0.58	0.62	1.1	1.1	1.2	36.3	32.3	33.3
	SD	2.6	2.9	3.1	0.00	0.20	0.21	0.1	0.2	0.2	2.6	3.8	4.0
	CV%	12	15	17	0	35	33	14	17	18	7	12	12
Naproxen + Sumatriptan (Phase II)	1101	18.0	19.3	20.2	0.50	0.50	0.50	1.1	1.3	1.0	45.0	38.0	33.4
	1102	19.0	18.8	18.4	0.50	0.50	0.50	1.0	0.8	0.9	31.6	26.2	27.7
	1104	15.8	18.0	15.8	0.50	0.50	0.50	1.1	1.1	1.2	28.0	31.5	30.2
	1105	18.5	17.4	18.1	0.50	0.50	0.50	1.0	1.2	1.2	30.9	31.2	34.6
	1107	21.2	21.3	21.8	0.50	0.53	0.50	1.0	1.1	1.1	34.2	38.7	36.6
	1108	21.2	18.1	19.2	0.50	0.50	0.50	1.2	1.2	1.5	38.9	35.1	43.7
	Mean	19.0	18.8	18.9	0.50	0.51	0.50	1.1	1.1	1.2	34.8	33.4	34.4
	SD	2.1	1.4	2.0	0.00	0.01	0.00	0.1	0.2	0.2	6.2	4.7	5.5
	CV%	11	7	11	0	3	0	8	15	18	18	14	16

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 926)

Table 5. Naproxen Toxicokinetic Parameters in Beagle Dogs During Phase II

Animal ID	C_{range}[a] Day 1 (µg/mL)	C_{range}[a] Day 2 (µg/mL)	C_{range} Day 3 (µg/mL)	$t_{1/2,z}$ (hr)	AUC_{last} (hr*µg/mL)	AUC_{inf} (hr*µg/mL)	AUC %Ext
1101	97.4 to 53.0	53.0 to 35.2	35.2 to 25.2	45.1	3226	4865	33.7
1102	74.2 to 37.7	37.7 to 23.2	23.2 to 16.3	37.9	2397	3287	27.1
1104	82.0 to 45.5	45.5 to 28.3	28.3 to 18.3	34.4	2746	3653	24.8
1105	79.4 to 42.5	42.5 to 29.7	29.7 to 21.7	39.4	2603	3835	32.1
1107	57.9 to 24.9	24.9 to 18.3	18.3 to 11.3	41.5	1620	2295	29.4
1108	84.1 to 41.5	41.5 to 23.5	23.5 to 10.9	29.9	2265	2735	17.2
Mean	79.2 to 40.9	40.9 to 26.4	26.4 to 17.3	38.0	2476	3445	27.4
SD	NR	NR	NR	5.3	536	901	6.0
CV%	NR	NR	NR	14	22	26	26

NR= Not reported

a. The final plasma concentrations for Days 1 and 2 were also used as the time 0 value for Days 2 and 3, respectively

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 927)

Reviewer's Comments:

The mean Cmax of SS in humans given one oral tablet of Trexima (Study MT400-101) was 74.9 ng/mL (Tmax = 1.0 hrs). The mean Cmax of NAP in Study MT400-101 was 69.7 ug/mL (Tmax = 6.0 hrs).

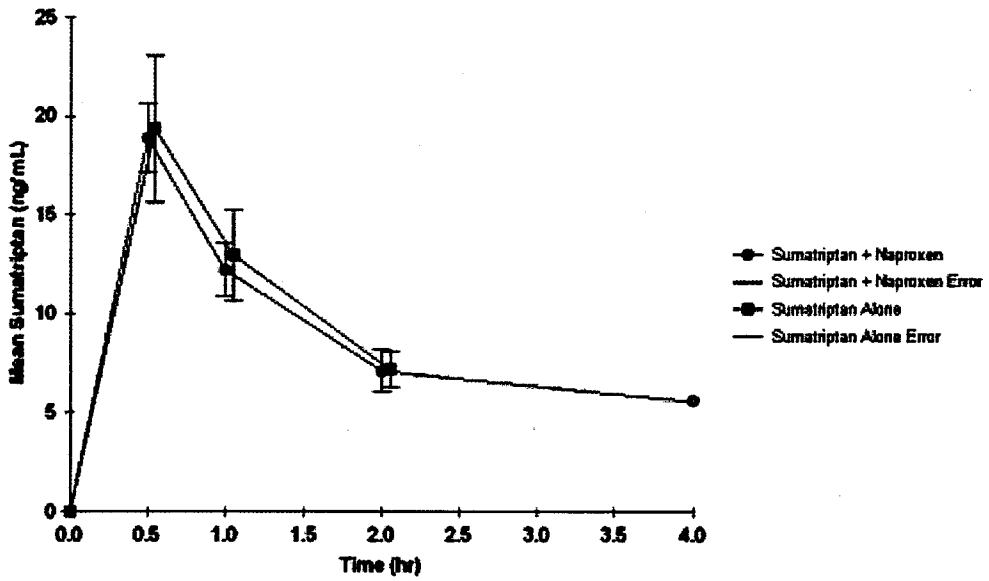
The 80 ug/kg IV SS dose used in Phases I and II of the dog study resulted in mean peak plasma levels of SS of ~19 ng/mL, well below mean peak plasma levels in humans after oral Trexima (74.9 ng/mL) (though the stated Cmax in the dog may be an underestimate since it represents the initial time point of 0.5 hrs after the IV dose). Thus, there is no margin of safety between plasma levels of SS in dogs exhibiting putatively SS-induced coronary artery vasoconstriction and those in humans after administration of one Trexima tablet.

The 20 mg/kg IV NAP dose used in the dog study resulted in mean peak plasma levels of 79.2 ug/mL NAP on Day 1 (range: 57.9-90.5 ug/mL), 40.9 ug/mL on Day 2, and 26.4 ug/mL on Day 3, compared with 69.7 ug/mL NAP in humans after oral Trexima. Thus, even if the Day 1 NAP Cmax value were to be considered a NOEL in dog for exacerbation of SS-induced coronary artery vasoconstriction, there is virtually no margin of safety above mean peak human NAP plasma levels after one Trexima tablet.

Comparison of Plasma Drug Levels Between Humans and Dogs		
	SS (ng/mL)	**NAP (ug/mL)**
Human Cmax after one Trexima Tablet	74.9	69.7
Dog Cmax at 80 ug/kg IV SS + 20 mg/kg NAP	19	79.2
Ratio of Dog Cmax to Human Cmax	0.25	1.1

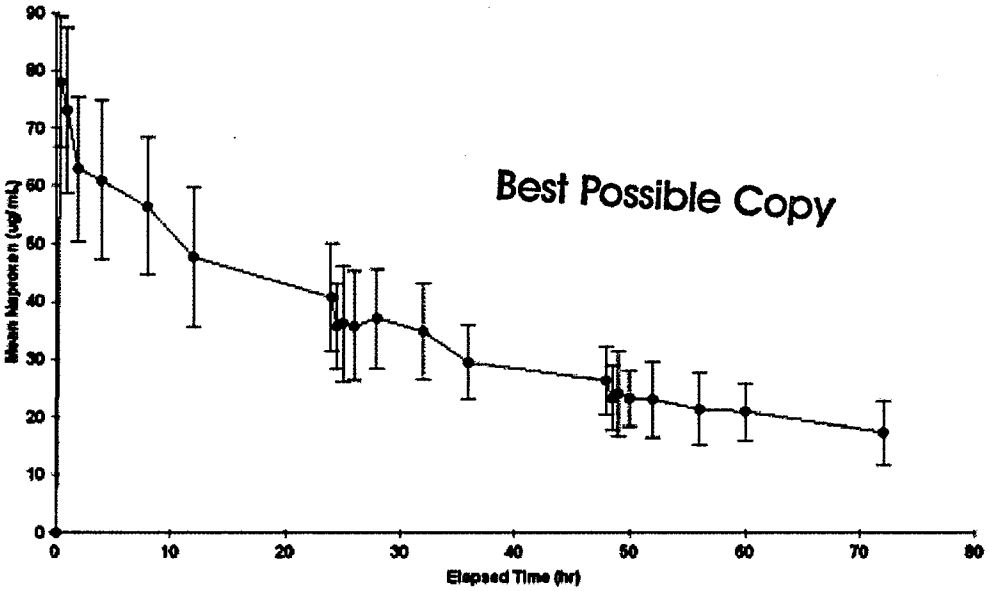
(Reviewer's Table; human Cmax values are geometric means from Study MT400-101)

Figure 1. Mean Plasma Sumatriptan Concentrations vs. Time in the Beagle Dog (n=6): Average of Days 1, 2 and 3 of Phase I and Phase II



(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 928)

Figure 2. Mean (±SD) Phase II Plasma Naproxen Concentration vs. Time in the Beagle Dog (n=6)



(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 929)

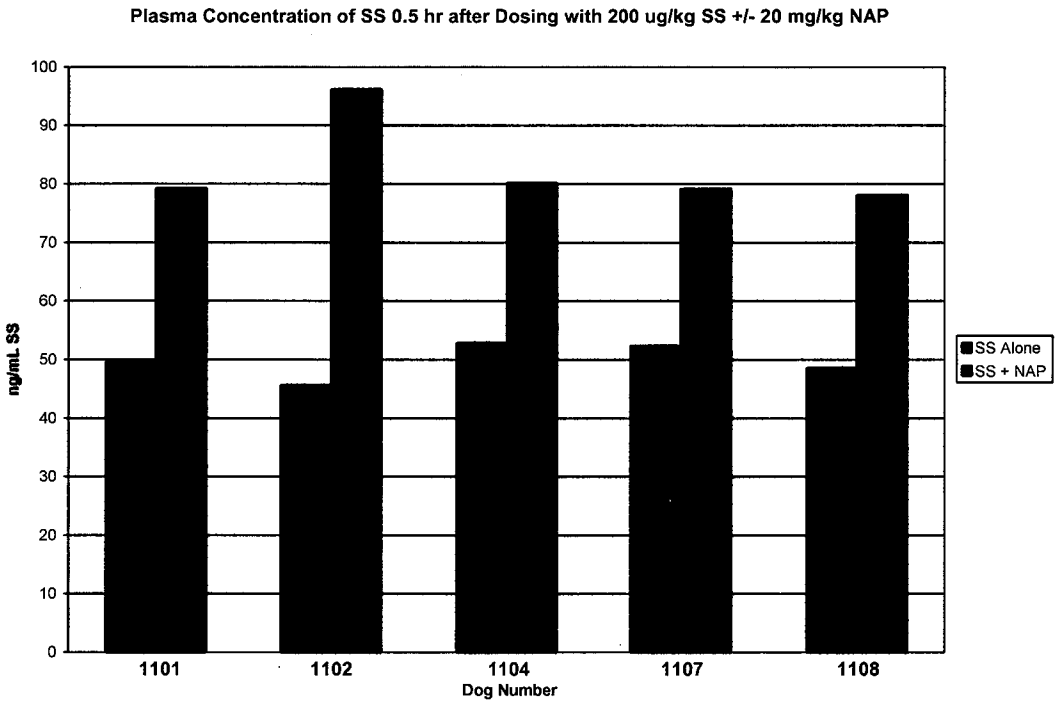
7 Data Listing - Individual Dog Concentration-time Data for Phase III

Animal ID	Phase	Day	Time (hr)	Sumatriptan (ng/mL)
1101	III		0	
1101	III		0.5[a]	
1101	III		1[a]	
1101	III		0.5[b]	
1101	III		1[b]	
1102	III		0	
1102	III		0.5[a]	
1102	III		1[a]	
1102	III		0.5[b]	
1102	III		1[b]	
1104	III		0	
1104	III		0.5[a]	
1104	III		1[a]	
1104	III		0.5[b]	
1104	III		1[b]	
1107	III		0	
1107	III		0.5[a]	
1107	III		1[a]	
1107	III		0.5[b]	
1107	III		1[b]	
1108	III		0	
1108	III		0.5[a]	
1108	III		1[a]	
1108	III		0.5[b]	
1108	III		1[b]	

a. Indicates sample time after the first dose of sumatriptan

b. Indicates sample time after the second dose of sumatriptan. Note that naproxen was administered about 1 minute prior to the second dose of sumatriptan.

(Reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 943)



(Reviewer's graph)

The graph above illustrates that the plasma concentration of SS 0.5 hrs after a dose of 200 ug/kg IV in Phase III increased dramatically in all 5 dogs tested when a second dose of 200 ug/kg IV SS was administered only one hour later, a minute after administration of 20 mg/kg IV NAP. The mean increase observed was 65.8 ± 10.6% (SEM). This effect was attributed to the incomplete clearance of SS from the systemic circulation in the short one hour interval between the two doses of SS. Any possible effect of NAP on the level of SS (or on any other parameters measured in this experiment) cannot be distinguished from this large effect due to accumulation of SS. Thus, the results of Phase III cannot be relied upon to demonstrate the presence or absence of an effect of NAP on SS-induced changes in cardiovascular parameters.

No effect of NAP on SS levels was seen in Phase II, where prior administration of 20 mg/kg IV NAP did not dramatically change the mean plasma level of SS 0.5 hrs after the lower dose of 80 ug/kg IV SS (21.4 ng/mL SS alone, vs. 19.0 ng/mL SS + NAP).

Cardiovascular Parameters:

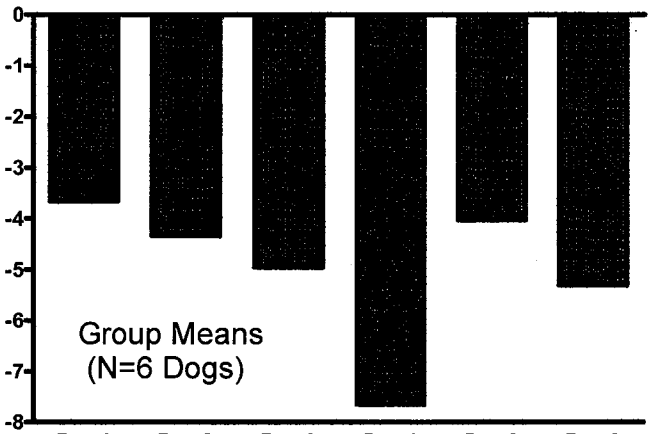
Left Circumflex Coronary Artery Diameter:

In Phase 1, a single IV bolus dose of 80 ug/kg SS given ~1 min after a one minute IV infusion of vehicle induced vasoconstriction of the LCX artery such that the arterial diameter was reduced 3.66% from baseline on Day 1, *(see graph below)*. In Phase 2, however, 80 ug/kg SS IV given ~1 min after a one minute IV infusion of 20 mg/kg NAP induced a 7.65% reduction in LCX arterial diameter. While this appears to show a dramatic ~2-fold enhancement of the SS-induced vasoconstrictive effect by NAP *(see graph below)*, the average reductions with and without NAP were not statistically significantly different (p=0.1399) according to a paired t-test. This apparent 2-fold increase in LCX vasoconstriction occurred at a mean Cmax of 79.2 ng/mL NAP, which is not much higher than the 48-53 ug/mL mean Cmax of Naproxen in human subjects given one oral tablet of Trexima. Mean SS Cmax in these subjects was 40-54 ng/mL, which is *higher* than the ~20 ng/mL mean Cmax observed here in the dogs.

Examination of the individual animal data *(see additional graphs below)* reveals that the SS-induced reduction in LCX diameter was 7-10% in 4/6 dogs in the presence of NAP (Phase II Day 1), but was only 1-4% in 4/6 dogs in the absence of NAP (Phase I Day 1). Furthermore, one of the 2 dogs that did not show an enhanced reduction of SS-induced LCX diameter with NAP (Dog #1107) had an anomalously high value for SS-induced reduction without NAP (7.83% vs. 1.21-5.00% in the other 5 dogs). However, it is difficult to argue that these data demonstrate a consistent NAP effect when both the intra-individual (day to day) and inter-individual variation are so great.

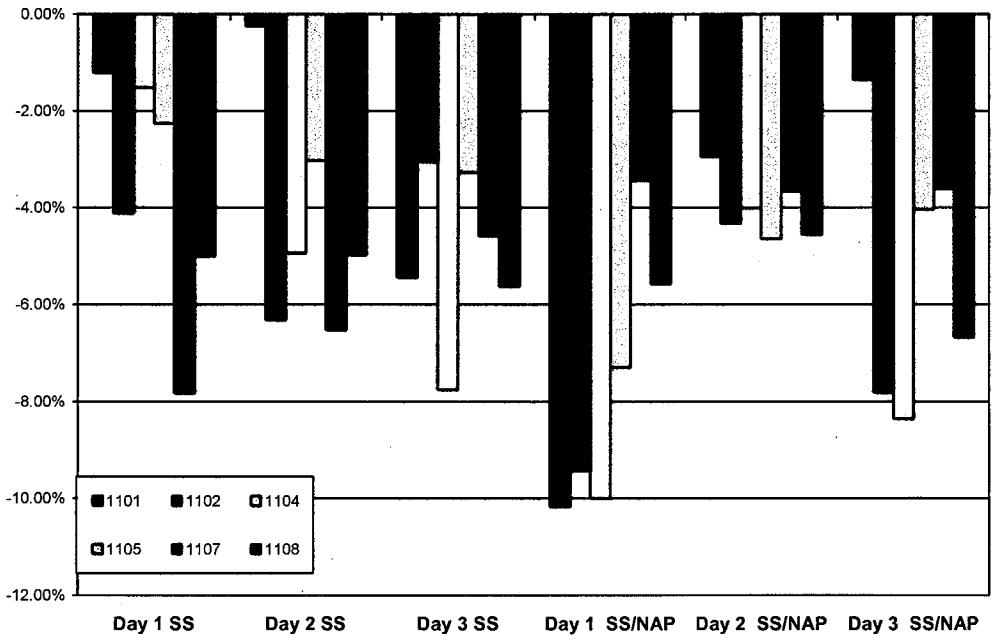
Data from Days 2 and 3 did not show significant differences (p=0.6186 and 0.6572, respectively) in SS-induced vasoconstriction of LCX in the presence vs. the absence **of NAP—mean values were all in the range** of 4-5% reduction in LCX diameter with or without NAP, which agrees with the 5.3 ± 0.9% reduction reported by Carel et al. (2001, *Br J Pharmacol* 132:1071-1083) in response to 100 ug/kg SS IV in a similar experiment. The baseline of ~2600 um for the external diameter of the LCX coronary artery reported by Carel et al., (2001) is lower than the range of 2.6 to 4.3 um in this experiment, but this could be because adult female beagles were used here instead of the mongrels used by Carel et al.

Percent Change in Coronary Artery Diameter



Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
SS 21.4 ng/mL	SS 19.6 ng/mL	SS 18.4 ng/mL	SS 19.0 ng/mL	SS 18.8 ng/mL	SS 18.9 ng/mL
			NAP 79.2 ng/mL	NAP 40.9 ng/mL	NAP 26.4 ng/mL

Change in Coronary Artery Diameter Induced by SS +/- NAP in Dog



Maximum Reductions in
Coronary Diameter (mm) on Day 1

	Phase I (Vehicle + 80 μg/kg Sumatriptan)			Phase II (20 mg/kg Naproxen + 80 μg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	3.6071	3.5633	-0.0438	3.5386	3.1785	-0.3601	-0.3163
1102	3.9718	3.8083	-0.1635	4.1781	3.7839	-0.3941	-0.2307
1104	3.2097	3.1610	-0.0487	3.4923 [d]	3.1430	-0.3493	-0.3006
1105	3.0507	2.9817	-0.0690	3.4085	3.1598	-0.2486	-0.1796
1107	3.9149	3.6082	-0.3067	3.1788	3.0694	-0.1094	0.1973
1108	2.7476	2.6101	-0.1375	2.8021	2.6460	-0.1561	-0.0185
						Mean	-0.1414
						STD	0.1976
						95% CI	(-0.3487, 0.0659)
						p-value	0.1399

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding vehicle (Phase I) or naproxen (Phase II) administration.
[b] Minimum Coronary Diameter during the first hour after sumatriptan administration.
[c] Minimum Coronary Diameter - Baseline Coronary Diameter.
[d] An outlier value of 5.4712 mm at the one minute pre-NAP sampling time was determined to be an outlier and was excluded from calculation of the baseline value

Maximum Reductions in
Coronary Diameter (mm) on Day 2

	Phase I (80 μg/kg Sumatriptan)			Phase II (80 μg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	3.3883	3.3797	-0.0086	3.4682	3.3657	-0.1024	-0.0938
1102	4.3101	4.0378	-0.2723	4.1128	3.9349	-0.1779	0.0943
1104	3.0521	2.9014	-0.1507	3.2629	3.1321	-0.1309	0.0199
1105	2.9889	2.8985	-0.0905	3.3656	3.2092	-0.1564	-0.0659
1107	4.0199	3.7575	-0.2624	3.1805	3.0639	-0.1166	0.1458
1108	2.7941	2.6550	-0.1391	2.6486	2.5279	-0.1207	0.0184
						Mean	0.0198
						STD	0.0913
						95% CI	(-0.0761, 0.1156)
						p-value	0.6186

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Minimum Coronary Diameter during the first hour after sumatriptan administration.
[c] Minimum Coronary Diameter - Baseline Coronary Diameter.

Maximum Reductions in
Coronary Diameter (mm) on Day 3

	Phase I (80 µg/kg Sumatriptan)			Phase II (80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	3.4381	3.2512	-0.1869	3.4491	3.4023	-0.0468	0.1402
1102	3.9887	3.8669	-0.1218	4.1699	3.8438	-0.3261	-0.2044
1104	3.1713	2.9252	-0.2461	3.5305	3.2353	-0.2952	-0.0491
1105	3.0257	2.9267	-0.0990	3.3221	3.1880	-0.1340	-0.0350
1107	3.3684	3.2138	-0.1546	3.1711	3.0566	-0.1145	0.0401
1108	2.8176	2.6591	-0.1585	2.7131	2.5319	-0.1812	-0.0227
						Mean	-0.0218
						STD	0.1134
						95% CI	(-0.1408, 0.0972)
						p-value	0.6572

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Minimum Coronary Diameter during the first hour after sumatriptan administration.
[c] Minimum Coronary Diameter - Baseline Coronary Diameter.

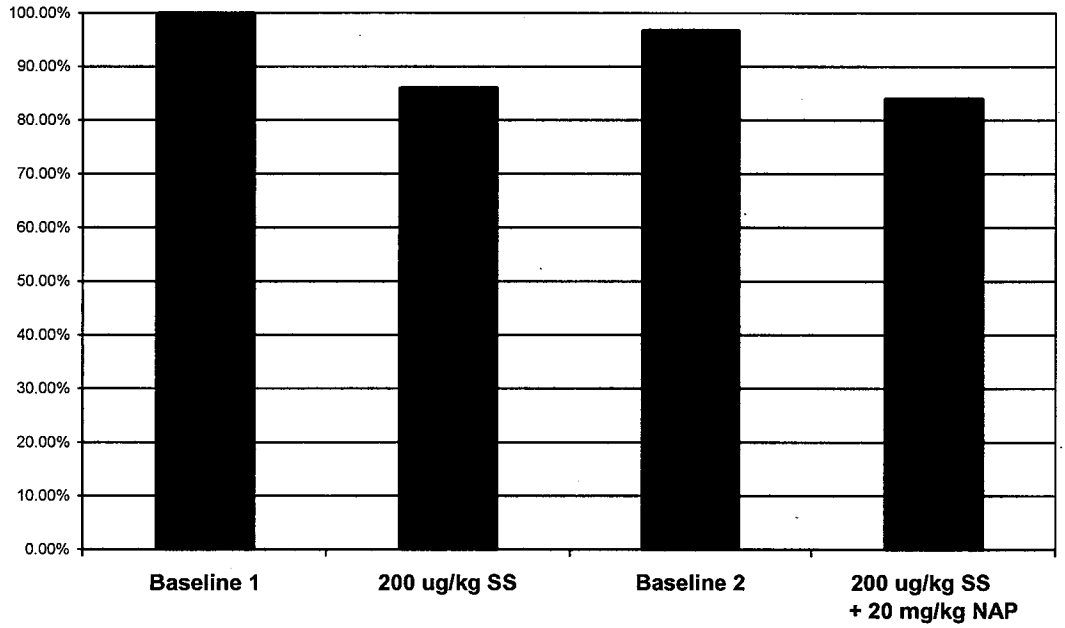
In Phase III, the mean reduction in LCX artery diameter induced by 200 ug/kg IV SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP (p=0.1642).

Study QCBW 106
Table 11
Maximum Reductions in
Coronary Diameter (mm)

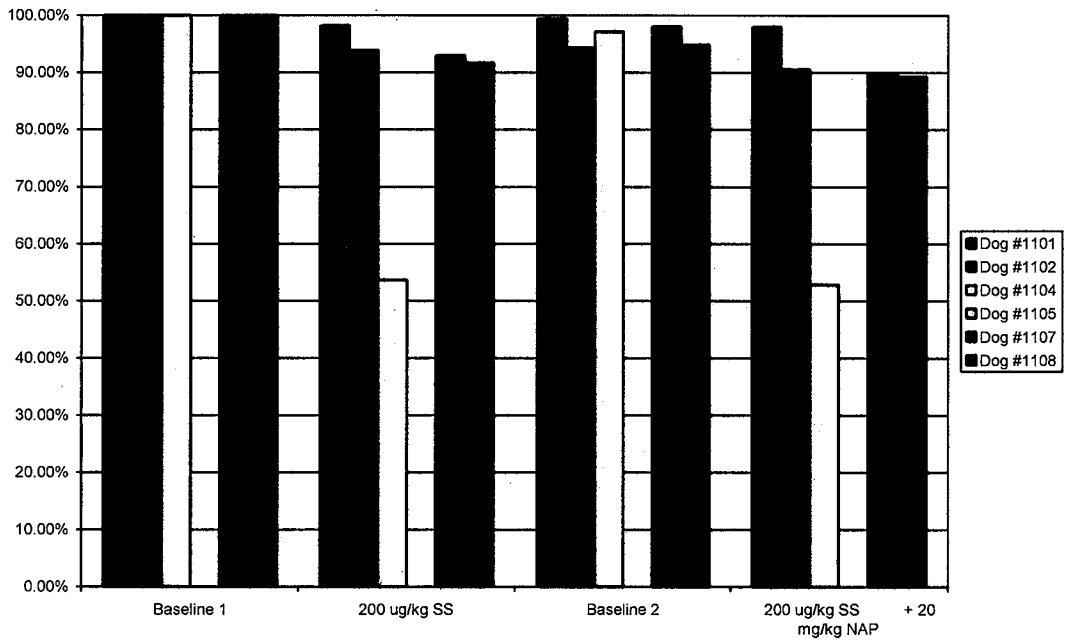
	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	3.7776	3.7092	-0.0684	3.7572	3.7024	-0.0548	0.0136
1102	4.4656	4.1917	-0.2740	4.2113	4.0439	-0.1674	0.1066
1104	5.9048	3.1692	-2.7356	5.7385	3.1216	-2.6169	0.1187
1105[d]	.	.	.	.	.	.	.
1107	3.9882	3.7073	-0.2809	3.9095	3.5798	-0.3297	-0.0488
1108	2.7702	2.5394	-0.2308	2.6282	2.4738	-0.1544	0.0764
						Mean	0.0533
						STD	0.0701
						95% CI	(-0.0337, 0.1403)
						p-value	0.1642

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Minimum Coronary Diameter during the first hour after sumatriptan administration.
[c] Minimum Coronary Diameter - Baseline Coronary Diameter.
[d] Period (.) denotes a missing value.

Mean Change in Coronary Artery Diameter Induced by SS +/- NAP

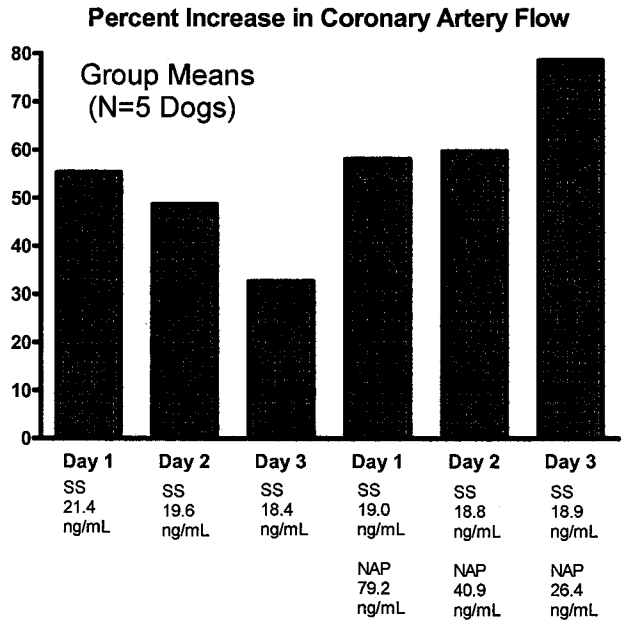


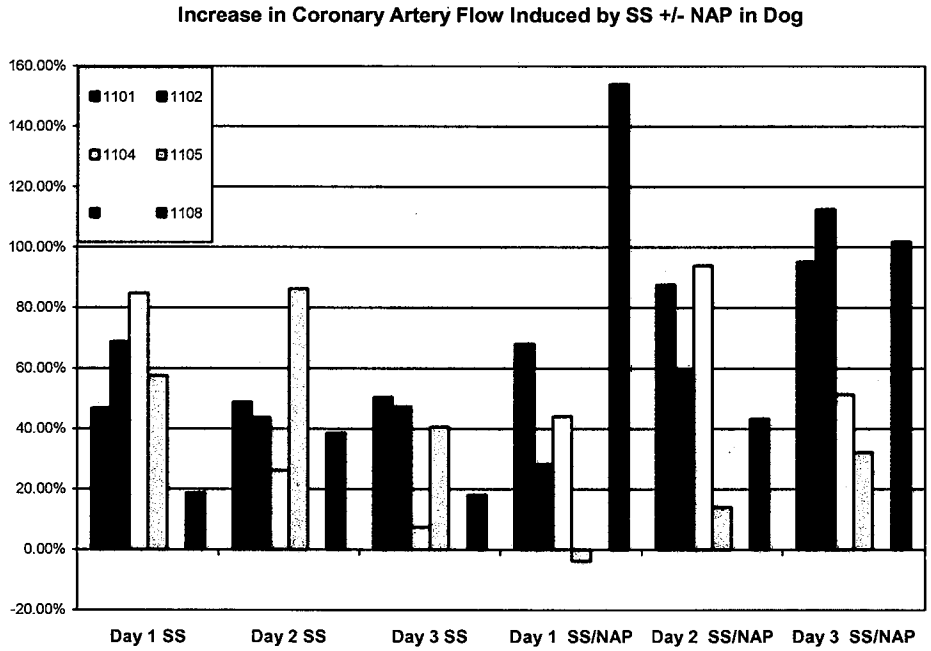
Change in Coronary Artery Diameter Induced by SS +/- NAP



Left Circumflex Coronary Artery Flow:

In Phases I and II, the mean increase in LCX artery flow induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.7154), Day 2 (p=0.2107), or Day 3 (p=0.0628).





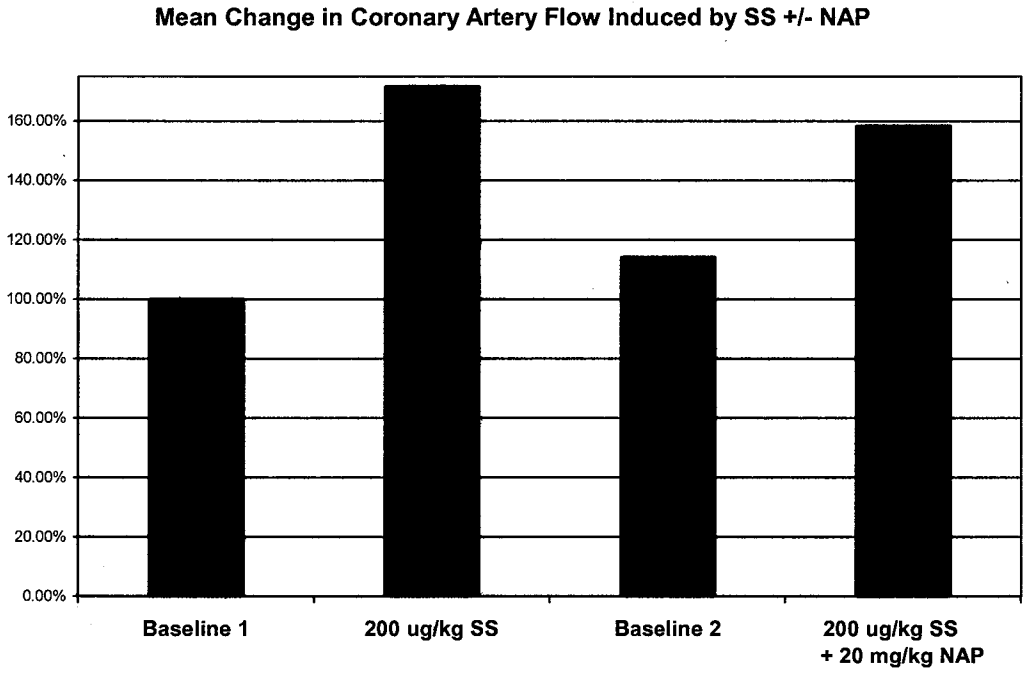
In Phase III, the mean increase in LCX artery flow induced by 200 ug/kg IV SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP (p=0.0665).

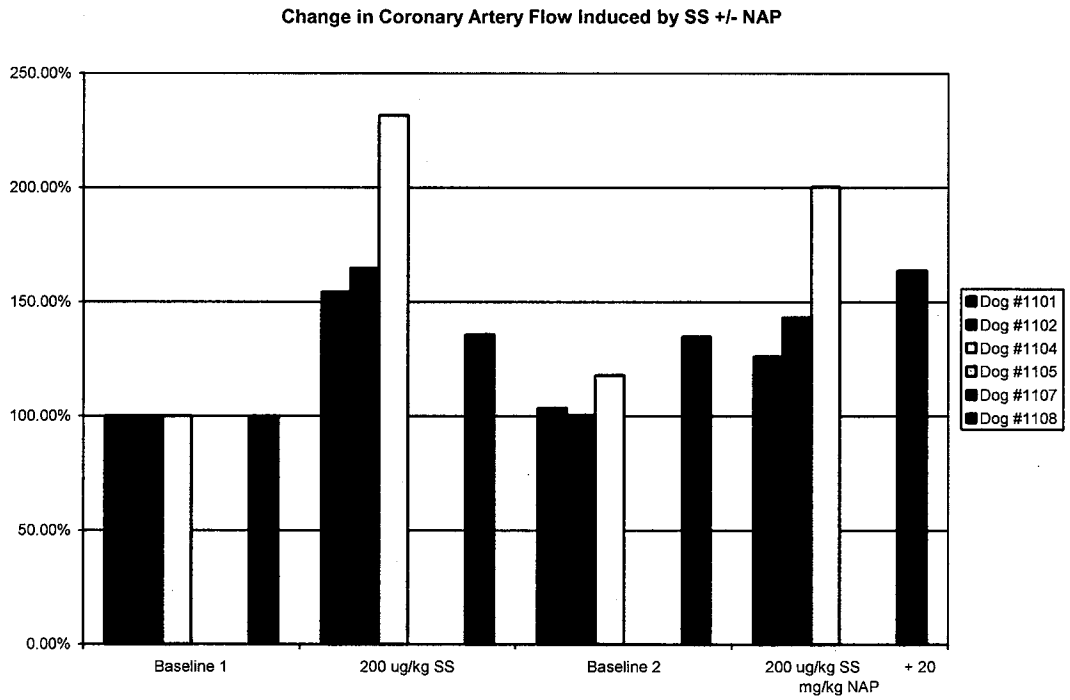
PHASE III

Study QCBW 106
Table 13
Maximum Increases in
Coronary Flow (mL/min)

	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Maximum Flow [b]	Change From Baseline [c]	Baseline [a]	Maximum Flow [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	17.108	26.420	9.312	17.714	21.570	3.856	-5.456
1102	54.936	90.560	35.624	55.152	78.690	23.538	-12.086
1104	15.598	36.120	20.522	18.398	31.250	12.852	-7.670
1105[d]	.	.	.	.	.	.	.
1107[d]	.	.	.	.	.	.	.
1108	13.982	18.970	4.988	18.868	22.900	4.032	-0.956
						Mean	-6.542
						STD	4.633
						95% CI	(-13.914, 0.830)
						p-value	0.0665

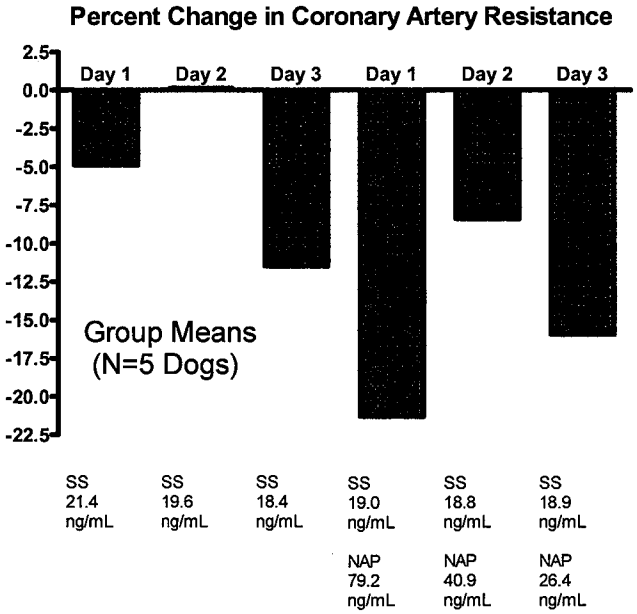
[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Maximum Coronary Flow during the first hour after sumatriptan administration.
[c] Maximum Coronary Flow - Baseline Coronary Flow.
[d] Period (.) denotes a missing value.

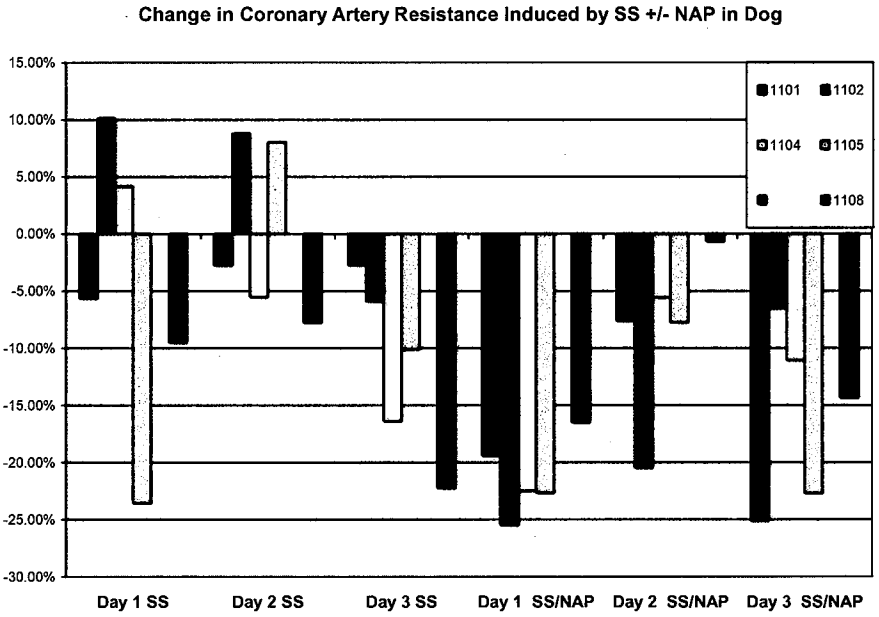




Left Circumflex Coronary Artery Resistance:

The mean reduction in LCX artery resistance induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 ($p=0.1617$), Day 2 ($p=0.3147$), or Day 3 ($p=0.5757$). However, there is an apparent trend toward increased reduction of coronary artery resistance with NAP compared to with SS alone (Day 1 Phase II vs. Day 1 Phase I), stemming from the 5 of 6 dogs showing changes in this parameter (see second graph below of individual animal data).


Percent Change in Coronary Artery Resistance
2.5
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
-15.0
-17.5
-20.0
-22.5
Day 1
Day 2
Day 3
Day 1
Day 2
Day 3
Group Means
(N=5 Dogs)
SS 21.4 ng/mL
SS 19.6 ng/mL
SS 18.4 ng/mL
SS 19.0 ng/mL
NAP 79.2 ng/mL
SS 18.8 ng/mL
NAP 40.9 ng/mL
SS 18.9 ng/mL
NAP 26.4 ng/mL


Change in Coronary Artery Resistance Induced by SS +/- NAP in Dog
15.00%
10.00%
5.00%
0.00%
-5.00%
-10.00%
-15.00%
-20.00%
-25.00%
-30.00%
1101
1102
1104
1105
1108
Day 1 SS
Day 2 SS
Day 3 SS
Day 1 SS/NAP
Day 2 SS/NAP
Day 3 SS/NAP

In Phase III, the mean reduction in LCX coronary artery resistance induced by 200 ug/kg IV SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP (p=0.7132).

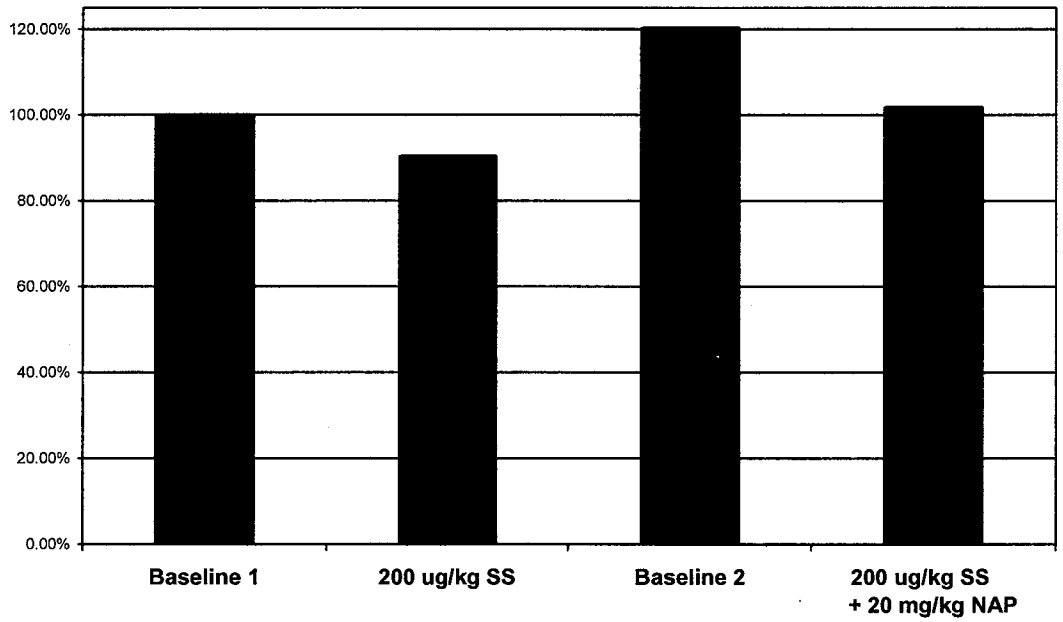
PHASE III

Study QCBW 106
Table 12
Maximum Reductions in
Coronary Resistance (mL/mmHg.min)

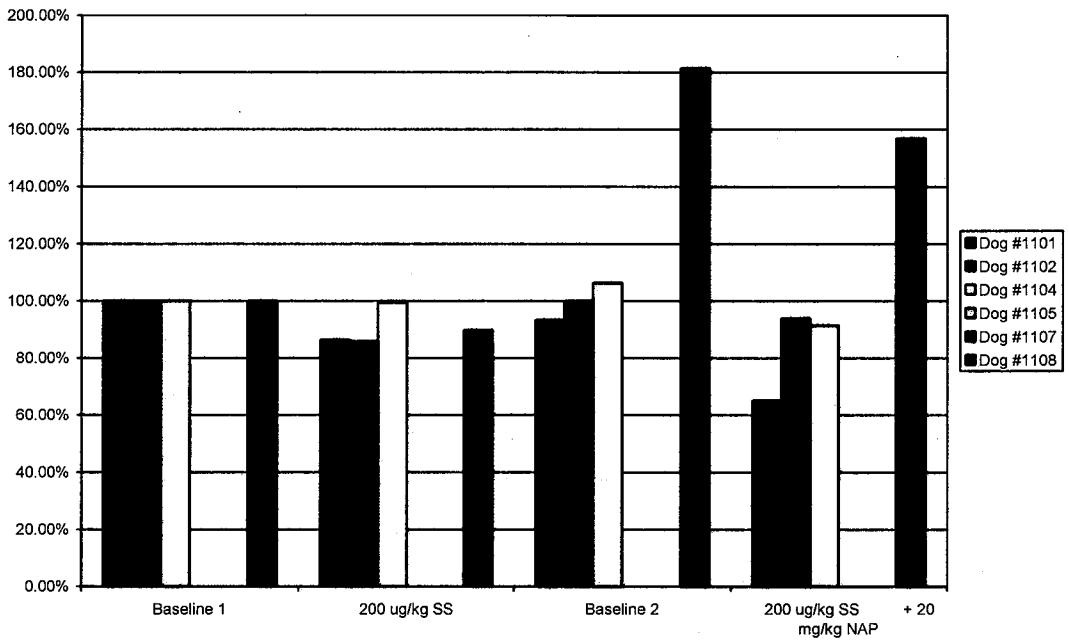
	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Minimum Resistance [b]	Change From Baseline [c]	Baseline [a]	Minimum Resistance [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	0.1681	0.1453	-0.0228	0.1569	0.1094	-0.0476	-0.0248
1102	0.4166	0.3575	-0.0591	0.4166	0.3911	-0.0255	0.0336
1104	0.1292	0.1284	-0.0008	0.1372	0.1181	-0.0191	-0.0183
1105[d]	.	.	.	.	.	.	.
1107[d]	.	.	.	.	.	.	.
1108	0.0842	0.0756	-0.0087	0.1528	0.1321	-0.0207	-0.0120
						Mean	-0.0054
						STD	0.0265
						95% CI	(-0.0476, 0.0368)
						p-value	0.7132

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Minimum Coronary Resistance during the first hour after sumatriptan administration.
[c] Minimum Coronary Resistance - Baseline Coronary Resistance.
[d] Period (.) denotes a missing value.

Mean Change in Coronary Artery Resistance Induced by SS +/- NAP

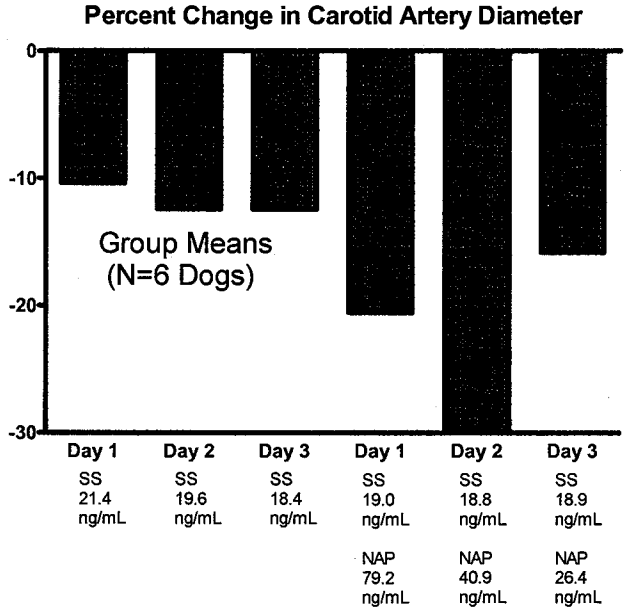


Change in Coronary Artery Resistance Induced by SS +/- NAP



Carotid Arterial Diameter:

The mean reduction in carotid artery diameter induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.0835), Day 2 (p=0.0924), or Day 3 (p=0.5286). However, examination of the individual animal data in the second graph below reveals that 5 of the 6 dogs showed enhancement of the SS-induced reduction in carotid artery diameter by NAP (Day 1 Phase II vs. Day 1 Phase I). This observation, combined with the wide variability of this measure, argues that an effect of NAP on SS-induced vasoconstriction of the carotid artery cannot be ruled out without further investigation.



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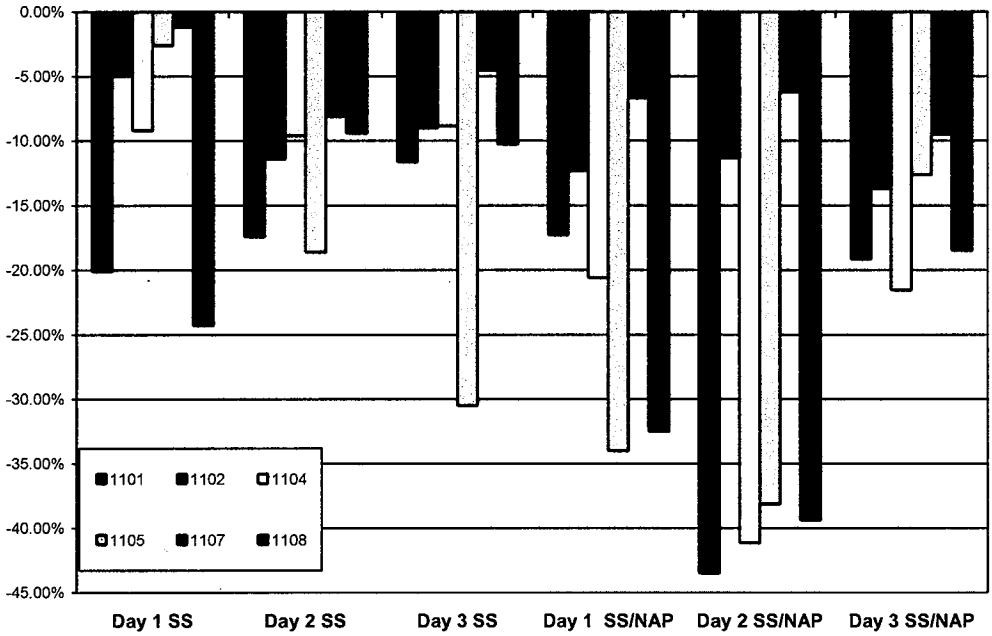
Change in Carotid Artery Diameter Induced by SS +/- NAP in Dog



Maximum Reductions in Carotid Diameter (mm) on Day 1

	Phase I (Vehicle + 80 μg/kg Sumatriptan)			Phase II (20 mg/kg Naproxen + 80 μg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	5.1569	4.1227	-1.0342	4.9081	4.0581	-0.8500	0.1842
1102	5.9945	5.7021	-0.2924	4.2856	3.7571	-0.5285	-0.2361
1104	2.9883	2.7129	-0.2754	3.6837	2.9264	-0.7573	-0.4818
1105	7.0062	6.8267	-0.1795	6.2442	4.1210	-2.1232	-1.9437
1107	6.5184	6.4419	-0.0765	6.6695	6.2246	-0.4449	-0.3684
1108	7.2671	5.5006	-1.7665	10.8691	7.3404	-3.5287	-1.7621
						Mean	-0.7680
						STD	0.8720
						95% CI	(-1.6831, 0.1471)
						p-value	0.0835

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding vehicle (Phase I) or naproxen (Phase II) administration.
[b] Minimum Carotid Diameter during the first hour after sumatriptan administration.
[c] Minimum Carotid Diameter - Baseline Carotid Diameter.

Maximum Reductions in
Carotid Diameter (mm) on Day 2

	Phase I (80 µg/kg Sumatriptan)			Phase II (80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	4.8989	4.0453	-0.8536	5.0104	3.4912	-1.5192	-0.6656
1102	5.8973	5.2248	-0.6725	4.6516	4.1798	-0.4718	0.2007
1104	3.0779	2.7831	-0.2948	4.2516	3.0123	-1.2393	-0.9445
1105	5.4824	4.4615	-1.0209	5.7116	4.1349	-1.5767	-0.5559
1107	6.5983	6.0631	-0.5352	6.7288	6.3346	-0.3942	0.1410
1108	6.8887	6.2406	-0.6481	7.7285	5.5450	-2.1835	-1.5354
						Mean	-0.5599
						STD	0.6604
						95% CI	(-1.2530, 0.1331)
						p-value	0.0924

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Minimum Carotid Diameter during the first hour after sumatriptan administration.
[c] Minimum Carotid Diameter - Baseline Carotid Diameter.

Maximum Reductions in
Carotid Diameter (mm) on Day 3

	Phase I (80 µg/kg Sumatriptan)			Phase II (80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	4.8403	4.2777	-0.5627	5.0929	4.1181	-0.9749	-0.4122
1102	4.0582	3.6919	-0.3663	4.6492	4.0117	-0.6374	-0.2711
1104	3.0864	2.8132	-0.2732	4.0423	3.1709	-0.8714	-0.5982
1105	6.1634	4.2834	-1.8800	5.4037	4.7223	-0.6814	1.1986
1107	6.5951	6.2973	-0.2978	6.7821	6.1394	-0.6427	-0.3449
1108	7.8122	7.0105	-0.8017	8.3397	6.7994	-1.5403	-0.7386
						Mean	-0.1944
						STD	0.7037
						95% CI	(-0.9328, 0.5441)
						p-value	0.5286

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Minimum Carotid Diameter during the first hour after sumatriptan administration.
[c] Minimum Carotid Diameter - Baseline Carotid Diameter.

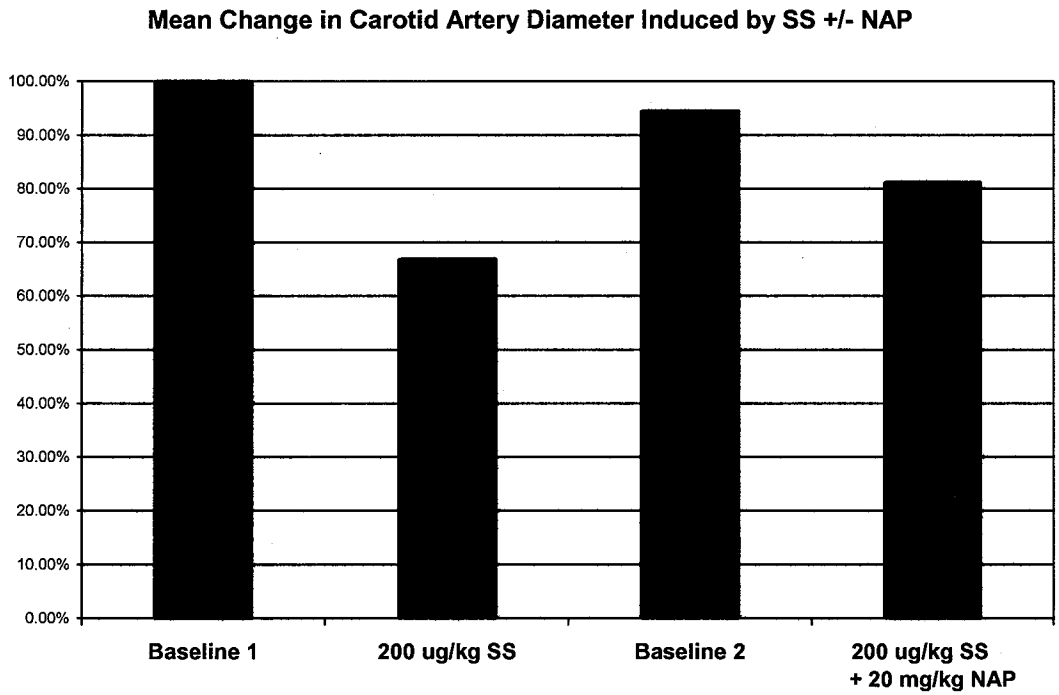
In Phase III, the mean reduction in carotid artery diameter induced by 200 ug/kg IV SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP (p=0.1920).

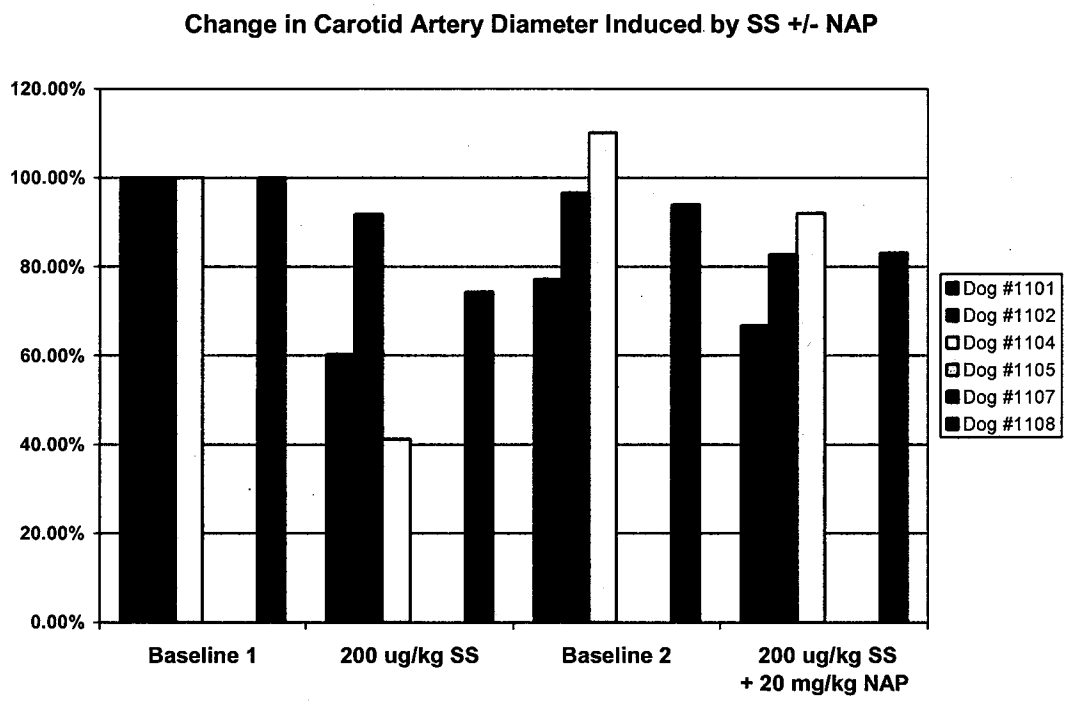
PHASE III

Study QC8W 106
Table 14
Maximum Reductions in
Carotid Diameter (mm)

	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	Baseline [a]	Minimum Diameter [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	4.4147	2.6639	-1.7508	3.4075	2.9477	-0.4598	1.2911
1102	6.5494	6.0121	-0.5373	6.3319	5.4227	-0.9092	-0.3719
1104	12.6987	5.2393	-7.4594	13.9821	11.6805	-2.3016	5.1578
1105[d]	.	.	.	.	.	.	.
1107	6.7759	6.4737	-0.3022	6.4804	6.2934	-0.1870	0.1152
1108	9.5325	7.0876	-2.4449	8.9538	7.9274	-1.0264	1.4185
						Mean	1.5221
						STD	2.1708
						95% CI	(-1.1732, 4.2175)
						p-value	0.1920

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Minimum Carotid Diameter during the first hour after Sumatriptan Succinate administration.
[c] Minimum Carotid Diameter - Baseline Carotid Diameter.
[d] Period (.) denotes a missing value.

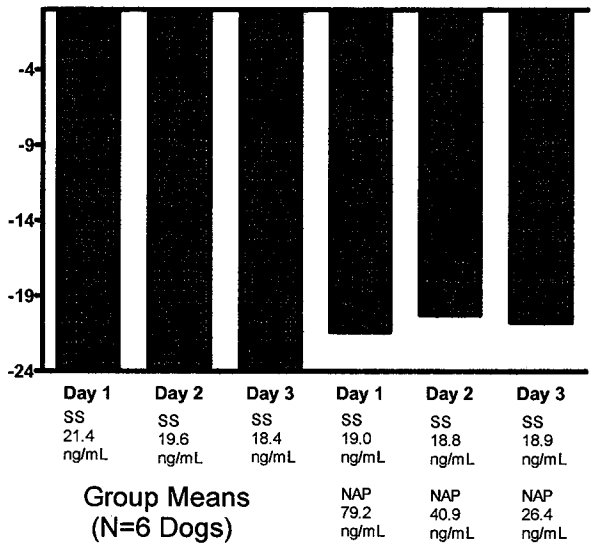




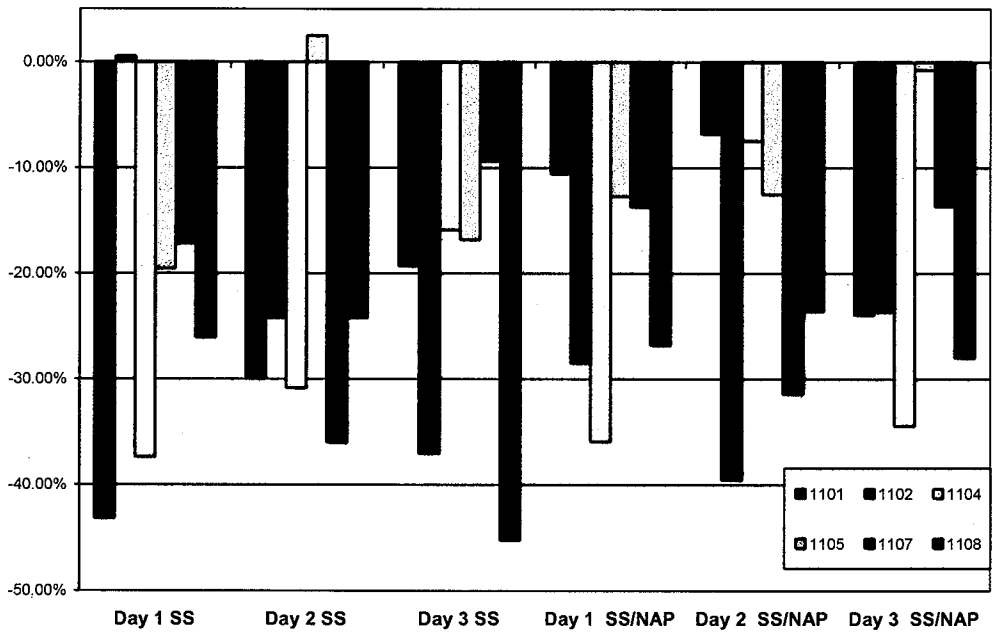
Carotid Artery Flow:

The mean reduction in carotid artery flow induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.5573), Day 2 (p=0.7522), or Day 3 (p=0.5808).

Percent Change in Carotid Artery Flow



Change in Carotid Artery Flow Induced by SS +/- NAP in Dog

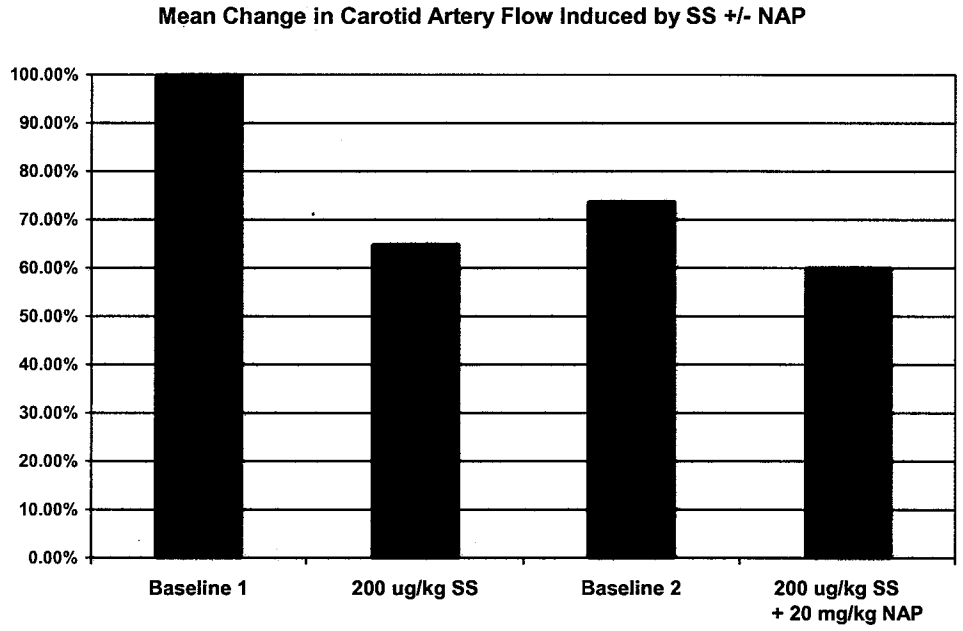


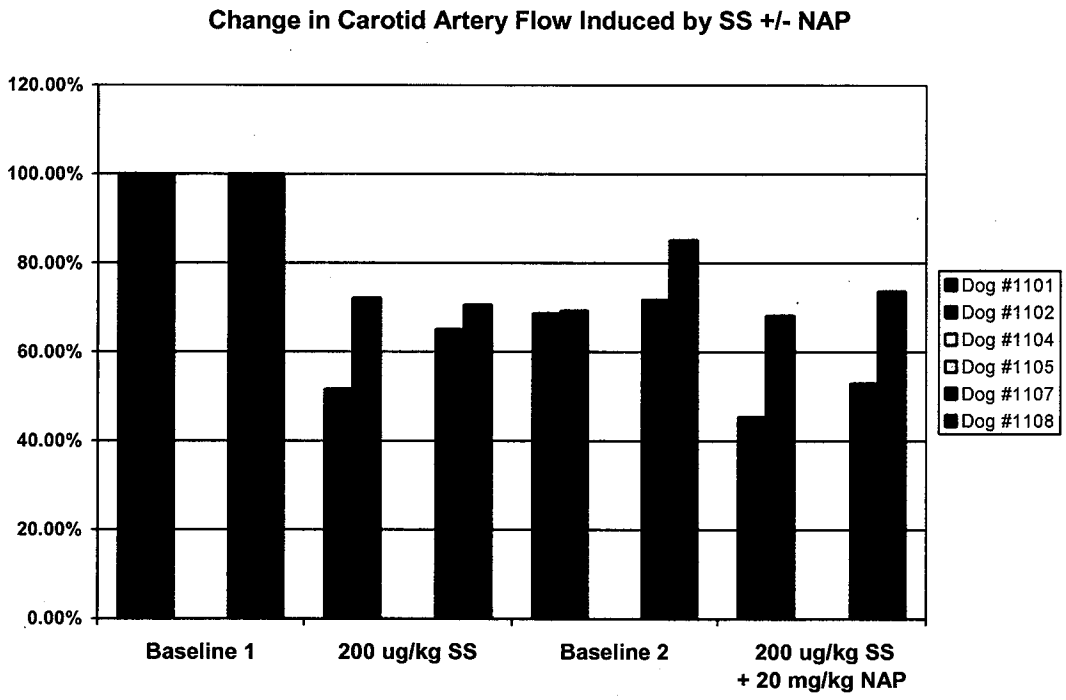
In Phase III, the mean reduction in carotid artery flow induced by 200 ug/kg IV SS was statistically significantly reduced with coadministration of 20 mg/kg IV NAP (p=0.00 95). However, as shown in the graphs below, this difference was attributable to failure to return to baseline after the first dose of SS.

Study QCBW 106
Table 16
Maximum Reductions in
Carotid Flow (mL/min)

	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Minimum Flow [b]	Change From Baseline [c]	Baseline [a]	Minimum Flow [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	55.348	28.560	-26.788	37.970	25.140	-12.830	13.958
1102	38.806	27.980	-10.826	26.872	26.460	-0.412	10.414
1104[d]	.	.	.	.	.	.	.
1105[d]	.	.	.	.	.	.	.
1107	44.838	29.180	-15.658	32.168	23.760	-8.408	7.250
1108	39.466	27.880	-11.586	33.618	29.067	-4.551	7.035
						Mean	9.664
						STD	3.253
						95% CI	(4.488, 14.840)
						p-value	0.0095

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Minimum Carotid Flow during the first hour after sumatriptan administration.
[c] Minimum Carotid Flow - Baseline Carotid Flow.
[d] Period (.) denotes a missing value.





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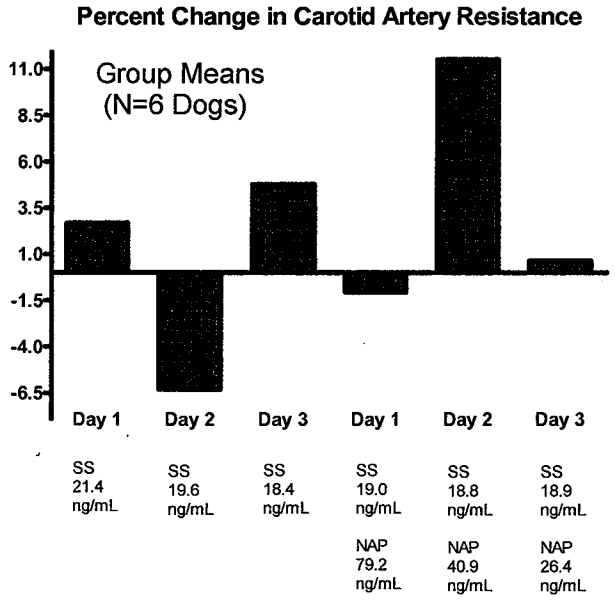
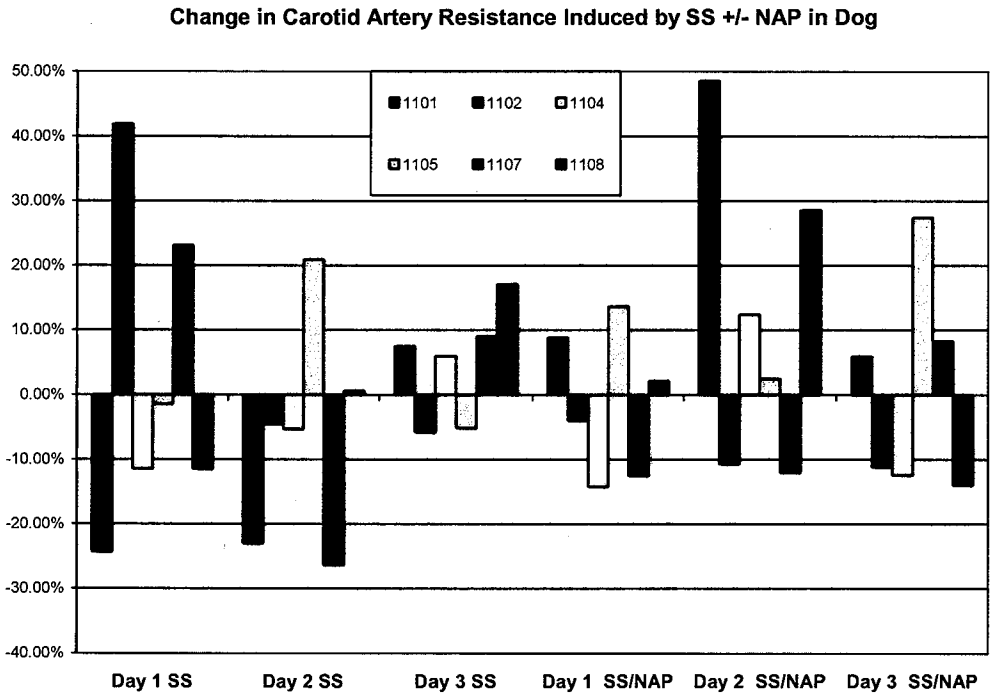
Carotid Artery Resistance:

The mean increase in carotid artery resistance induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.9218), Day 2 (p=0.2004), or Day 3 (p=0.6694).





In Phase III, the mean increase in carotid artery resistance induced by 200 ug/kg IV SS was statistically significantly different with coadministration of 20 mg/kg IV NAP (p=0.0225).

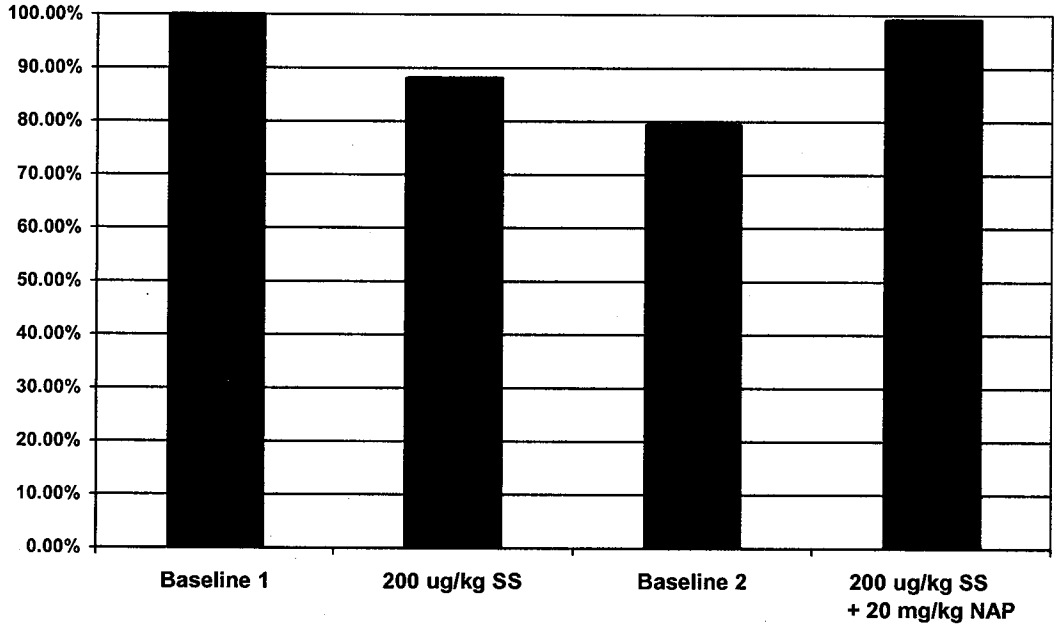
PHASE III

Study QCBW 106
Table 15
Maximum Increases in
Carotid Resistance (mL/mmHg.min)

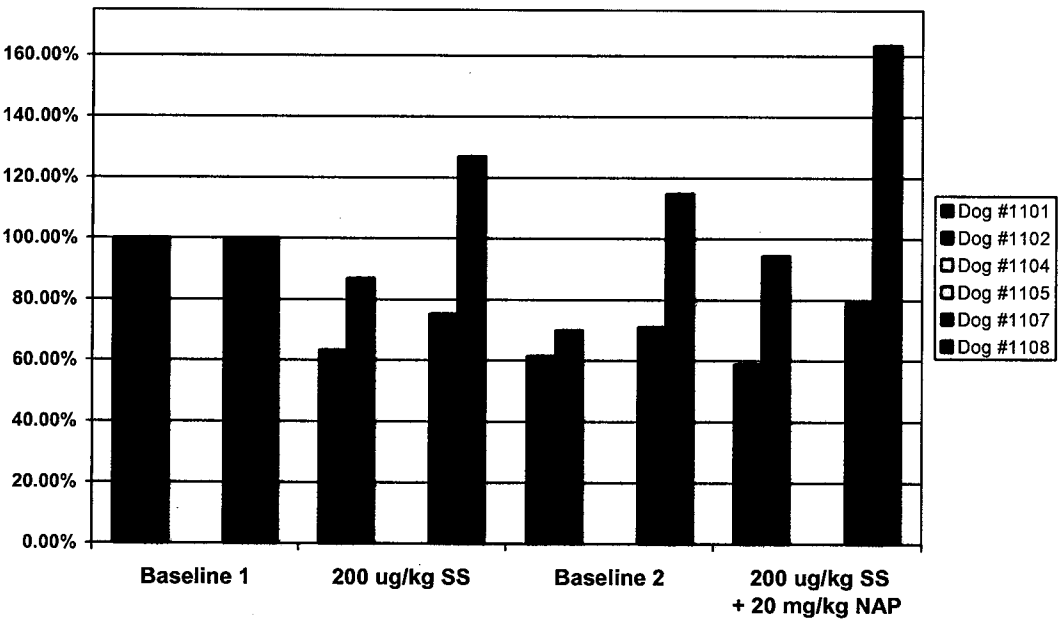
	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Maximum Resistance [b]	Change From Baseline [c]	Baseline [a]	Maximum Resistance [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	0.5449	0.3449	-0.2000	0.3357	0.3217	-0.0141	0.1859
1102	0.2935	0.2552	-0.0383	0.2055	0.2774	0.0719	0.1101
1104[d]	.	.	.	.	.	.	.
1105[d]	.	.	.	.	.	.	.
1107	0.4116	0.3099	-0.1016	0.2924	0.3264	0.0340	0.1356
1108	0.2377	0.3016	0.0639	0.2727	0.3887	0.1161	0.0521
						Mean	0.1210
						STD	0.0557
						95% CI	(0.0324, 0.2095)
						p-value	0.0225

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Maximum Carotid Resistance during the first hour after sumatriptan administration.
[c] Maximum Carotid Resistance - Baseline Carotid Resistance.
[d] Period (.) denotes a missing value.

Mean Change in Carotid Artery Resistance Induced by SS +/- NAP

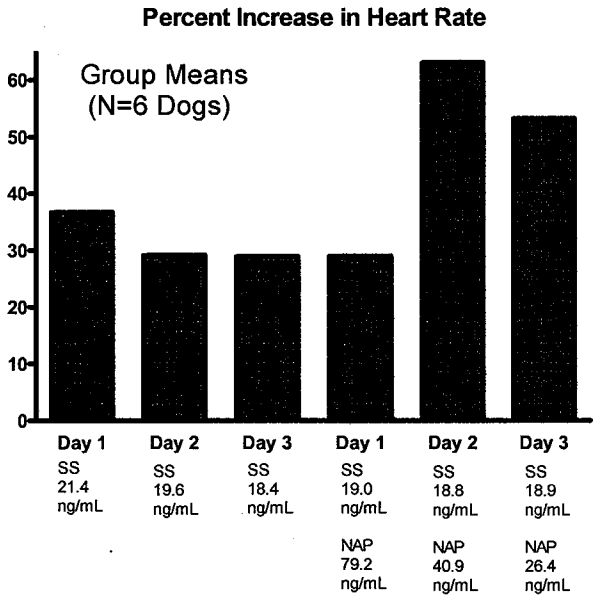


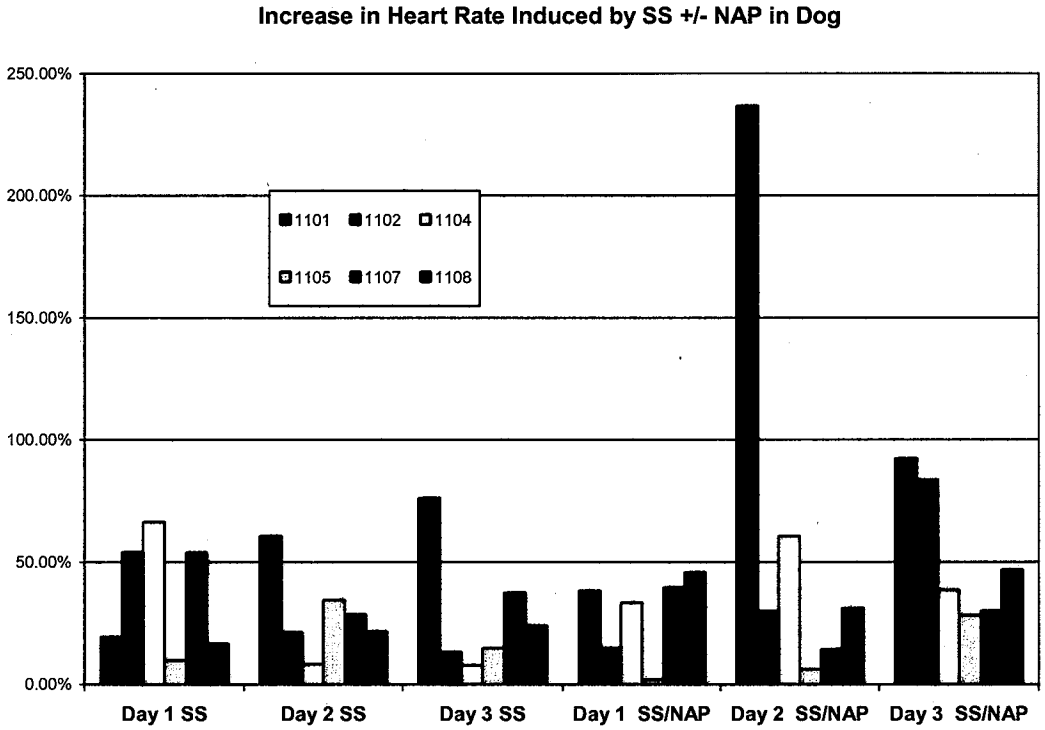
Change in Carotid Artery Resistance Induced by SS +/- NAP



Heart Rate:

The mean increase in heart rate induced by SS was not statistically significantly affected by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.3833), Day 2 (p=0.3471), or Day 3 (p=0.0903).





In Phase III, the mean increase in heart rate induced by 200 ug/kg IV SS was not statistically significantly different with coadministration of 20 mg/kg IV NAP (p=0.7404).

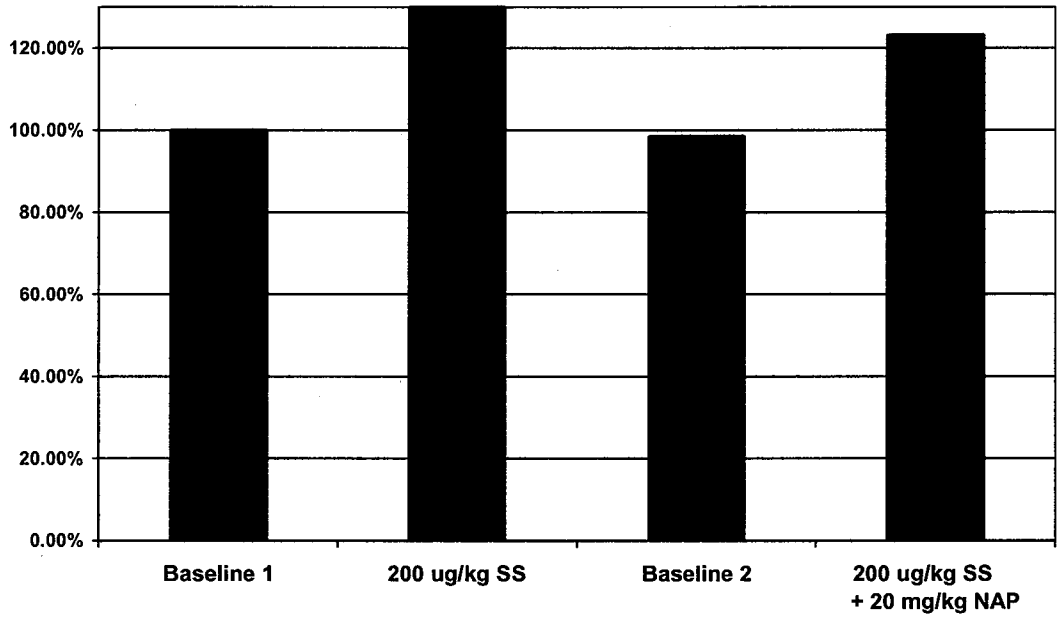
PHASE III

Study QCBW 106
Table 17
Maximum Increases in
Heart Rate (bpm)

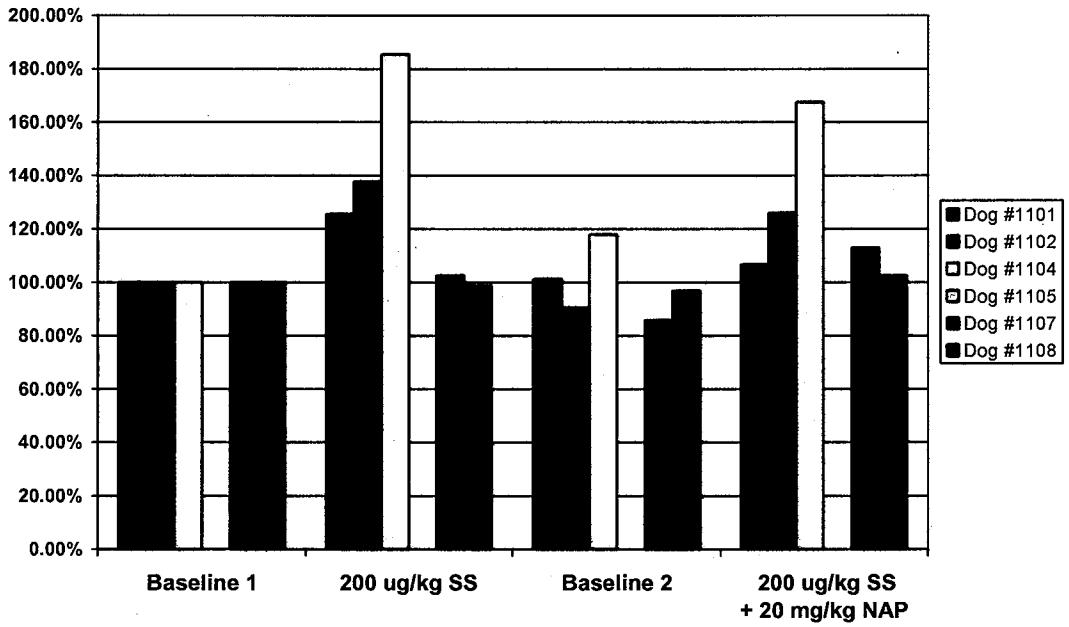
	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Maximum Rate [b]	Change From Baseline [c]	Baseline [a]	Maximum Rate [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	126.6	159.0	32.4	128.2	135.0	6.8	-25.6
1102	101.6	140.0	38.4	92.0	128.0	36.0	-2.4
1104	83.6	155.0	71.4	98.6	140.0	41.4	-30.0
1105[d]	.	.	.	.	.	.	.
1107	125.8	129.0	3.2	108.0	142.0	34.0	30.8
1108	105.4	104.0	-1.4	102.0	108.0	6.0	7.4
						Mean	-4.0
						STD	24.9
						95% CI	(-34.9, 27.0)
						p-value	0.7404

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Maximum Heart Rate during the first hour after sumatriptan administration.
[c] Maximum Heart Rate - Baseline Heart Rate.
[d] Period (.) denotes a missing value.

Mean Change in Heart Rate Induced by SS +/- NAP

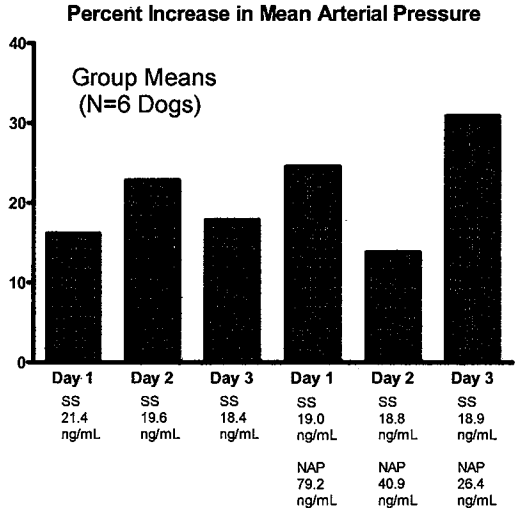


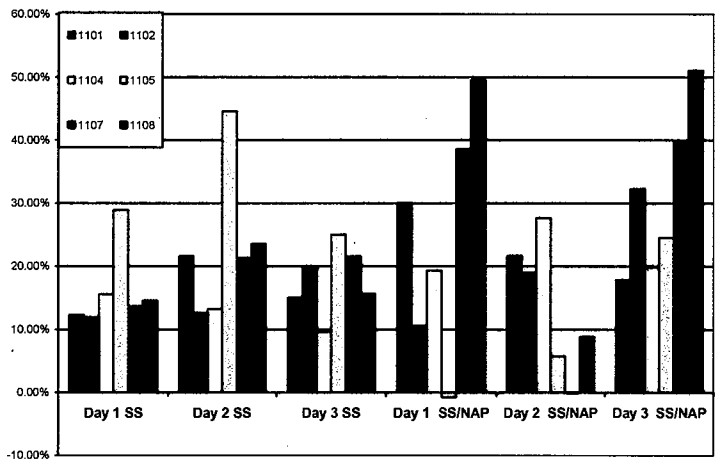
Change in Heart Rate Induced by SS +/- NAP



Mean Arterial Pressure:

The mean increase in mean arterial pressure induced by SS was not statistically significantly enhanced by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.4931), Day 2 (p=0.3061), or Day 3 (p=0.0648). However, examination of the individual animal data in the second graph below reveals that three of the six dogs showed dramatic (~3-fold) increases in the SS-induced MAP increase with NAP (Day 1 Phase II vs. Day 1 Phase I). Two other dogs showed little or no increase, and one anomaly failed to show the expected SS-induced increase in MAP at all in Phase II. The two dogs showing the greatest increase in SS-induced MAP increase with NAP (~3-fold), showed similar large increases on Day 3, when NAP levels had decreased 3-fold, and no increases on Day 2. These data suggest that the increases in MAP were due to technical problems, or unknown factors, rather than to the presence of SS/NAP.





Maximum Increases in
MAP (mmHg) on Day 1

	Phase I (Vehicle + 80 µg/kg Sumatriptan)			Phase II (20 mg/kg Naproxen + 80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	105.66	118.68	13.02	103.83	135.01	31.18	18.16
1102	115.55	129.29	13.74	113.19	125.18	11.99	-1.75
1104	117.23	135.54	18.31	108.25	129.18	20.93	2.62
1105	108.86	140.36	31.50	136.53	135.46	-1.07	-32.56
1107	136.14	154.77	18.63	112.12	155.38	43.26	24.63
1108	134.51	154.09	19.58	101.19	151.38	50.19	30.61
						Mean	6.95
						STD	23.04
						95% CI	(-17.23, 31.13)
						p-value	0.4931

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding vehicle (Phase I) or naproxen (Phase II) administration.
[b] Maximum MAP during the first hour after sumatriptan administration.
[c] Maximum MAP - Baseline MAP.

Maximum Increases in
MAP (mmHg) on Day 2

	Phase I (80 µg/kg Sumatriptan)			Phase II (80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	104.14	126.70	22.56	99.88	121.53	21.65	-0.92
1102	115.61	130.21	14.60	113.57	135.13	21.56	6.96
1104	115.65	130.99	15.34	108.69	138.75	30.06	14.72
1105	89.69	129.67	39.98	124.93	132.12	7.19	-32.79
1107	109.79	133.24	23.45	141.82	141.68	-0.14	-23.59
1108	114.16	141.07	26.91	123.25	134.17	10.92	-15.99
						Mean	-8.60
						STD	18.49
						95% CI	(-28.01, 10.80)
						p-value	0.3061

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Maximum MAP during the first hour after sumatriptan administration.
[c] Maximum MAP - Baseline MAP.

Maximum Increases in
MAP (mmHg) on Day 3

	Phase I (80 µg/kg Sumatriptan)			Phase II (80 µg/kg Sumatriptan)			
Dog ID	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Change Phase II - Phase I
1101	106.23	122.20	15.97	101.37	119.47	18.10	2.13
1102	106.51	127.70	21.19	111.42	147.39	35.97	14.78
1104	136.20	149.33	13.13	106.81	128.02	21.21	8.08
1105	108.11	135.23	27.12	100.62	125.31	24.69	-2.43
1107	138.80	168.78	29.98	116.29	162.53	46.24	16.25
1108	116.92	135.21	18.29	98.85	149.35	50.50	32.21
						Mean	11.84
						STD	12.29
						95% CI	(-1.06, 24.73)
						p-value	0.0648

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan administration.
[b] Maximum MAP during the first hour after sumatriptan administration.
[c] Maximum MAP - Baseline MAP.

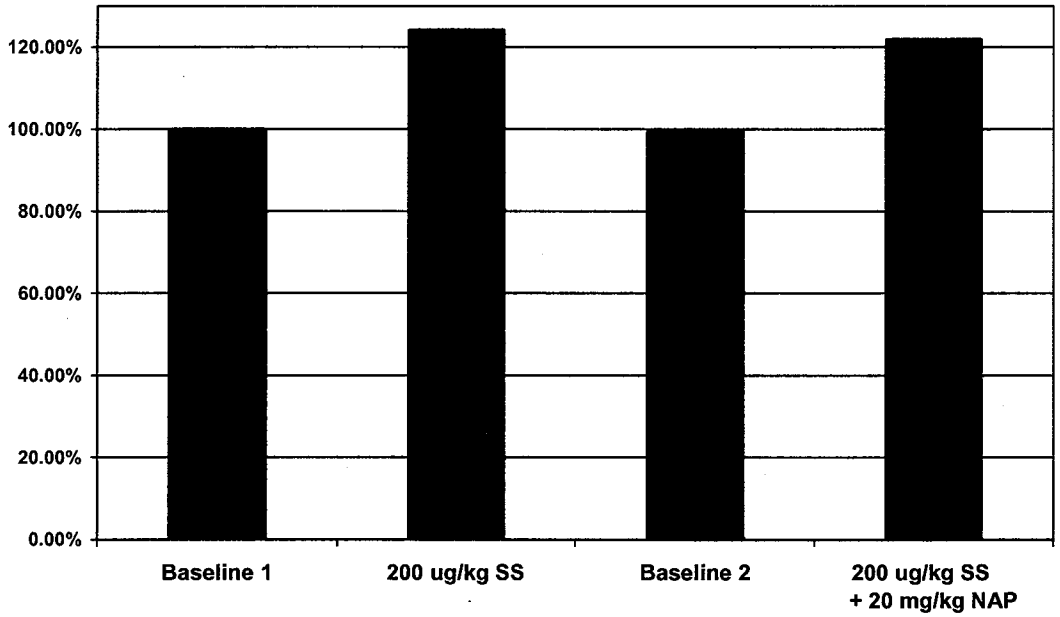
In Phase III, the mean increase in heart rate induced by 200 ug/kg IV SS was not statistically significantly different with coadministration of 20 mg/kg IV NAP (p=0.7551).

Study QCBW 106
Table 18
Maximum Increases in
MAP (mmHg)

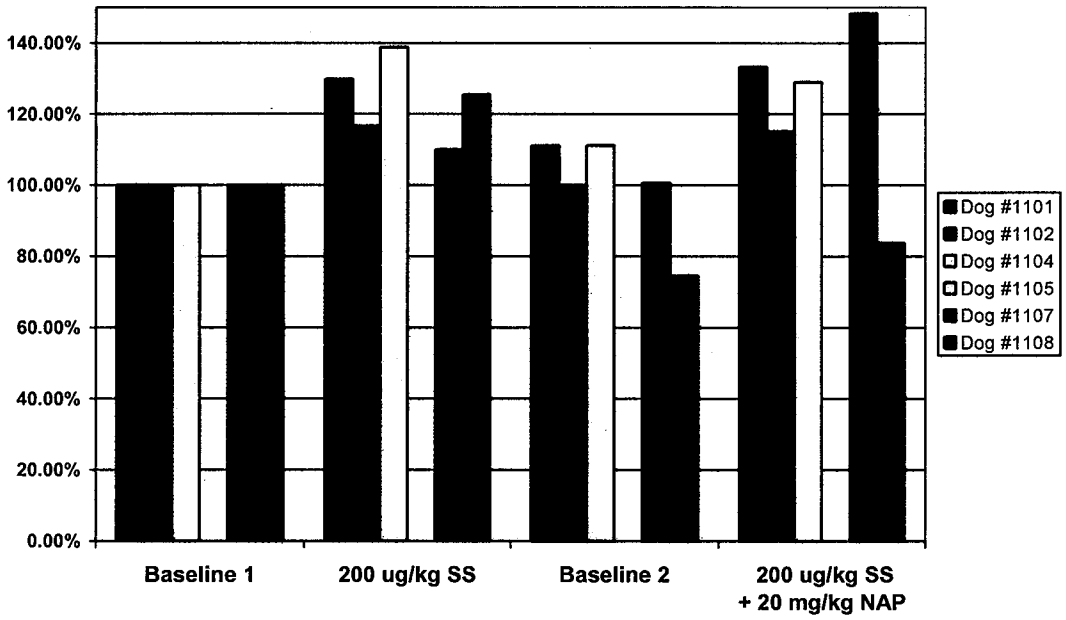
	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	Baseline [a]	Maximum MAP [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101	101.746	132.030	30.284	112.982	135.480	22.498	-7.786
1102	131.562	153.550	21.988	131.566	151.470	19.904	-2.084
1104	120.556	167.360	46.804	134.008	155.460	21.452	-25.352
1105[d]	.	.	.	.	.	.	.
1107	109.878	120.850	10.972	110.538	162.910	52.372	41.400
1108	165.926	208.190	42.264	123.566	139.000	15.434	-26.830
						Mean	-4.130
						STD	27.641
						95% CI	(-38.452, 30.191)
						p-value	0.7551

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Maximum MAP during the first hour after sumatriptan administration.
[c] Maximum MAP - Baseline MAP.
[d] Period (.) denotes a missing value.

Mean Change in Mean Arterial Pressure Induced by SS +/- NAP

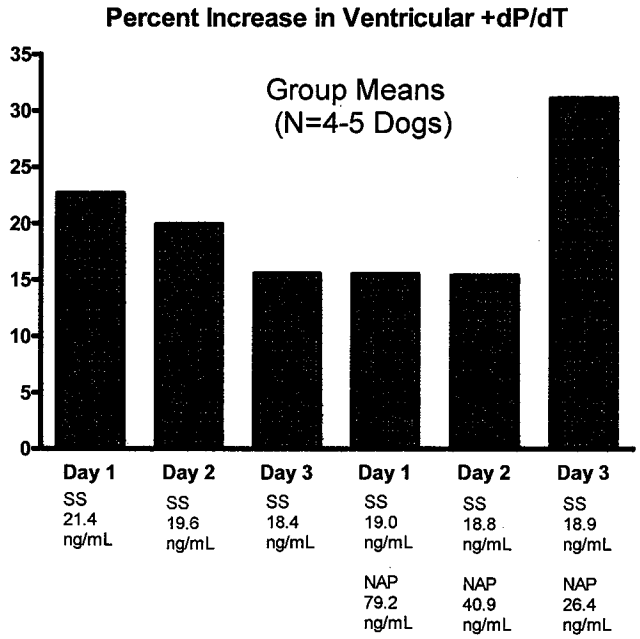


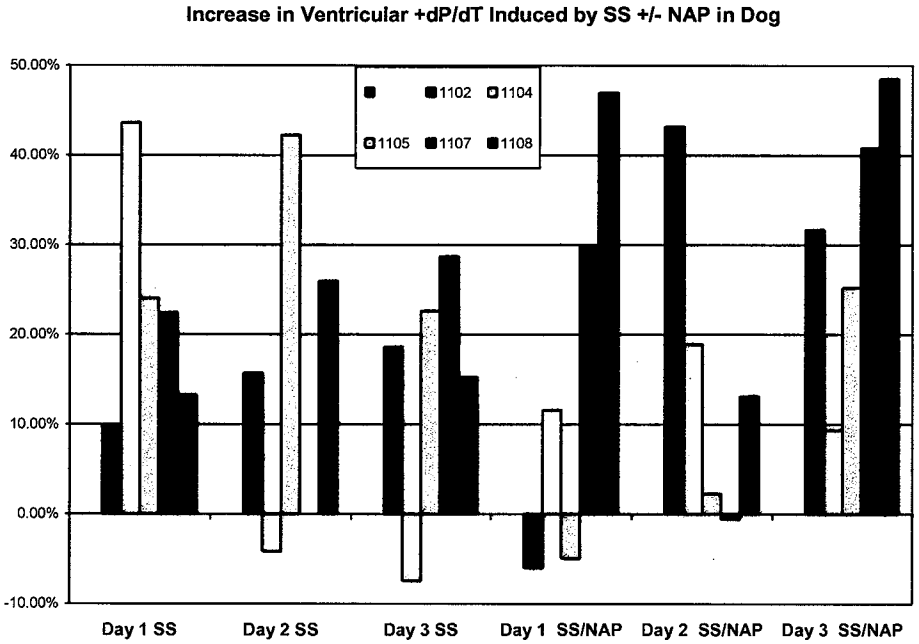
Change in Mean Arterial Pressure Induced by SS +/- NAP



Left Ventricular Pressure (+dP/dT):

The mean increase in +dP/dT induced by SS was not statistically significantly affected by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.3905), Day 2 (p=0.7230), or Day 3 (p=0.0502).





In Phase III, the mean increase in +dP/dT induced by 200 ug/kg IV SS was not statistically significantly different with coadministration of 20 mg/kg IV NAP (p=0.2093).

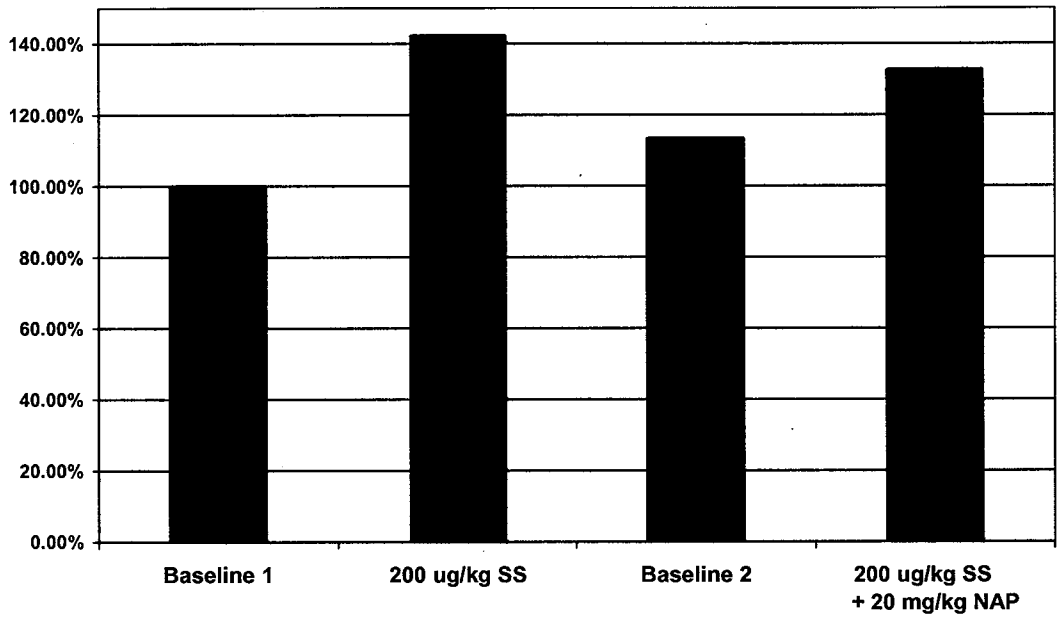
PHASE III

Study QCBW 106
Table 19
Maximum Increases in
+dP/dT (mmHg/sec)

	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			
Dog ID	Baseline [a]	Maximum +dP/dT [b]	Change From Baseline [c]	Baseline [a]	Maximum +dP/dT [b]	Change From Baseline [c]	(Nap+Suma) - Suma
1101[d]	.	.	.	.	.	.	.
1102	8313.0	10916.0	2603.0	9448.2	9862.0	413.8	-2189.2
1104	2464.6	4507.0	2042.4	2767.2	3936.0	1168.8	-873.6
1105[d]	.	.	.	.	.	.	.
1107[d]	.	.	.	.	.	.	.
1108	2440.2	2745.0	304.8	2788.2	2919.0	130.8	-174.0
						Mean	-1078.9
						STD	1023.2
						95% CI	(-3620.6, 1462.8)
						p-value	0.2093

[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceeding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).
[b] Maximum +dP/dT during the first hour after sumatriptan administration.
[c] Maximum +dP/dT - Baseline +dP/dT.
[d] Period (.) denotes a missing value.

Mean Change in +dP/dT Induced by SS +/- NAP



Change in +dP/dT Induced by SS +/- NAP