

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-198

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-198
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: June 29, 2007, Electronic Submission
PRODUCT: SANCUSO® (Granisetron Transdermal System)
INTENDED CLINICAL POPULATION: Chemotherapy-induced nausea and vomiting (CINV)
SPONSOR: Strakan International Ltd., Galashiels TD1 1QH, UK.
DOCUMENTS REVIEWED: Electronic submission of the NDA
REVIEW DIVISION: Division of Gastroenterology Products (HFD-180)
PHARM/TOX REVIEWER: Sushanta Chakder, Ph.D.
ACTING PHARM/TOX SUPERVISOR: Sushanta Chakder, Ph.D.
DIVISION DIRECTOR: Donna Griebel, M.D.
PROJECT MANAGER: Tom Moreno, M.S.

Date of review submission to Division File System (DFS): June 11, 2008

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EXECUTIVE SUMMARY

I. Recommendations

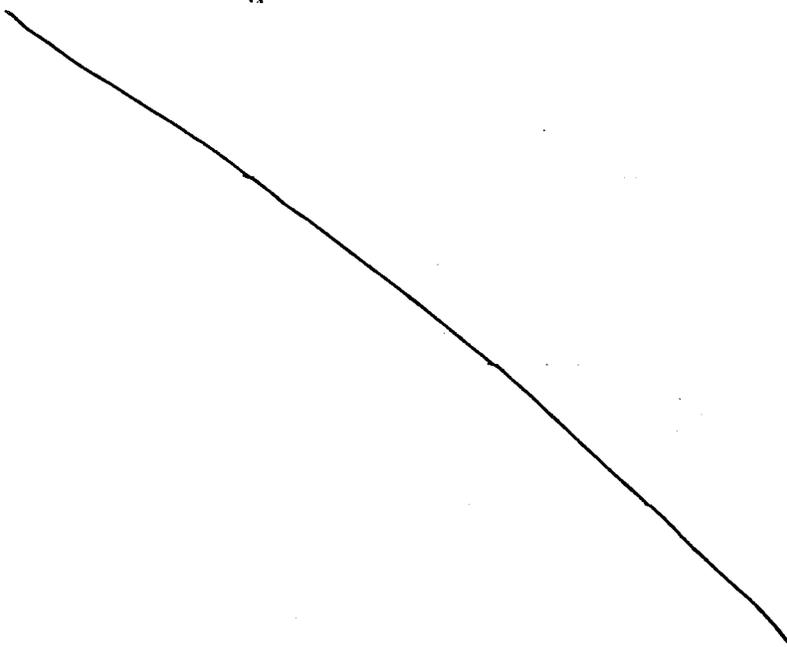
Recommendation on approvability: From a nonclinical standpoint, approval of the NDA application is recommended.

A. Recommendation for nonclinical studies: None.

B. Recommendations on labeling: Following changes in the sponsor's proposed labeling is recommended:

8.1 Pregnancy

Proposed version:



b(4)

Evaluation:

Recommended version: The sponsor's proposed labeling is based on the approved labeling of Kytril. However some changes in the language are recommended. In addition, the comparison of doses between humans and animals should be based on daily dose of 3.1 mg/day for an average body weight of 50 kg.

Recommended version:

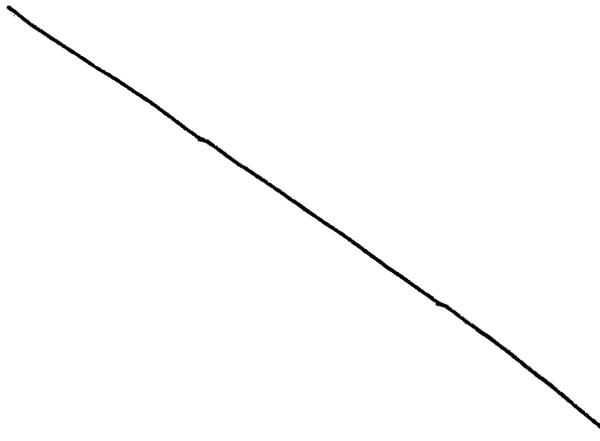
PREGNANCY CATEGORY B

Reproduction studies with granisetron hydrochloride have been performed in pregnant rats at intravenous doses up to 9 mg/kg/day (54 mg/m²/day, about 24 times the recommended human

dose delivered by the SANCUSO[®] patch, based on body surface area) and oral doses up to 125 mg/kg/day (750 mg/kg/day, about 326 times the recommended human dose with SANCUSO[®] based on body surface area). Reproduction studies have been performed in pregnant rabbits at intravenous doses up to 3 mg/kg/day (36 mg/m²/day, about 16 times the human dose with SANCUSO[®] based on body surface area) and at oral doses up to 32 mg/kg/day (384 mg/m²/day, about 167 times the human dose with SANCUSO[®] based on body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to granisetron. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, SANCUSO[®] should be used during pregnancy only if clearly needed.

13 NONCLINICAL TOXICOLOGY

Proposed version:



b(4)

Page(s) Withheld

 Trade Secret / Confidential (b4)

 ✓ Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

13.3 Phototoxicity¹¹⁰

Granisetron was not phototoxic when tested *in vitro* in a mouse fibroblast cell line. When tested for potential photogenotoxicity *in vitro* in a Chinese hamster ovary (CHO) cell line, at 200 and 300 µg/ml, granisetron increased the percentage of cells with chromosome damage following photoirradiation. When tested *in vivo* in guinea-pigs, SANCUSO[®] patches did not show any potential for photoirritation or photosensitivity.¹¹¹

Evaluation: The comparison of doses between humans and animals should be based on 3.1 mg/day _____ and on the basis of an average body weight of 50 kg. _____

_____ Moreover, there are human data on the safety of SUNCUSO.

b(4)

Recommended version:

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

In a 24-month carcinogenicity study, rats were treated orally with granisetron 1, 5 or 50 mg/kg/day (6, 30 or 300 mg/m²/day). The 50 mg/kg/day dose was reduced to 25 mg/kg/day (150 mg/m²/day) during week 59 due to toxicity. For a 50 kg person of average height (1.46 m² body surface area), these doses represent about 2.6, 13 and 65 times the recommended clinical dose (3.1 mg/day, 2.3 mg/m²/day, delivered by SANCUSO[®] patch, on a body surface area basis). There was a statistically significant increase in the incidence of hepatocellular carcinomas and adenomas in males treated with 5 mg/kg/day (30 mg/m²/day, about 13 times the recommended human dose with SANCUSO[®], on a body surface area basis) and above, and in females treated with 25 mg/kg/day (150 mg/m²/day, about 65 times the recommended human dose with SANCUSO[®], on a body surface area basis). No increase in liver tumors was observed at a dose of 1 mg/kg/day (6 mg/m²/day, about 2.6 times the recommended human dose with SANCUSO[®], on a body surface area basis) in males and 5 mg/kg/day (30 mg/m²/day, about 13 times the recommended human dose with SANCUSO[®], on a body surface area basis) in females. In a 12-month oral toxicity study, treatment with granisetron 100 mg/kg/day (600 mg/m²/day, about 261 times the recommended human dose with SANCUSO[®], on a body surface area basis) produced hepatocellular adenomas in male and female rats while no such tumors were found in the control rats. A 24-month mouse carcinogenicity study of granisetron did not show a statistically significant increase in tumor incidence, but the study was not conclusive.

Because of the tumor findings in rat studies, SANCUSO[®] should be prescribed only at the dose and for the indication recommended (see INDICATIONS AND USAGE, and DOSAGE AND ADMINISTRATION).

Granisetron was not mutagenic in an *in vitro* Ames test and mouse lymphoma cell forward mutation assay, and *in vivo* mouse micronucleus test and *in vitro* and *ex vivo* rat hepatocyte UDS assays. It, however, produced a significant increase in UDS in HeLa cells *in vitro* and a significant increased incidence of cells with polyploidy in an *in vitro* human lymphocyte chromosomal aberration test.

Granisetron at subcutaneous doses up to 6 mg/kg/day (36 mg/m²/day, about 16 times the recommended human dose of SANCUSO[®], on a body surface area basis), and oral doses up to 100 mg/kg/day (600 mg/m²/day, about 261 times the recommended human dose of SANCUSO[®], on a body surface area basis) was found to have no effect on fertility and reproductive performance of male and female rats.

13.2 Phototoxicity

Granisetron was not phototoxic when tested *in vitro* in a mouse fibroblast cell line. When tested for potential photogenotoxicity *in vitro* in a Chinese hamster ovary (CHO) cell line, at 200 and 300 µg/ml, granisetron increased the percentage of cells with chromosomal aberration following photoirradiation. When tested *in vivo* in guinea-pigs, SANCUSO[®] patches did not show any potential for photoirritation or photosensitivity.

II. Summary of nonclinical findings

Strakan International Ltd. submitted the NDA according to 505(b)(2) of the Federal Food, Drug and Cosmetic Act, and as such is relying on the Agency's previous findings of safety and effectiveness of the Reference Listed Drugs. The Reference Listed Drugs for this product are the three dosage forms of Kytril (granisetron HCl) approved under NDA 20-239 (Injectable), NDA 20-305 (Tablets) and NDA 21-238 (Oral Solution). Several new toxicology studies have been conducted to address the safety of the new route of administration. In addition, published pharmacology, PK and toxicology studies with granisetron are provided in the NDA submission.

A. Pharmacology:

Granisetron is a selective 5-hydroxytryptamine-3 (5-HT₃) receptor antagonist with little or no affinity for other serotonin receptors, including 5-HT₁, 5-HT_{1A}, 5-HT_{1B/C}, 5-HT₂, alpha₁, alpha₂ or beta-adrenoreceptors, dopamine D₂, histamine-H₁, benzodiazepine, picrotoxin or opioid receptors. Serotonin receptors of the 5-HT₃ type are located peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. Stimulation of 5-HT₃ receptors in these areas induces vomiting. Animal studies indicate that granisetron, by binding to 5-HT₃ receptors, blocks serotonin stimulation and subsequent vomiting after emetogenic stimuli such as cisplatin.

B. ADME:

Following two consecutive applications for 7 days of the granisetron patch (6%) to rats, the maximum plasma concentration was reached in 24 hours. The AUC was higher than that following i.v. administration of a 9 mg/kg/day dose; however, the C_{max} was lower than that following the i.v. administration. The exposure levels in female rats were higher than that in males. Similar to rats, in dogs, granisetron patch group had higher mean daily AUC compared to that following a 3 mg/kg/day i.v. dose. The C_{max} values for the granisetron patch group were similar to that of the i.v. dose group. No apparent sex differences in the exposure levels were observed between male and female dogs. Following application of the patches, the maximum plasma concentration was reached in about 19 hours in male dogs and 48 hours in female dogs.

B. Toxicology:

Two-week bridging toxicology studies comparing granisetron patches with i.v. and orally administered granisetron HCl were conducted in rats and dogs. Application of granisetron patches produced increased severity of edema at the application sites compared to placebo patches. In rats, lymphocytic infiltration in the heart was observed in groups receiving the patch, and oral or i.v. granisetron, and interstitial nephritis in the kidneys was observed in groups receiving the patch and the i.v. dose. In dogs, fatty infiltration in the liver and increased ALT levels were observed in groups receiving all three dosage forms. Thus, sustained exposure of granisetron to rats and dogs for 2 weeks through application of granisetron patch or continuous i.v. administration of granisetron hydrochloride showed similar toxicity profiles. No new target organs of toxicity were identified following application of the patch in rats and dogs.

C. Nonclinical safety issues relevant to clinical use: Granisetron was positive in the *in vitro* chromosome aberration assay in Chinese hamster ovary cells in the presence of UV irradiation. It was negative in the absence of UV irradiation. Thus, patients should avoid exposure to sunlight or any artificial sunlight while wearing and for at least 10 days after removing the patch.

APPEARS THIS WAY ON ORIGINAL

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-198

Review number: 01

Sequence number/date/type of submission: 000/June 29, 2007/Original (Electronic submission).

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Strakan International Ltd., Galashiels TD1 1QH, UK.

Manufacturer for drug substance: _____

b(4)

Reviewer name: Sushanta Chakder, Ph.D.

Division name: Division of Gastroenterology Products

HFD #: 180

Review completion date: June 11, 2008

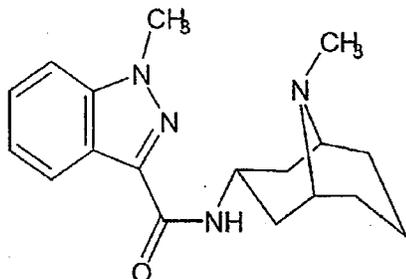
Drug:

Trade name: SANCUSO® Transdermal System

Generic name: Granisetron

Code name: N/A

Chemical name: 1-methyl-N-[(1R, 3r, 5S)-9-methyl-9-azabicyclo[3, 3, 1]non-3-yl]-1H-indazole-3-carboxamide.

Chemical Structure:

CAS registry number: 1098889-09-0

Molecular weight: 212.4 Da

Relevant INDs/NDAs/DMFs:

IND 70,582, Granisetron Patch, Strakan International Ltd.

NDA 20-239, Kytril (granisetron HCl) Injection, Roche

NDA 20-305, Kytril (Granisetron HCl) Tablets, Roche

NDA 21-238, Kytril (Granisetron HCl) Oral Solution, Roche

Drug class: 5-Hydroxytryptamine (5-HT₃) receptor antagonist.

Indication: For the prevention of chemotherapy-induced nausea and vomiting.

Clinical formulation: Granisetron transdermal delivery system (TDS) or patch consists of an active matrix spread on to a printed _____ backing. The active matrix has an area weight of 110 g/m² (527 mg/patch). The active matrix consists of a _____ adhesive containing _____ granisetron base. The total amount of granisetron per patch is 34.3 mg.

b(4)

Route of administration: Transdermal

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

Studies reviewed within this submission: Strakan International Ltd. submitted the NDA according to 505(b)(2) of the Federal Food, Drug and Cosmetic Act, and as such is relying on the Agency's previous findings of safety and effectiveness of the Reference Listed Drugs. Several new toxicology studies have been conducted to address the safety of the new route of administration. In addition, published pharmacology, PK and toxicology studies with granisetron

are provided in the NDA submission. Two-week toxicity studies with granisetron patch in rats and dogs (and relevant studies), and skin sensitization study in guinea pigs were reviewed earlier under IND 70,582, and the reviews are incorporated. The *in vitro* phototoxicity and photogenotoxicity studies, *in vivo* photosensitization study and published pharmacology and toxicology studies were reviewed.

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Granisetron is a selective 5-hydroxytryptamine-3 (5-HT₃) receptor antagonist with little or no affinity for other serotonin receptors, including 5-HT₁, 5-HT_{1A}, 5-HT_{1B/C}, 5-HT₂, alpha₁, alpha₂ or beta-adrenoreceptors, dopamine D₂, histamine-H₁, benzodiazepine, picrotoxin or opioid receptors. Serotonin receptors of the 5-HT₃ type are located peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. Stimulation of 5-HT₃ receptors in these areas induces vomiting. Animal studies indicate that granisetron, by binding to 5-HT₃ receptors, blocks serotonin stimulation and subsequent vomiting after emetogenic stimuli such as cisplatin.

2.6.2.2 Primary pharmacodynamics

The following published pharmacology study was submitted.

General Pharmacology Study of Granisetron Hydrochloride. Spa Z, Rapalli S, Caremi I, Neil C, Kelvin AS, Sanger G, Nishioka Y, Ishii R. The Clinical Report 1990, 24(13): 269.

The general pharmacological study of granisetron was conducted in mice, rats, guinea pigs, dogs and rabbits. The highest dose tested was 6 mg/kg, and the route of administration was via intravenous in all tests except for the exploratory locomotor activity test and the passive avoidance test which used subcutaneous route of administration.

Observation of general symptoms yielded no notable findings. Reduced frequency of phenylquinone-induced writhing was noted following treatment with granisetron at doses of 3 mg/kg and above. No other effects, such as increase in depth of anesthesia or anti-convulsive effect were observed. Furthermore, body temperature and brain wave effects were unaffected. High concentrations of granisetron suppressed contraction of the diaphragm. Application of 0.1% solution to the eyes reduced the corneal reflex. High concentrations of granisetron induced nonspecific suppression of contraction of isolated ileum under stimulation with acetylcholine, histamine, 5-HT or barium chloride. It inhibited the contractions of isolated trachea by histamine, and isolated uterus by 5-HT.

Granisetron, at doses of 3 mg/kg or above reduced the blood pressure and heart rate of anesthetized dogs with reductions of aortic pressure, contractility, aortic blood flow, total peripheral resistance, and left ventricular work. Granisetron had no effects on gastrointestinal transit or gastric motility. At high concentrations, granisetron suppressed platelet aggregation induced by arachidonic acid or ADP, but had no effects on hematological parameters, renal or hepatic function.

Mechanism of Action:

Granisetron is a selective 5-hydroxytryptamine-3 (5-HT₃) receptor antagonist. Serotonin receptors of the 5-HT₃ type are located peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. Stimulation of 5-HT₃ receptors in these areas induces vomiting. Animal studies indicate that granisetron, by binding to 5-HT₃ receptors, blocks serotonin stimulation and subsequent vomiting after emetogenic stimuli such as cisplatin.

2.6.2.3 Secondary pharmacodynamics: No studies were submitted.

2.6.2.4 Safety pharmacology: No studies were submitted.

2.6.2.5 Pharmacodynamic drug interactions: N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

The sponsor submitted following published ADME studies of granisetron.

Pharmacokinetics of Granisetron Hydrochloride- Distribution, Metabolism and Elimination Following Intravenous Administration to Rats and Dogs. Haddock, RE, Higham FC, Pope JA, Woods FR, Allen A, Pience DM, Zussman BD, Nishioka Y, Kumakura, H, Ito S. The Clinical Report 1990, 24(13), 287.

The distribution, metabolism and excretion of granisetron were studied in rats and dogs following intravenous dosing using a combination of radiotracer and chromatographic assay techniques. In rats, ¹⁴C-granisetron was administered at an i.v. dose of 0.3 to 3 mg/kg, and in dogs the doses were 0.03, 0.3 or 3 mg/kg. Whole body autoradiography was conducted in rats following administration of

an i.v. dose of 3 mg/kg. To determine the fetal transfer of the radioactivity, ^{14}C -granisetron was administered to pregnant rats on day 15 of pregnancy.

In rats, blood radioactivity concentrations readily after a single intravenous administration of ^{14}C -granisetron at 0.3 mg/kg. A proportionally higher profile was found at a dose level of 3 mg/kg. In dogs, plasma radioactivity concentration also increased proportionally with dose. The plasma profiles of granisetron were parallel over a 100-fold dose range, indicating linear kinetics. Following a single, concentrations of radioactivity were maximal in most tissues at 0.25 hours after dosing. The concentrations of radioactivity were generally greater than corresponding concentrations in blood except for tissues of the central nervous system. The concentration of the radioactivity declined with time, and at 72 hours, the radioactivity concentrations decreased below 2% of the maximum concentration in any tissues. In rats, the radioactivity was excreted in both urine and feces. Urinary excretion was not altered by a 10-fold increase in dose, and excretion occurred mainly during the first 24 hours after dosing. Urinary excretion accounted for 2% of the dose. Biliary excretion of the radioactivity was approximately 50% of the dose in chronic cannulated rats, and some re-absorption of the radioactivity in the bile was observed. Granisetron itself was not excreted into the bile and therefore is not a subject to enterohepatic circulation.

In dogs, the radioactivity was also excreted in both urine and feces, and urinary excretion was similar over 100-fold dose range. Urinary excretion accounted for 6% of the dose.

In pregnant rats, approximately 1% of the radioactivity was reversibly transferred to the fetus. The transfer of the radioactivity via breast milk to suckling pups was less than 1% of the dose.

The extent of *in vitro* plasma protein binding of granisetron was 46-65% in rats, and 36-52% in dogs. The extent of *in vivo* binding was 52.8-59.3% in rats and 25.6%-30.7% in dogs. The metabolite pattern in the rat urine was dominated by conjugate of 5-phenol (metabolite E) accompanied by its des-methyl counterpart (metabolite P). In rat bile, conjugates of metabolite E and P were the principal metabolites. In dog urine, five metabolites (metabolite E, D, H, A and C) were identified.

In rats, steady state concentrations were reached on day four of daily oral administration. Minimal accumulation in the blood and tissues were observed and the radioactivity was readily excreted on cessation of dosing.

Metabolism and Disposition of ^{14}C -Granisetron in Rat, Dog and Man after Intravenous and Oral Dosing. Clarke SE, Austin NE, Bloomer JC, Haddock RE, Higham FC, Hollis FJ, Nash M, Shardlow PC, Tasker TC, Woods FR and Allen GD. *Xenobiotica* 1994, 24(11), 1119-1131.

The disposition and metabolism of ^{14}C -granisetron were studied in Sprague-Dawley rats, beagle dogs and male human volunteers following intravenous and oral administration. In rats, single intravenous doses of 0.3 and 3 mg/kg, and single oral doses of 0.25 and 5 mg/kg were used. In dogs, single intravenous doses of 0.03, 0.3 and 3 mg/kg, and single oral doses of 0.25, 1.5 and 10 mg/kg were used. In humans, single doses of ^{14}C -granisetron were administered intravenously and orally 3 months apart to three healthy male volunteers at a dose of 100 μg /kg equivalent.

Complete absorption of the drug occurred from the gastrointestinal tract following oral dosing, but bioavailability was reduced in all three species by first-pass metabolism. No sex-differences in the metabolism of granisetron were observed in rats and dogs, and there was no appreciable change in disposition of the radioactivity with doses between 0.25 and 5 mg/kg in rats and between 0.25 and 10 mg/kg in dogs. No differences in the disposition were observed in any species with the three routes of administration. In rats and dogs, 35-41% of the dose was excreted in the urine and 52-62% in the feces via the biliary route. Metabolites were primarily present as glucuronide and sulfate conjugates, together with numerous minor polar metabolites. In humans, about 60% of the administered radioactivity was excreted in the urine and 36% in the feces after both intravenous and oral dosing. Unchanged granisetron was only excreted in the urine (5-25% of dose). In rats, the dominant routes of metabolism after both intravenous and oral administration were 5-hydroxylation and N1-demethylation, followed by formation of conjugates. These were the major metabolites in urine, bile and plasma. In dogs, and humans, the major metabolite was 7-hydroxy-granisetron with lesser quantities of the 6,7-dihydrodiol and/or their conjugates.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.4 TOXICOLOGY

Dose-Range-Finding Study for a 2-Week Combined Local and Systemic Tolerance and Subchronic Toxicity Study of Granisetron Base Patches by Epicutaneous Administration in Rats

Key Study Findings: granisetron base patches were tolerated at sizes of 2.5%, 5%, and 10% of body surface area; increased patch size was associated with increased plasma drug levels

Study # 19137/05

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: June 7, 2005 (report dated December 1, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: ycs (x) no ()

Drug: lot # C017GRATDS/SNR800646 (granisetron base patches); purity of drug was not indicated

METHODS: Male CrI:CD@ (SD) rats (age 50 days, 234-263 g) were treated with granisetron using epicutaneous (transdermal) administration via granisetron base patches. The patches contained 0.65 mg granisetron base/cm². The inactive matrix included an adhesive, the components of which were not stated. The study groups were administered descending patch sizes, with a 7-day application period used for each patch size. The patches were applied to a shaved region on the back of the animals, and were held in place with a cohesive fixative bandage. The patch sizes were 10%, 5%, and 2.5% of the body surface area, as calculated using the Meeh formula (3 rats/patch size). New animals were used for each patch size. The application site was shaved after patch removal to facilitate skin examination. The animals were sacrificed on day 8. The following parameters were recorded:

Observations and Times:

Mortality: daily

Clinical Signs: daily

Bodyweight: days 1 and 8

Gross Pathology: at sacrifice

Toxicokinetics: Blood samples were collected at 10, 24, 72, 120, and 144 hr after application of the patch, and at approximately 24 hr after patch removal (3 rats/group/time-point). Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

Other: Local effects were evaluated immediately after and 1 hr after patch removal.

RESULTS:

Mortality: None.

Clinical Signs: None.

Bodyweight: The effects of granisetron on bodyweight cannot be evaluated due to the absence of a control group. However, loss of bodyweight occurred in all animals. The mean bodyweight was decreased by 21.3, 6, and 19.3 g in groups treated with 10%, 5%, and 2.5% body surface area patches, respectively.

Gross Pathology: No abnormalities were observed.

Toxicokinetics: Plasma concentrations of granisetron were measured. The results are shown in the table below.

Patch Size (%Body Surface Area)	t_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-144\text{ hr}}$ (ng·hr/ml)	$AUC_{0-t\text{ last}}$ (ng·hr/ml)	C_{avg} (ng/ml)
2.5%	24	5.1 ± 1.5	376 ± 57	373 ± 60	2.6 ± 0.4
5%	24	6.5 ± 3.2	491 ± 148	516 ± 190	3.4 ± 3.7
10%	24	18.6 ± 5.0	1394 ± 530	1524 ± 600	9.7 ± 3.7

Values are the mean ± S.D. of 3 rats/group, except for t_{max} (median value).

The AUC, C_{avg} , and C_{max} values in the 5% body surface area group were disproportionately small relative to the dose increment over the 2.5% body surface area group. However, the values in the 10% body surface area group were approximately proportional relative to the 2.5% body surface area group. At 24 hr after patch removal, granisetron was below the limit of quantification (0.5 ng/ml) in the 2.5% body surface area group, whereas low levels (0.66-1.69 ng/ml) were still present in the 5% and 10% body surface area groups.

Other: The patch application site was evaluated for erythema, eschar formation, and edema. Scoring of these evaluations was based on the Draize scale, as shown below.

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing erythema reading	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

For all patch sizes, the erythema scores immediately after patch removal were 1 or 2. At 1 hr after patch removal, the erythema scores in all animals were 1. No edema was observed in any animal.

Conclusions: Granisetron base patches were tolerated at sizes of 2.5%, 5%, and 10% of body surface area. Increased patch size was associated with increased plasma drug levels.

1-Week Dose-Range-Finding Study of Granisetron HCl by 24-H Continuous I.V. Infusion to Rats

Key Study Findings: granisetron HCl was tolerated at 1, 3, and 9 mg/kg/day, given as a continuous intravenous infusion

Study # 19292/05

Vol. 7

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: August 31, 2005 (report dated December 12, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # FX5103; 100.3% pure

METHODS: Male Crl:CD®(SD) rats (age 63 days, 248.7-300.1 g) were treated intravenously with 1, 3, or 9 mg/kg/day granisetron HCl for one week (3 rats/group). At seven days prior to study initiation, catheters were implanted in the jugular vein and saline was continuously infused (60 ml/kg/day) to acclimatize the animals to the infusion conditions. The test and control articles were administered using continuous (24 hr/day) infusion via the implanted catheter. The dose volume was 60 ml/kg/day, and the vehicle was saline. The animals were sacrificed on day 8. Dose selection was based on information reported in the Summary Basis for Approval for NDA

20,239. The selected doses were within the range of doses administered in previous toxicology studies in rats. The present study included the following parameters:

Observations and Times:

Mortality: twice daily

Clinical Signs: twice daily

Bodyweight: days 1 and 8

Food Consumption: weekly

Toxicokinetics: Blood samples were collected at 0.5, 2, 24, 72, 120, and 168 hr after the start of infusion (3 rats/group/time-point). Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

RESULTS:

Mortality: None.

Clinical Signs: None.

Bodyweight: The effects of granisetron on bodyweight cannot be evaluated due to the absence of a control group. The mean bodyweight was increased by 9.8, 25.2, and 23.6 g in the 1, 3, and 9 mg/kg/day groups, respectively.

Food Consumption: The results were reported only as g/kg bodyweight/day. Due to the absence of a control group, the effects of granisetron on food intake cannot be evaluated. No obvious effect on food consumption was discernible from the reported data.

Gross Pathology: Enlarged spleen was observed in the 1, 3, and 9 mg/kg/day groups (2/3, 2/3, and 3/3 rats, respectively). The authors considered this effect as a secondary immune response caused by the continuous infusion procedure.

Toxicokinetics: Plasma concentrations of granisetron were measured. The results are shown in the table below.

Dose (mg/kg/day)	t _{max} (hr)	C _{max} (ng/ml)	AUC _{0-168 hr} (ng•hr/ml)	Mean Daily AUC (ng•hr/ml)	C _{avg} (ng/ml)
1	120	10.6 ± 2.8	1192 ± 302	170 ± 43	7.1 ± 1.8
3	120	30.4 ± 12.4	3728 ± 903	533 ± 129	22.2 ± 5.4
9	120	93.2 ± 20.7	10,424 ± 881	1489 ± 126	62.0 ± 5.2

Values are the mean of 3 rats/group/time-point, except for t_{max} (median value).

The increases in AUC, C_{avg}, and C_{max} values with dose level were proportional to the dose increment.

Conclusions: Granisetron HCl was tolerated at doses of 1, 3, and 9 mg/kg/day, given as a continuous intravenous infusion.

2-Week Combined Local and Systemic Tolerance and Subchronic Toxicity Study of Granisetron Base Patches Applied Epicutaneously Compared to Granisetron HCl Applied Intravenously or Orally to Rats

Note: This study was submitted as a draft report in the initial submission. The final report was submitted in amendment # 005.

Key Study Findings: granisetron patch produced an increased severity of erythema, as compared to the placebo patch; heart appears to have been a target organ of toxicity in the granisetron patch, 9 mg/kg/day iv, and 50 mg/kg/day po groups; the systemic toxicity of transdermally-administered granisetron was similar to that observed with intravenous and oral administration

Study # 19138/05

Amendment # 005, Vol. 1

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: July 26, 2005 (report dated June 29, 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # C017GRATDS/SNR800646 (granisetron base patches); purity of drug was not stated

lot # FX4379 (granisetron HCl); 99.5% pure

METHODS: Crl:CD®(SD) rats were treated as described in the following table.

Group	Route	Dose	Frequency of Administration
1	Epicutaneous (patch)	Placebo patch	2 consecutive applications for 7 days each
2		Granisetron 6% laminate patch	
3	Intravenous	Vehicle control	Daily for 14 days
4		1 mg/kg/day Granisetron HCl	
5		9 mg/kg/day Granisetron HCl	
6	Oral	50 mg/kg/day Granisetron HCl	

Groups 1-2: Males were 50 days old, 231-269 g
Females were 58 days old, 196-220 g

Groups 3-6: Males were 34 days old, 127-164 g
Females were 35 days old, 119-145 g

All groups included 10 rats/sex for the main study. Groups 2, 4, 5, and 6 also included 6 rats/sex for measurement of toxicokinetics. For groups 1 and 2, the patch size was equal to 10% of the body surface area, as calculated using the Meeh formula. In group 2, the patch size for males and females was 36 cm² and 31.4 cm², respectively. Similar patch sizes were used in group 1. The granisetron patch contained 0.65 mg granisetron base/cm². The inactive matrix included an adhesive, the components of which were not stated. The patches were applied to a shaved area of the back in groups 1 and 2. The final patch was removed at approximately 1 hr prior to sacrifice. The granisetron patches were analyzed for residual drug content after removal from the animals. The residual granisetron content was 13-56% for the first week of treatment, and 10-36% for the second week of treatment. The estimated granisetron dose delivered by the patches was in the range of 10.2 to 19 mg/animal. Although the patches were protected with fixation bandages during the treatment period, some of the rats managed to chew on the patches, resulting in an irregular shape at the time of removal. Therefore, the accuracy of the calculated residual drug content in patches and the estimated delivered dose is questionable due to the loss of patch area during the treatment period. Intravenous administration in groups 3-5 was performed using a dose volume of 5 ml/kg, with saline as the vehicle. Oral administration in group 6 was performed using a dose volume of 10 ml/kg, with 1% methylcellulose as the vehicle. Most animals were sacrificed on day 15. Some animals in groups 3-6 were dosed on day 15 and sacrificed on day 16. The selection of patch size (10% of body surface area) was based on results of a 7-day dose range-finding study in rats (study # 19137/05) and pharmacokinetic data from humans. The largest patch size in the dose range-finding study was equal to 10% of the body surface area, which is the area of application recommended in OECD

guidelines for dermal toxicity studies. The group that was treated with the 10% body surface area patch exhibited no adverse effects other than erythema at the application site. In addition, the authors claim that the systemic exposure observed with this patch size was similar to that seen in humans at the clinically active dose. The dose levels used for intravenous and oral administration were selected on the basis of information reported in the Summary Basis for Approval for NDA 20,239 and were within the range of doses administered in previous toxicology studies in rats. The present study included the following parameters:

Observations and Times:

Mortality: twice daily

Clinical Signs: pre-dose and post-dose on all days

Bodyweight: days 1, 8, and 15

Food Consumption: weekly

Ophthalmoscopy: day 14

Hematology: blood samples were collected at termination (day 15 or 16); bone marrow smears were prepared from the first five animals/sex in groups 1, 2, 5, and 6

Clinical Chemistry: blood samples were collected at termination

Urinalysis: urine samples were collected at termination

Gross Pathology: at termination

Organ Weights: adrenals, brain, heart, kidneys, liver, lungs, lymph node (cervical and mesenteric), ovaries, pituitary, spleen, testes, thymus, thyroid/parathyroid

Histopathology: The following organs/tissues were examined in groups 1, 2, 3, 5, and 6: adrenals, aorta, brain (cerebrum, cerebellum, brain stem), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), femur (with joint and marrow), gross lesions, Harderian glands, heart, ileum, injection site (groups 3-5), jejunum, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin (left flank), skin (patch application site and untreated site in groups 1 and 2), spinal cord (3 sections), spleen, sternum (with marrow), stomach, testes, thymus, thyroid/parathyroid, tissue masses or tumors (including regional lymph nodes), tongue, trachea, ureters, urinary bladder, uterus, vagina. All tissues were stained with hematoxylin-eosin. Additional sections of heart, kidney, and liver were stained with scarlet R.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected from the granisetron patch-treated group at 10, 24, 48, 96, 120, 144, and 168 hr after application of the first patch (3 rats/sex/time-point). Blood was collected from the placebo patch-treated group at 48 hr after the first application (3 rats/sex). The blood sampling time-points for the intravenous drug treatment groups were 2 min, 15 min, 30 min, 2 hr, and 5 hr post-dose on day 7 (3 rats/sex/time-point). Blood was collected from the intravenous vehicle control group at 2 min post-dose on day 7 (3 rats/sex). The blood sampling time-points for the oral drug treatment group were 15 min, 30 min, 1 hr, 2 hr, and 4 hr post-dose on day 7 (3 rats/sex/time-point). Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

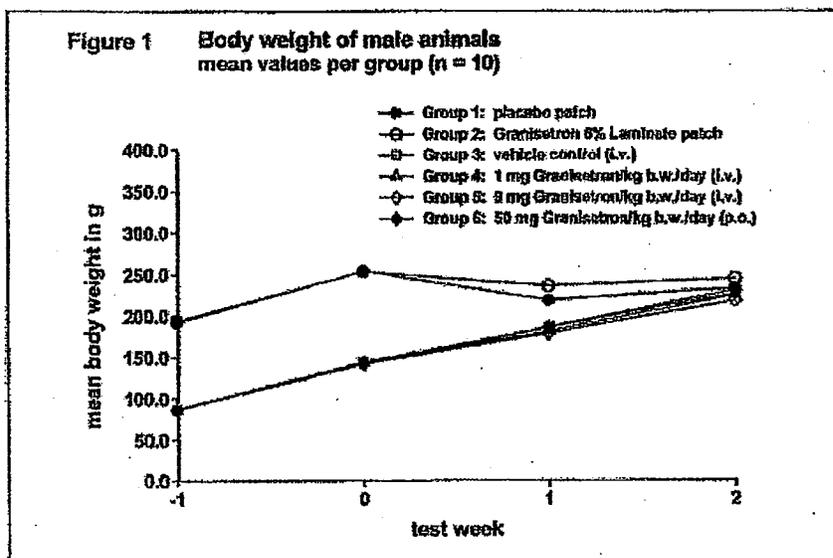
Other: Local effects were evaluated in groups 1 and 2 immediately after and at 1 hr after patch removal on days 8 and 15. A hearing test was performed on day 15.

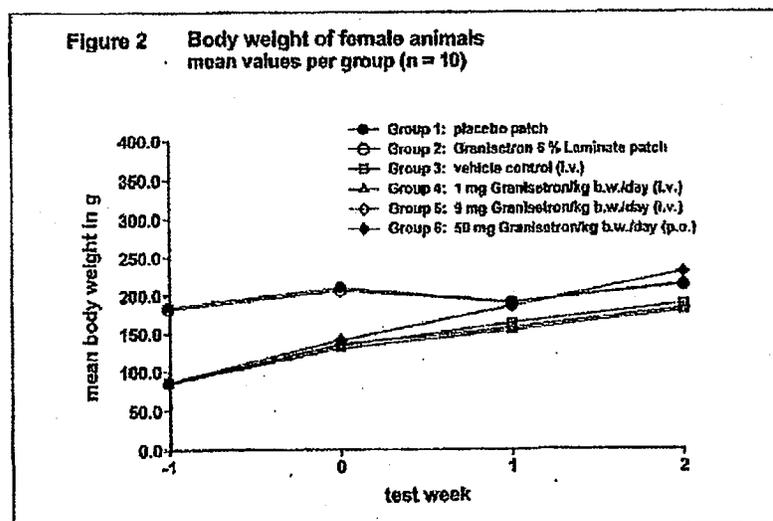
RESULTS:

Mortality: None.

Clinical Signs: Tonicoclonic convulsions were observed sporadically in group 5 (9 mg/kg/day iv) immediately after dosing. The duration of this effect was 0.5-5 min.

Bodyweight: The effects on bodyweight are shown in the figures below (taken from the study report).





It should be noted that the patch-treated groups were 2-3 weeks older than the intravenous and oral administration groups. Therefore, the bodyweight of the patch-treated groups was higher at study initiation. Weight loss was observed in both the placebo patch- and granisetron patch-treated males (-20.71 g and -8.5 g, respectively, based on the mean bodyweights at study initiation and termination). In contrast, slight weight gain occurred in the placebo patch- and granisetron patch-treated females (4.52 g and 7.23 g, respectively). Weight gain in group 3 (iv vehicle) was relatively robust (83.22 g and 54.81 g in males and females, respectively). Weight gain was reduced by 11% and 15% in the 1 and 9 mg/kg/day iv females, respectively, and by 8% in the 9 mg/kg/day iv males. Weight gain was unaffected in the 50 mg/kg/day po group, relative to the intravenous vehicle control group. The data indicates that weight gain was strongly inhibited by both the placebo and granisetron patches, with loss of bodyweight observed in males. The magnitude of this effect was similar for the placebo and granisetron patches, whereas the intravenous granisetron groups exhibited only minimal inhibition of weight gain. Therefore, the marked impairment of weight gain in the granisetron patch-treated group does not appear to be a drug-related effect.

Food Consumption: The results were reported only as g/kg bodyweight/day. A 10% decrease occurred in the 9 mg/kg/day iv group (not significant). Since the absolute amount of food consumed (g/animal/day) was not reported, it is difficult to evaluate the effects on food intake in the patch-treated groups, in which weight gain was markedly impaired.

Ophthalmoscopy: No abnormalities were observed.

ECG: Not performed.

Hematology: The results are shown in the table below. Changes in the granisetron patch-treated group are expressed relative to values from the placebo patch group. Changes in the intravenous and oral granisetron-treated groups are expressed relative to values from the intravenous vehicle control group.

Parameter	Group	Change	Day
WBC	Granisetron 6% laminate patch ^m	60% increase (ns)	15
	1 ^f , 9 ^f mg/kg/day iv	29%, 34% increase (ns)	45
	50 mg/kg/day po ^f	60% increase (ns)	15
Neutrophils	Granisetron 6% laminate patch ^m	107% increase (ns)	15
	50 mg/kg/day po ^f	39% increase (ns)	15
Lymphocytes	Granisetron 6% laminate patch ^m	35% increase (ns)	15
	50 mg/kg/day po ^f	66% increase (ns)	15
Monocytes	Granisetron 6% laminate patch ^m	109% increase (ns)	15
	50 mg/kg/day po ^m	29% increase (ns)	15
	50 mg/kg/day po ^f	56% increase (ns)	15
LUC	Granisetron 6% laminate patch ^m	100% increase (ns)	15
Basophils	Granisetron 6% laminate patch ^m	180% increase (ns)	15

LUC: large unstained cells

ns: not significant

m: males

f: females

Males in the granisetron patch group exhibited increases in neutrophils, lymphocytes, monocytes, large unstained cells, and basophils. Females in the 50 mg/kg/day po group exhibited increases in neutrophils, lymphocytes, and monocytes. TPT (thromboplastin time) and APTT (activated partial thromboplastin time) were unaffected. Bone marrow smears were examined in groups 1, 2, 5, and 6. The myeloid:erythroid ratio was unaffected.

Clinical Chemistry: ALT in one male in the granisetron patch group was increased by 3.4-fold relative to the mean control value (placebo patch group). No drug-related lesions in liver were observed in the microscopic examination of this animal. Total protein was reduced by 7% in males in the 50 mg/kg/day po group.

Urinalysis: The following parameters were reported: volume, specific gravity, appearance, color, glucose, bilirubin, ketones, hemoglobin, pH, protein, urobilinogen, nitrite, WBC, RBC, epithelial cells, casts, crystals, bacteria, sperm, and yeast. No parameters were affected.

Organ Weights: Absolute and relative (g/kg bodyweight) weights were reported. Changes in the granisetron patch-treated group are expressed relative to values from the placebo patch group. Changes in the intravenous and oral granisetron-treated groups are expressed relative to values from the intravenous vehicle control group.

Liver: Relative weight in the 50 mg/kg/day po males was increased by 17%.

Lungs: Absolute weight was decreased by 14% and 15% in the 9 mg/kg/day iv males and the 50 mg/kg/day po females, respectively.

Gross Pathology: No abnormalities were observed.

Histopathology: Groups 1, 2, 3, 5, and 6 were examined.

Heart: Lympho-histiocytic infiltration was observed in the granisetron patch-treated group (3/20 rats), the 9 mg/kg/day iv group (2/20 rats), and the 50 mg/kg/day po groups (1/20 rats). Fatty infiltration and epicarditis occurred in the granisetron patch-treated group (1/20 rats for each lesion).

Injection Site (early, groups 3 and 5): Increased incidence of hemorrhage was observed in the 9 mg/kg/day iv group (7/20 rats, as compared with 3/20 rats in the control group).

Injection Site (last, groups 3 and 5): Increased incidence of perivascular granulation tissue occurred in the 9 mg/kg/day iv group (15/20 rats, as compared with 6/20 rats in the control group). Hemorrhage was also increased in the 9 mg/kg/day iv group (10/20 rats, as compared with 2/20 rats in the control group).

Kidneys: Interstitial nephritis occurred in the granisetron patch-treated group and the 9 mg/kg/day iv group (1/20 rats in both groups). Lympho-histiocytic infiltration was observed in the 9 mg/kg/day iv and 50 mg/kg/day po groups (1/20 and 2/20 rats, respectively). Basophilic tubular cells and pyelitis occurred in the 9 mg/kg/day iv group (1/20 rats for each lesion).

Liver: Focal necrosis and microgranuloma was observed in the granisetron patch-treated group (1/20 rats for each lesion). Vacuolization occurred in the 50 mg/kg/day po group (1/20 rats).

Thyroid: Lymphocytic infiltration was observed in the granisetron patch-treated group (1/20 rats).

Toxicokinetics: Plasma levels of granisetron were measured. The results are shown in the table below.

Dose	Sex	t _{max} (hr)	C _{max} (ng/ml)	AUC (ng•hr/ml)	Mean Daily AUC (ng•hr/ml)	C _{avg} (ng/ml)
6% granisetron patch Days 1-7	M	24.0	20.4	1531.3 ^a	218.8	9.1
	F	24.0	53.5	3080.7 ^a	440.1	18.3
1 mg/kg/day IV Day 7	M	0.03	325.7	111.3 ^b	na	na
	F	0.03	298.3	99.6 ^b	na	na
9 mg/kg/day IV Day 7	M	0.03	2403.3	1018.8 ^b	na	na
	F	0.03	2305.0	855.5 ^b	na	na
50 mg/kg/day PO Day 7	M	0.25	658.3	1792.7 ^c	na	na
	F	0.25	695.0	1674.9 ^c	na	na

Values were determined from 3 rats/sex/group/time-point.

a: 0-168 hr

b: 0-5 hr

c: 0-4 hr

na: not applicable

The mean daily plasma exposure (AUC) in the granisetron patch group was within the range of the AUC_{0-5 hr} values in the 1 and 9 mg/kg/day iv groups, whereas the C_{max} values in the granisetron patch group were substantially lower than those of the intravenous groups. Granisetron levels in the intravenous groups were near the limit of quantification (0.5 ng/ml) at 5 hr post-dose, the final time-point. On days 4-7, the drug concentrations in the patch-treated group were ≤ 10 ng/ml, whereas higher concentrations were present during the initial 48 hr of application. Thus, the daily plasma exposure produced by the patch appears to have varied during the 7-day period of application. The AUC, C_{max}, and C_{avg} values for the patch-treated females were approximately 2-fold greater than those of the patch-treated males. In contrast, no sex-related differences in pharmacokinetic parameters were observed with intravenous or oral administration.

Other: The patch application site was evaluated for erythema, eschar formation, and edema. Scoring of these evaluations was based on the Draize scale, as shown below.

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing erythema reading	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Day 8: The severity of erythema immediately after patch removal was scored as 3 in all of the group 2 (granisetron patch) animals, as compared with a score of 2 in all of the group 1 (placebo patch) animals. The severity of erythema was slightly diminished in both groups at 1 hr after patch removal, although the severity was still greater in the granisetron-treated group. Edema was not observed in any animal.

Day 15: A high incidence of erythema scores of 4 (all with eschar) occurred in group 2 (9/20 rats) immediately after patch removal, whereas the scores for group 1 were either 2 or 3. The severity of erythema was diminished in both groups at 1 hr after patch removal, but the severity remained higher in the granisetron-treated group. Edema was not observed in any animal.

No drug-related effects were observed in the microscopic examination of the application sites from the granisetron patch group. No signs of local irritation were observed in the intravenously treated groups. Auditory testing results were normal in all animals.

Conclusions: The granisetron patch produced an increased severity of erythema, as compared to the placebo patch. No signs of drug-related irritation were observed in the microscopic examination of the patch application sites. The estimated mean daily AUC value for the granisetron patch-treated group was comparable to the AUC values in the intravenously-treated groups, but substantially lower than that of the orally-treated group. Heart appears to have been a target organ of toxicity in the granisetron patch, 9 mg/kg/day iv, and 50 mg/kg/day po groups, based on a low incidence of lympho-histiocytic infiltration. Lesions in other organs (e.g., kidneys and liver) that occurred exclusively in drug-treated groups were sporadic, and are not considered to be treatment related. Under the study conditions, the systemic toxicity of transdermally-administered granisetron in rats was similar to that observed with intravenous or oral administration. Intravenous administration of 9 mg/kg/day granisetron HCl produced an increased incidence of hemorrhage and perivascular granulation tissue at the injection site, as compared with the vehicle control. Weight gain was markedly impaired in both the placebo patch and granisetron patch-treated groups. The reason for this effect is unknown. Granisetron was not detected in plasma samples from the placebo patch-treated group, which excludes the possibility of an unintended administration of drug in this group. The absolute amount of food consumed (g/animal/day) was not reported, so the effect of patch treatment on food intake is uncertain.

2-Week Subchronic Toxicity Study of Granisetron HCl by 24-H Continuous I.V. Infusion to Rats

Key Study Findings: liver appeared to be a target organ of toxicity in the 9 mg/kg/day group, based on the incidence of bile duct proliferation

Study # 19293/05

Vol. 7

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: September 19, 2005 (report dated December 9, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # FX5103; 100.3% pure

METHODS: CrI:CD®(SD) rats (males: age 59 days, 246-320 g; females: age 72 days, 223-284 g) were treated intravenously with 0 (vehicle), 1, 3, or 9 mg/kg/day granisetron HCl for two weeks (10 rats/sex/group). The toxicokinetic groups were treated with 1, 3, or 9 mg/kg/day granisetron HCl (6 rats/sex/group). At 5-8 days prior to study initiation, catheters were implanted in the jugular vein and saline was continuously infused (60 ml/kg/day) to acclimatize the animals to the infusion conditions. The test and control articles were administered using continuous (24 hr/day) infusion via the implanted catheter. The dose volume was 60 ml/kg/day, and the vehicle was saline. The animals were sacrificed on day 15. Dose selection was based on the results of a 1-week dose range-finding study in rats using continuous iv infusion (study # 19292/05, reviewed above). The dose levels used in this study were 1, 3, and 9 mg/kg/day (no control group was included). The only observed abnormality was slight enlargement of the spleen. The authors stated that 9 mg/kg/day was the maximum intravenous bolus dose used in previous subchronic toxicity studies of granisetron HCl in rats (NDA 20,239). The present study included the following parameters:

Observations and Times:

Mortality: twice daily

Clinical Signs: multiple times during each day

Bodyweight: days 1, 8, and 15

Food Consumption: weekly

Ophthalmoscopy: day 15

Hematology: blood samples were collected at termination (day 15); bone marrow smears were prepared from the first five animals/sex in the control and high-dose groups

Clinical Chemistry: blood samples were collected at termination

Urinalysis: urine samples were collected at termination

Gross Pathology: at termination

Organ Weights: adrenals, brain, heart, kidneys, liver, lungs, lymph node (cervical and mesenteric), ovaries, pituitary, spleen, testes, thymus, thyroid/parathyroid

Histopathology: The following organs/tissues were examined in control and high-dose groups: adrenals, aorta, brain (cerebrum, cerebellum, brain stem), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), femur (with joint and marrow), gross lesions, Harderian glands, heart, ileum, infusion site, jejunum, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin (left flank), spinal cord (3 sections), spleen, sternum (with marrow), stomach, testes, thymus, thyroid/parathyroid, tissue masses or tumors (including regional lymph nodes), tongue, trachea, ureters, urinary bladder, uterus, vagina. All tissues were stained with hematoxylin-cosin. Additional sections of heart, kidney, and liver were stained with scarlet R.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected from the granisetron-treated groups at 0.5, 2, 24, 72, 120, and 168 hr after the start of infusion (3 rats/sex/time-point). Blood was collected from the control group at 168 hr (3 rats/sex). Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

Other: A hearing test was performed on day 15.

RESULTS:

Mortality: No deaths occurred.

Clinical Signs: No clinical signs were observed.

Bodyweight: Weight gain was unaffected. The mean weight (\pm S.D.) of the control males and females was 275.0 ± 15.9 g and 236.3 ± 8.9 g, respectively, on day 1, and 316.2 ± 23.5 g and 254.5 ± 14.9 g, respectively, on day 15.

Food Consumption: The results were reported only as g/kg bodyweight/day. No effects were observed.

Ophthalmoscopy: No abnormalities were observed.

ECG: Not performed.

Hematology: No effects on hematology parameters were observed. TPT and APTT were unaffected. Bone marrow smears were examined in the control and high-dose groups. The myeloid:erythroid ratio was unaffected.

Clinical Chemistry: Granisetron had no effect on clinical chemistry parameters.

Urinalysis: The following parameters were reported: specific gravity, volume, color, glucose, bilirubin, ketones, hemoglobin, pH, protein, urobilinogen, nitrite, WBC, RBC, epithelial cells, casts, crystals, bacteria, sperm, and yeast. A small but statistically significant reduction in specific gravity occurred in the 3 and 9 mg/kg/day females. Urine volume was increased by 46-75% in the 1, 3, and 9 mg/kg/day females.

Organ Weights: Absolute weight and relative weight (g/kg bodyweight) were reported. There were no effects.

Gross Pathology:

Infusion Site: Purulent tissue was observed in the 1, 3, and 9 mg/kg/day groups (1/20, 1/20, and 2/20 rats, respectively). Tissue enlargement occurred in the 3 mg/kg/day group (2/20 rats). Heart: Thickening of the auricle was observed in the 3 and 9 mg/kg/day groups (1/20 and 2/20 rats, respectively).

Histopathology:

Heart: Granulation tissue was observed in the 9 mg/kg/day group (1/20 rats). Lympho-histiocytic infiltration occurred in the 9 mg/kg/day group (1/20 rats). Purulent pericarditis was observed in the 9 mg/kg/day group (1/20 rats). Each of these lesions occurred in different animals. Given the low incidence and the absence of data from the 1 and 3 mg/kg/day groups, the relationship of these lesions to drug treatment is uncertain. However, lympho-histiocytic infiltration in heart was observed in the 2-week toxicity study in rats using transdermal, IV bolus, and oral administration (study # 19138/05, reviewed above). Therefore, it is reasonable to assume that lympho-histiocytic infiltration in the present study was drug-related.

Liver: Bile duct proliferation was observed in the 9 mg/kg/day group (4/20 rats).

Urinary Bladder: Proteinaceous content was observed in the 9 mg/kg/day group (3/20 rats).

Toxicokinetics: Plasma levels of granisetron were measured. The results are shown in the table below.

Dose (mg/kg/day)	Sex	t _{max} (hr)	C _{max} (ng/ml)	AUC _{0-168 hr} (ng•hr/ml)	Mean Daily AUC (ng•hr/ml)	C _{avg} (ng/ml)
1	M	24	8.5	1148	164	6.8
	F	2	8.7	978	140	5.8
3	M	72	24.7	3514	502	20.9
	F	72	19.3	2701	386	16.1
9	M	120	72.0	11323	1617	67.4
	F	24	61.1	7599	1086	45.2

Values were determined from 3 rats/sex/group/time-point.

The AUC, C_{max}, and C_{avg} values were increased with the dose level. The increase in these parameters was proportional to the dose increment. Males exhibited higher AUC, C_{max}, and C_{avg} values in comparison to the female values, particularly at 3 and 9 mg/kg/day.

Other: All animals tested normal in the hearing test.

Conclusions: Liver appears to be a target organ of toxicity in the 9 mg/kg/day group, based on the incidence of bile duct proliferation. Heart is also considered as a target organ of toxicity in the 9 mg/kg/day group, based on the incidence of lympho-histiocytic infiltration and the previous observation of this lesion in the 2-week toxicity study in rats using transdermal, IV bolus, and oral administration. A NOAEL (no observed adverse effect level) was not established due to the effects in liver and heart, and the absence of histopathology data from the 1 and 3 mg/kg/day groups.

Dose-Range-Finding Study for a 2-Week Combined Local and Systemic Tolerance and Subchronic Toxicity Study of Granisetron Base Patches by Epicutaneous Administration to Dogs

Key Study Findings: granisetron base patches were tolerated at sizes of 5% and 10% of body surface area; plasma drug levels were similar for both patch sizes

Study # 19139/05

Vol. 9

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: June 7, 2005 (report dated December 1, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # C017GRATDS/SNR800646 (granisetron base patches); purity of drug was not indicated

METHODS: Male Beagle dogs (age 6 months, 7.5-7.9 kg) were treated with granisetron using epicutaneous (transdermal) administration via granisetron base patches. The patches contained 0.65 mg granisetron base/cm². The inactive matrix included an adhesive, the components of which were not stated. Three dogs were administered ascending patch sizes, with a 7-day application period used for each patch size. The patch sizes were 5% and 10% of the body surface area, as calculated using the Meeh formula. A 3-day washout period was allowed between the first and second patch application periods. The patches were applied to a shaved region on the back of the animals, and were held in place with a cohesive fixative bandage. The application site was shaved after patch removal to facilitate skin examination. A scheduled sacrifice was not included in the study protocol. The following parameters were recorded:

Observations and Times:

Mortality: twice daily

Clinical Signs: daily

Bodyweight: at the beginning and end of each 7-day treatment period

Food Consumption: daily

Toxicokinetics: Blood samples were collected from each animal at 10, 24, 48, 72, 96, 120, 144, and 168 hr after application of the patch, and at approximately 24 hr after patch removal. Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

Other: Local effects were evaluated immediately after and at 1 and 24 hr after patch removal. In addition, the skin area designated for patch application was evaluated after shaving and prior to application.

RESULTS:

Mortality: None.

Clinical Signs: None.

Bodyweight: The effects of granisetron on bodyweight cannot be evaluated due to the absence of a control group. The mean (\pm S.D.) bodyweight was 7.73 ± 0.21 , 7.77 ± 0.32 , 7.87 ± 0.32 , and 8.4 ± 0.36 kg on days 1, 8, 11, and 18, respectively.

Food Consumption: The effects of granisetron on food intake cannot be evaluated due to the absence of a control group. Each animal consumed the entire daily allotment of food (40 g/kg bodyweight) during the study.

Toxicokinetics: Plasma concentrations of granisetron were measured. The results are shown in the table below.

Patch Size (%Body Surface Area)	t _{max} (hr)	C _{max} (ng/ml)	AUC _{0-168 hr} (ng•hr/ml)	AUC _{0-t last} (ng•hr/ml)	C _{avg} (ng/ml)
5%	48 ± 24	27.8 ± 1.3	2404 ± 419	2390 ± 438	14.3 ± 2.4
10%	120 ± 41.6	20.3 ± 9.0	2285 ± 1272	2460 ± 1434	13.6 ± 7.6

Values are the mean ± S.D. of 3 dogs/group.

The pharmacokinetic parameters obtained with the 5% and 10% body surface area patch sizes were similar. The reason for this is uncertain.

Other: The patch application site was evaluated for erythema, eschar formation, and edema. Scoring of these evaluations was based on the Draize scale, as shown below.

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing erythema reading	4

Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Very slight erythema (score of 1) was observed in 2/3 dogs at all observation times after removal of the 5% body surface area patch. All dogs exhibited very slight erythema immediately after and at 1 hr after removal of the 10% body surface area patch, whereas no erythema was observed at 24 hr. No edema was observed after treatment with either patch size. Prior to application of the patches, no erythema or edema was observed (days 1 and 11).

Conclusions: Granisetron base patches were tolerated at sizes of 5% and 10% of body surface area. Plasma drug exposure levels were similar for both patch sizes.

1-Week Dose-Range-Finding Study of Granisetron HCl by 24-H Continuous I.V. Infusion to Beagle Dogs

Key Study Findings: granisetron HCl was tolerated at 0.3, 1, and 3 mg/kg/day, given as a continuous intravenous infusion

Study # 19335/05

Vol. 12

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: August 31, 2005 (report dated December 12, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # FX5103; 100.3% pure

METHODS: Male Beagle dogs (age 9 months, 9.8-10.3 kg) were treated intravenously with 0.3, 1, or 3 mg/kg/day granisetron HCl for seven days (1 dog/group). The test article was administered using continuous (24 hr/day) infusion via an implanted catheter in the vena cava caudalis. The dose volume was 24 ml/kg/day, and the vehicle was saline. At seven days prior to study initiation, the catheters were implanted and saline was continuously infused (24 ml/kg/day) to acclimatize the animals to the infusion conditions. A scheduled sacrifice was not included in the study protocol. Dose selection was based on information reported in the Summary Basis for Approval for NDA 20,239. The selected doses were within the range of doses administered in previous toxicology studies in dogs. The present study included the following parameters:

Observations and Times:

Mortality: twice daily

Clinical Signs: daily

Bodyweight: days 1 and 8

Food Consumption: daily

Toxicokinetics: Blood samples were collected from each animal at 1, 4, 24, 72, 120, and 168 hr after the start of infusion. Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

RESULTS:**Mortality:** None.**Clinical Signs:** The 0.3 mg/kg/day animal exhibited liquid around the entrance of the catheter on day 6.**Bodyweight:** The effects of granisetron on bodyweight cannot be evaluated due to the absence of a control group. In all animals, the bodyweight at study termination was identical to that measured on day 1.**Food Consumption:** The results were reported only as g/kg bodyweight/day. The effects of granisetron on food intake cannot be evaluated due to the absence of a control group. The mean values for food consumption were 24.3, 29.8, and 27.5 g/kg/day in the 0.3, 1, and 3 mg/kg/day groups, respectively. The daily food allotment was 40 g/kg.**Toxicokinetics:** Plasma concentrations of granisetron were measured. The results are shown in the table below.

Dose (mg/kg/day)	t _{max} (hr)	C _{max} (ng/ml)	AUC _{0-168 hr} (ng•hr/ml)	Mean Daily AUC (ng•hr/ml)	C _{avg} (ng/ml)
0.3	168	3.2	395	56	2.3
1	168	8.2	1235	176	7.3
3	24	44.7	6608	944	39.3

Values were determined from 1 dog/group.

AUC, C_{avg}, and C_{max} values were increased with dose level.**Conclusions:** Granisetron HCl was tolerated at doses of 0.3, 1, and 3 mg/kg/day, given as a continuous intravenous infusion.**2-Week Combined Local and Systemic Tolerance and Subchronic Toxicity Study of Granisetron Base Patches Applied Epicutaneously Compared to Granisetron HCl Applied Intravenously or Orally to Beagle Dogs****Note:** This study was submitted as a draft report in the initial submission. The final report was submitted in amendment # 005.**Key Study Findings:** the granisetron patch produced subepithelial edema at the application site (observed microscopically); systemic toxicity in the granisetron patch group, intravenous granisetron groups, and the oral granisetron group was minimal**Study #** 19140/05**Amendment # 005, Vol. 3**

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: August 10, 2005 (report dated June 29, 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # C017GRATDS/SNR800646 (granisetron base patches); purity of drug was not stated
lot # FX4379 (granisetron HCl); 99.5% pure

METHODS: Beagle dogs (age 6-6.5 months; males: 6.7-8.0 kg; females: 5.8-7.0 kg) were treated as described in the following table.

Group	Route	Dose	Frequency of Administration
1	Epicutaneous (patch)	Placebo patch	2 consecutive applications for 7 days each
2		Granisetron 6% laminate patch	
3	Intravenous	Vehicle control	Daily for 14 days
4		0.3 mg/kg/day Granisetron HCl	
5		3 mg/kg/day Granisetron HCl	
6	Oral	2 mg/kg/day Granisetron HCl	

Each group included 3 dogs/sex. For groups 1 and 2, the patch size was equal to 10% of the body surface area, as calculated using the Meeh formula. The patch size in the group 2 males and females was 438-480 cm² and 398-442 cm², respectively. Similar patch sizes were used in group 1. The granisetron patch contained 0.65 mg granisetron base/cm². The inactive matrix included an adhesive, the components of which were not stated. The patches were applied to a shaved area of the back in groups 1 and 2, and were held in place with a cohesive fixative bandage. The final patch was removed at approximately 1 hr prior to sacrifice. The granisetron patches were analyzed for residual drug content after removal from the animals. The residual granisetron content was 0-15.4% for the first week of treatment, and 1.2-29.9% for the second week of treatment. The estimated granisetron dose delivered by the patches was in the range of 211 to 312 mg/animal. Intravenous administration in groups 3-5 was performed using a dose volume of 2.5 ml/kg, with saline as the vehicle. Gelatin capsules were used for oral administration in group 6. The animals were sacrificed on day 15. The selection of patch size (10% of body surface area) was based on results of a dose range-finding study in dogs (study # 19139/05, reviewed above) and pharmacokinetic data from humans. The largest patch size in the dose range-finding study was 10% of the body surface area, which is the area of application

recommended in OECD guidelines for dermal toxicity studies. The dogs were initially treated with a 5% body surface area patch containing granisetron for seven days. After a 3-day washout period, the dogs were then treated with a 10% body surface area patch for an additional seven days. Both patch sizes were tolerated. In addition, the authors claim that the systemic exposure observed with the 10% body surface area patch was similar to that seen in humans at the clinically active dose. The dose levels used for intravenous and oral administration were selected on the basis of information reported in the Summary Basis for Approval for NDA 20,239 and are within the range of doses administered in previous toxicology studies in dogs. The present study included the following parameters:

Observations and Times:

Mortality: twice daily

Clinical Signs: pre-dose and post-dose on all days

Bodyweight: days 1, 8, and 15

Food Consumption: daily (mean weekly values were reported)

Ophthalmoscopy: prior to study initiation and on week 2

ECG: For groups 1 and 2, recordings were taken before and approximately 2 hr after patch application on day 1. ECG was repeated on day 14 in groups 1 and 2. For groups 3-5 (intravenous administration), recordings were taken before and at approximately 5 min after dosing on days 1 and 14. For group 6 (oral administration), ECG was performed before and at approximately 2 hr post-dose on days 1 and 14.

Hematology: blood samples were collected prior to study initiation and on day 15; bone marrow smears were prepared during necropsy

Clinical Chemistry: blood samples were collected prior to study initiation and on day 15

Urinalysis: urine samples were collected prior to study initiation and on day 15

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, heart, kidneys, liver, lungs, lymph node (cervical and mesenteric), ovaries, pituitary, spleen, testes, thymus, thyroid/parathyroid

Histopathology: The following organs/tissues were examined in groups 1, 2, 3, 5, and 6: adrenals, aorta, brain (cerebrum, cerebellum, brain stem, hippocampus), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), femur (with joint and marrow), gall bladder, gross lesions, heart, ileum, injection site (groups 3-5), jejunum, kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes (cervical, mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual),

sciatic nerve, seminal ducts, skeletal muscle, skin (left flank), skin (patch application site and untreated site in groups 1 and 2), spinal cord (3 sections), spleen, stomach, testes, thymus, thyroid/parathyroid, tissue masses or tumors (including regional lymph nodes), tongue, trachea, ureters, urinary bladder, uterus, vagina. All tissues were stained with hematoxylin-cosin. Additional sections of heart, kidney, and liver were stained with scarlet R.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected from the granisetron patch-treated group at 10, 24, 48, 96, 120, 144, and 168 hr after application of the first patch (3 dogs/sex/time-point). Blood was collected from the placebo patch-treated group at 48 hr after the first application (3 dogs/sex). The blood sampling time-points for the intravenous drug treatment groups were 2 min, 15 min, 30 min, 2 hr, 5 hr, and 8 hr post-dose on day 7 (3 dogs/sex/time-point). Blood was collected from the intravenous vehicle control group at 2 min post-dose on day 7 (3 dogs/sex). The blood sampling time-points for the oral drug treatment group were 0.5, 1, 1.5, 3, 5, and 8 hr post-dose on day 7 (3 dogs/sex/time-point). Plasma concentrations of granisetron were measured using a validated HPLC method with _____.

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Other: Local effects were evaluated in groups 1 and 2 immediately after and at 1 hr after patch removal on days 8 and 15. Local effects were evaluated daily in the intravenously treated groups. A hearing test was performed prior to study initiation and on week 2.

RESULTS:

Mortality: None.

Clinical Signs: Salivation occurred sporadically in the 3 mg/kg/day iv group. This effect appeared during administration and continued for up to 5-20 min.

Bodyweight: Transdermal, intravenous, and oral administration of granisetron had no effect on weight gain. The mean weight (\pm S.D.) of placebo patch-treated males and females was 7.33 ± 0.58 kg and 6.33 ± 0.61 kg, respectively, on day 1, and 7.30 ± 0.36 kg and 6.53 ± 0.81 kg, respectively, on day 15. The mean weight (\pm S.D.) of the intravenous vehicle control males and females was 6.97 ± 0.25 kg and 6.20 ± 0.40 kg, respectively, on day 1, and 7.53 ± 0.40 kg and 6.70 ± 0.46 kg, respectively, on day 15.

Food Consumption: Results were reported as g/kg bodyweight/day. No effects were observed.

Ophthalmoscopy: No abnormalities were observed.

ECG: The timing of ECG recordings is stated above in the "METHODS" section. The following parameters were reported: heart rate, P segment, PQ, QRS, QT, and QT_c (van de Water formula). Systolic and diastolic pressure was measured on day 1 and at the end of week 2, after completion of the ECG. Mean heart rate was increased by 20.6 bpm in the 3 mg/kg/day iv males on day 1, relative to the vehicle control value, and by 34.3 bpm relative to the baseline

value. Similar effects were observed in this group on week 2 (+34 bpm relative to the vehicle control value, +39 bpm relative to the baseline value). The increase in heart rate occurred at 5 min post-dose, but disappeared by 30 min. Systolic pressure was increased by 11% and 18% in the 2 mg/kg/day po males and females, respectively, on day 1, relative to the intravenous vehicle control value (13% and 12% increase, respectively, relative to the baseline values). Diastolic pressure in the 2 mg/kg/day po females was decreased by 15% relative to the intravenous vehicle control value on week 2 (20% decrease relative to the baseline value).

The PQ interval in the granisetron patch-treated males was decreased by 15% relative to the placebo patch value, and by 13% relative to the baseline value on week 2. QRS in the granisetron patch-treated males was decreased by 17% relative to the placebo patch value, and by 22% relative to the baseline value on day 1. In the intravenous groups, granisetron (3 mg/kg/day) produced prolongation of QRS in females. On week 2, this increase was up to 39% relative to the vehicle control value, and 27% relative to the baseline value. One female in the granisetron patch-treated group exhibited a 36 msec increase in QT_c on day 1, relative to the baseline value. Two males in the 2 mg/kg/day po group exhibited QT_c increases of 22-24 msec on week 2, relative to the mean control value. These increases were 19-27 msec relative to the baseline values. Given that QT_c was unaffected in the 3 mg/kg/day iv group, which had the highest C_{max} value, the relationship of the QT_c increases to drug treatment is uncertain. The P segment was unaffected.

Hematology: The results are shown in the table below. Changes in the granisetron patch-treated group are expressed relative to values from the placebo patch group. Changes in the intravenous and oral granisetron-treated groups are expressed relative to values from the intravenous vehicle control group.

Parameter	Group	Change	Day
Eosinophils	Granisetron 6% laminate patch ^m	69% decrease	15
	2 mg/kg/day po ^m	2.2-fold increase (ns)	15
Basophils	3 mg/kg/day iv	75% increase (ns)	15
ESR (mm/hr)	Granisetron 6% laminate patch ^m	2.1-fold increase (ns)	15
	Granisetron 6% laminate patch ^f	3.9-fold increase (ns)	15

ESR: erythrocyte sedimentation rate

ns: not significant

m: males

f: females

The observed effects in the granisetron patch-treated group included a decrease in eosinophils (males only) and an increase in erythrocyte sedimentation rate. In contrast, eosinophils were increased in the 2 mg/kg/day po males. Basophils were increased in the 3 mg/kg/day iv group.

TPT and APTT were unaffected. Bone marrow smears were examined in all groups. The myeloid:erythroid ratio was unaffected.

Clinical Chemistry: One female in the 2 mg/kg/day po group exhibited a 5.9-fold increase in ALT on day 15, relative to the mean intravenous vehicle control value (4.2-fold increase relative to the baseline value). No hepatic lesions were observed in the microscopic evaluation of this animal.

Urinalysis: The following parameters were reported: specific gravity, color, glucose, bilirubin, ketones, hemoglobin, pH, protein, urobilinogen, nitrite, WBC, RBC, epithelial cells, casts, crystals, bacteria, sperm, and yeast. No parameters were affected.

Organ Weights: Absolute and relative (g/kg bodyweight) weights were reported. Changes in the granisetron patch-treated group are expressed relative to values from the placebo patch group. Changes in the intravenous and oral granisetron-treated groups are expressed relative to values from the intravenous vehicle control group.

Adrenals: Absolute and relative weight of right adrenal was reduced by 25% and 28%, respectively, in the 3 mg/kg/day iv females.

Liver: Absolute weight was increased by 24% in the 2 mg/kg/day po males.

Lungs: Absolute weight was increased by 32% in the 3 mg/kg/day iv males, and by 30% in the 2 mg/kg/day po females. Relative weight was increased by 22% in the granisetron patch males, by 28% in the 3 mg/kg/day iv males, and by 29% in the 2 mg/kg/day po females.

Lymph Nodes (cervical): Absolute weight was increased by 2.1- and 2.8-fold in the granisetron patch males and females, respectively. Relative weight was increased by 2.3- and 2.8-fold in the granisetron patch males and females, respectively. Absolute weight was reduced by 40-43% in the 0.3 and 3 mg/kg/day iv males, by 33% in the 2 mg/kg/day po males, and by 22% in the 2 mg/kg/day po females. Relative weight was decreased by 44-46% in the 0.3 and 3 mg/kg/day iv males, and by 39% in the 2 mg/kg/day po males.

Lymph Nodes (mesenteric): Absolute weight was increased by 63% and 31% in the granisetron patch males and females, respectively. Relative weight was increased by 74% and 35% in the granisetron patch males and females, respectively. Absolute weight was decreased by 30% in the 3 mg/kg/day iv males. Relative weight was decreased by 32% in the 3 mg/kg/day iv males.

Spleen: Absolute weight was increased by 27% in the 2 mg/kg/day po males. Absolute and relative weight were decreased by 36% and 39%, respectively, in the 3 mg/kg/day iv females.

Thymus: Absolute weight was reduced by 44% in the granisetron patch males, by 39% and 26% in the 0.3 and 3 mg/kg/day iv males, respectively, and by 21% in the 2 mg/kg/day po males. Relative weight was reduced by 39% in the granisetron patch males, by 40% and 31% in the 0.3 and 3 mg/kg/day iv males, respectively, and by 26% in the 2 mg/kg/day po males.

Thyroid: Absolute weight was reduced by 23% in the granisetron patch-treated males, by 28% and 23% in the 0.3 and 3 mg/kg/day iv males, respectively, and by 32% in the 2 mg/kg/day po males. Relative weight was decreased by 24% in the 0.3 and 3 mg/kg/day iv males, and by 38% in the 2 mg/kg/day po males.

Gross Pathology:

Lungs: Emphysematous appearance was observed in the 3 mg/kg/day iv group (1/6 dogs). Nodular indurated focus (10 mm diameter) occurred in the 2 mg/kg/day po group (1/6 dogs).

Spleen: Scarred induration (10 x 30 mm) was observed in the 2 mg/kg/day po group (1/6 dogs).

Thyroids: Size reduction of right thyroid occurred in the 3 mg/kg/day iv group (1/6 dogs).

Histopathology:

Brain: Hydrocephalus in frontal lobe of cerebrum and hippocampus occurred in the 2 mg/kg/day po group (1/6 dogs).

Early Injection Site: Granular tissue in vessel wall was observed in the 3 mg/kg/day iv group (1/6 dogs). Perivascular hemorrhage occurred in the 3 mg/kg/day iv group (1/6 dogs).

Last Injection Site: Granular tissue in vessel wall was observed in the 3 mg/kg/day iv group (1/6 dogs).

Liver (scarlet R stain): Peripheral fatty infiltration occurred in the granisetron patch group and the 3 mg/kg/day iv group (2/6 dogs in each group).

Lungs: Alveolar emphysema was observed in the 3 mg/kg/day iv group (1/6 dogs). Foamy macrophages occurred in the 3 mg/kg/day iv group (1/6 dogs). These lesions occurred in two different animals. Purulent bronchopneumonia was observed in the 2 mg/kg/day po group (1/6 dogs).

Lymph Node (cervical): Hemorrhage occurred in the granisetron patch group (4/6 dogs).

Skin (patch application site): Subepithelial edema occurred in the granisetron patch group (3/6 dogs).

Toxicokinetics: Plasma levels of granisetron were measured. The results are shown in the table below.

Dose	Sex	t _{max} (hr)	C _{max} (ng/ml)	AUC (ng•hr/ml)	Mean Daily AUC (ng•hr/ml)	C _{avg} (ng/ml)
6% granisetron patch Days 1-7	M	19.3 ± 8.1	101.4 ± 23.6	7697 ± 1147 ^a	1100 ± 164	45.8 ± 6.8
	F	48.0 ± 0.0	57.1 ± 14.0	4657 ± 906 ^a	665 ± 129	27.7 ± 5.4
0.3 mg/kg/day IV Day 7	M	0.03 ± 0.0	67.8 ± 8.4	75.4 ± 7.3 ^b	na	na
	F	0.03 ± 0.0	56.7 ± 24.0	61.5 ± 4.4 ^b	na	na
3 mg/kg/day IV Day 7	M	0.03 ± 0.0	715 ± 126	812 ± 44 ^b	na	na
	F	0.03 ± 0.0	695 ± 175	626 ± 38 ^b	na	na
2 mg/kg/day PO Day 7	M	1.33 ± 0.29	90.2 ± 62.1	243 ± 171 ^b	na	na
	F	1.00 ± 0.0	56.7 ± 28.3	139 ± 71 ^b	na	na

Values are the mean ± S.D. of 3 dogs.

a: 0-168 hr

b: 0-8 hr

na: not applicable

Males in the granisetron patch group exhibited a higher mean daily AUC in comparison to the 3 mg/kg/day iv males (AUC_{0-8 hr}), whereas no difference was observed in females. The C_{max} values in the granisetron patch group were similar to those of the 0.3 mg/kg/day iv group.

Granisetron levels in the intravenous and oral treatment groups were near the limit of quantification (0.5 ng/ml) at 8 hr post-dose, the final time-point. Substantial variation in plasma

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Edema Formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Day 8: Erythema in the placebo and granisetron patch-treated groups occurred with similar severity. Scores of 1 or 2 were obtained for each observation time. Edema scores of 0 or 1 were observed for the placebo and granisetron patch-treated groups. A complete absence of edema was observed in 2/3 drug-treated females, whereas all the placebo-treated females exhibited very slight edema (score of 1). In males, the severity of edema was not increased by the drug.

Day 15: Erythema occurred in the placebo and granisetron patch-treated groups, as described above for day 8. A complete absence of edema was observed in 5/6 dogs in the granisetron patch-treated group, whereas all dogs in the placebo group had edema scores of 1. Only one of the drug-treated dogs had an edema score of 1.

No signs of local irritation were observed in the intravenous groups. Auditory testing results were normal in all animals.

Conclusions: The granisetron patch produced no gross signs of drug-related irritation at the application site. However, microscopic examination revealed the presence of subepithelial edema at the application site in the granisetron patch-treated group, whereas the placebo patch did not produce this effect. The estimated mean daily AUC value for the granisetron patch-treated group was comparable to the AUC value in the 3 mg/kg/day iv group. Systemic toxicity in the granisetron patch group, intravenous granisetron HCl groups, and the oral granisetron HCl group was minimal. Liver appeared to be a target organ of toxicity in the granisetron patch group, the 3 mg/kg/day iv group, and the 2 mg/kg/day po group, based on the incidence of peripheral fatty infiltration and a marked increase in ALT. The only lesion that occurred exclusively and with high incidence in the granisetron patch group was hemorrhage in the cervical lymph nodes. The significance of this observation is uncertain. Lungs appeared to be a target organ of toxicity in the 3 mg/kg/day iv and 2 mg/kg/day po groups.

2-Week Subchronic Toxicity Study of Granisetron HCl by 24-H Continuous I.V. Infusion to Beagle Dogs

Key Study Findings: pulmonary lesions (interstitial pneumonia, alveolar emphysema, and foamy macrophages) and spleen congestion (red pulp) occurred in all treatment groups

Study # 19336/05

Vol. 12

Conducting Laboratory and Location:

b(4)

Date of Study Initiation: April 10, 2005 (report dated December 9, 2005)**GLP Compliance:** A statement of compliance was included.**QA Report:** yes (x) no ()**Drug:** lot # FX5103; 100.3% pure

METHODS: Beagle dogs (males: 6.5-8.5 months old, 6.4-9.7 kg; females: 7.0-8.5 months old, 6.1-8.7 kg) were treated intravenously with 0 (vehicle), 0.3, 1, or 3 mg/kg/day granisetron HCl for two weeks (3 dogs/sex/group). The test and control articles were administered using continuous (24 hr/day) infusion via an implanted catheter in the vena cava caudalis. The dose volume was 24 ml/kg/day, and the vehicle was saline. At seven days prior to study initiation, the catheters were implanted and saline was continuously infused (24 ml/kg/day) to acclimatize the animals to the infusion conditions. The animals were sacrificed on day 15. Dose selection was based on the results of a 1-week dose range-finding study in dogs using continuous iv infusion (study # 19335/05, reviewed above). The dose levels used in that study were 0.3, 1, and 3 mg/kg/day (no control group was included). No signs of local or systemic toxicity were observed. The authors stated that the systemic exposure at 3 mg/kg/day was similar to that observed for the maximum intravenous bolus dose (3 mg/kg/day) in previous subchronic toxicity studies in dogs (NDA 20,239). The present study included the following parameters:

Observations and Times:**Mortality:** twice daily**Clinical Signs:** daily**Bodyweight:** days 1, 8, and 15**Food Consumption:** daily (mean weekly values were reported)**Ophthalmoscopy:** prior to study initiation and on week 2**ECG:** Recordings were taken before and at 4 hr after the start of infusion on day 1. ECG was repeated at the end of week 2. Systolic and diastolic pressure was measured after completion of each ECG recording.**Hematology:** blood samples were collected prior to study initiation and on day 15; bone marrow smears were prepared during necropsy**Clinical Chemistry:** blood samples were collected prior to study initiation and on day 15

Urinalysis: urine samples were collected prior to study initiation and at the end of week 2

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, heart, kidneys, liver, lungs, lymph node (cervical and mesenteric), ovaries, pituitary, spleen, testes, thymus, thyroid/parathyroid

Histopathology: The following organs/tissues were examined in all groups: adrenals, aorta, brain (cerebrum, cerebellum, brain stem, hippocampus), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), femur (with joint and marrow), gall bladder, gross lesions, heart, ileum, infusion site (tip of catheter in vena cava caudalis), jejunum, kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes (cervical, mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal ducts, skeletal muscle, skin (left flank), spinal cord (three sections), spleen, stomach, testes, thymus, thyroid/parathyroid, tissue masses or tumors (including regional lymph nodes), tongue, trachea, ureters, urinary bladder, uterus, vagina. All tissues were stained with hematoxylin-cosin. Additional sections of heart, kidney, and liver were stained with scarlet R.

Adequate Battery: yes (x) no ()

Peer Review: yes () no (x)

Toxicokinetics: Blood samples were collected from the treatment groups at 1, 4, 24, 72, 120, and 168 hr after the start of infusion (3 dogs/sex/group/time-point). Blood was collected from the control group at 168 hr after the start of infusion (3 dogs/sex). Plasma concentrations of granisetron were measured using a validated HPLC method with fluorescence detection.

Other: A hearing test was performed prior to study initiation and on week 2.

RESULTS:

Mortality: No deaths occurred.

Clinical Signs: No signs were observed.

Bodyweight: Weight gain was reduced by 46% and 82% in the 1 and 3 mg/kg/day males, respectively. Weight loss occurred in the control and 3 mg/kg/day females (-0.57 and -0.3 kg, respectively). The mean weight (\pm S.D.) of the control males and females was 8.33 ± 1.19 kg and 7.60 ± 0.98 kg, respectively, on day 1, and 9.07 ± 1.51 kg and 7.03 ± 1.21 kg, respectively, on day 15.

Food Consumption: Results were reported as g/kg bodyweight/day. Food intake in the 0.3, 1, and 3 mg/kg/day males was decreased by 20-24% on week 1, and by 11-18% on week 2.

Ophthalmoscopy: No abnormalities were observed.

ECG: The following parameters were reported: systolic and diastolic pressure, heart rate, P segment, PQ, QRS, and QT. QT_c was calculated using the van de Water formula. Heart rate, P segment, PQ, QRS, and QT_c were unaffected. Systolic pressure in the 3 mg/kg/day females was decreased by 28% on week 2, relative to the vehicle control value (22% decrease relative to the baseline value on day 1). Diastolic pressure in the 3 mg/kg/day females was decreased by 20% on week 2, relative to the vehicle control value (14% decrease relative to the baseline value on day 1).

Hematology: Erythrocyte sedimentation rate (mm/hr) was increased by 25-fold in one of the 3 mg/kg/day females on day 15, compared to either the vehicle control or baseline value. TPT and APTT were unaffected. Myeloid:erythroid ratio in bone marrow was unaffected.

Clinical Chemistry: No parameters were affected.

Urinalysis: The following parameters were reported: specific gravity, color, glucose, bilirubin, ketones, hemoglobin, pH, protein, urobilinogen, nitrite, WBC, RBC, epithelial cells, casts, crystals, bacteria, sperm, and yeast. No parameters were affected.

Organ Weights: Absolute weight and relative weight (g/kg bodyweight) were reported.

Adrenals: Absolute weight of left and right adrenals was reduced by 17% and 22%, respectively, in the 3 mg/kg/day females. Relative weight of left and right adrenals was increased by 24% in the 3 mg/kg/day females, an effect that was due to loss of bodyweight.

Brain: Relative weight was increased by 21% in the 3 mg/kg/day females.

Kidneys: Absolute weight of left kidney was reduced by 19%, 14%, and 21% in the 0.3, 1, and 3 mg/kg/day males, respectively. Absolute weight of left and right kidneys was reduced by 13% and 16%, respectively, in the 3 mg/kg/day females.

Liver: Absolute weight was reduced by 19% in the 3 mg/kg/day males. Absolute weight was reduced by 14% and 17% in the 0.3 and 3 mg/kg/day females, respectively.

Lungs: Absolute weight was reduced by 48%, 22%, and 22% in the 0.3, 1, and 3 mg/kg/day females, respectively.

Lymph Nodes (cervical): Absolute weight was reduced by 43% and 14% in the 1 and 3 mg/kg/day males, respectively. Absolute weight was reduced by 31% in the 3 mg/kg/day females.

Lymph Nodes (mesenteric): Absolute weight was increased by 89%, 60%, and 57% in the 0.3, 1, and 3 mg/kg/day females, respectively. Relative weight was increased by 87%, 72%, and 91% in the 0.3, 1, and 3 mg/kg/day females, respectively.

Spleen: Absolute weight was reduced by 48% and 25% in the 1 and 3 mg/kg/day males, respectively.

Testes: Absolute weight of left and right testes was increased by 20-53% in the 0.3, 1, and 3 mg/kg/day groups. Relative weight of left and right testes was increased by 32-102% in the 0.3, 1, and 3 mg/kg/day groups.

Thymus: Absolute weight was reduced by 16% and 34% in the 1 and 3 mg/kg/day males, respectively.

Thyroids: Absolute weight of left and right thyroids was reduced by 13-32% in the 0.3, 1, and 3 mg/kg/day males. Absolute weight of left thyroid was reduced by 30% and 23% in the 1 and 3 mg/kg/day females.

Gross Pathology:

Spleen: Dark discoloration was observed in the 0.3, 1, and 3 mg/kg/day groups (4/6, 5/6, and 2/6 dogs, respectively).

Lungs: Emphysematous appearance occurred in the 1 and 3 mg/kg/day groups (1/6 dogs in each group). Multiple dark foci were observed in the 3 mg/kg/day group (1/6 dogs).

Histopathology:

Gall Bladder: Subepithelial lymphocytic infiltration occurred in the 3 mg/kg/day group (1/6 dogs).

Lungs: Alveolar emphysema occurred in the 1 and 3 mg/kg/day groups (1/6 dogs in each group). Interstitial pneumonia was observed in the 0.3, 1, and 3 mg/kg/day groups (4/6, 1/6, and 2/6 dogs, respectively). Foamy macrophages were observed in the 0.3 and 3 mg/kg/day groups (3/6 and 1/6 dogs, respectively).

Pancreas: Lymphocytic infiltration occurred in the 3 mg/kg/day group (1/6 dogs).

Spleen: Congestion of red pulp was observed in the 0.3, 1, and 3 mg/kg/day groups (4/6, 5/6, and 2/6 dogs, respectively).

Toxicokinetics: Plasma levels of granisetron were measured. The results are shown in the table below.

Dose (mg/kg/day)	Sex	t _{max} (hr)	C _{max} (ng/ml)	AUC _{0-168 hr} (ng·hr/ml)	Mean Daily AUC (ng·hr/ml)	C _{avg} (ng/ml)
0.3	M	168	4.0 ± 2.0	520 ± 296	74.4 ± 42.2	3.1 ± 1.8
	F	120	3.5 ± 0.6	524 ± 100	74.9 ± 14.2	3.1 ± 0.6
1	M	72	10.8 ± 0.6	1657 ± 120	237 ± 17	9.9 ± 0.7
	F	72	8.8 ± 1.9	1293 ± 282	185 ± 40	7.7 ± 1.7
3	M	72	38.7 ± 16.2	5288 ± 1372	755 ± 196	31.5 ± 8.2
	F	120	32.2 ± 8.2	4050 ± 1133	579 ± 162	24.1 ± 6.7

Values are the mean ± S.D. of 3 dogs, except for t_{max} (median value).

The AUC and C_{avg} values in the 1 and 3 mg/kg/day males were 28-31% higher than those of the females. Plasma drug levels were increased with dose level. The C_{max} values in the 1 and 3 mg/kg/day males were 20-23% higher than those of the females. The increases in AUC, C_{avg}, and C_{max} values in males were proportional to the dose increment, whereas the increases in females were slightly lower in relation to dose increment.

Other: All animals responded normally in the hearing test.

Conclusions: A NOAEL was not demonstrated due to the incidence of pulmonary lesions (interstitial pneumonia, alveolar emphysema, and foamy macrophages) and spleen congestion (red pulp) in all treatment groups.

Published Toxicology Studies:

Toxicological Study of Granisetron Hydrochloride- Single Dose Toxicity in Mice and Rats and Repeated Dose Toxicity in Rats and Dogs. Hahansson S, Artus JA, Kelvin AS, Abdi M, Nishioka Y, Iwabuchi M. *The Clinical Report* 1990, 24(10): 371.

Single dose toxicity studies of granisetron were conducted in rats and mice and repeated dose toxicity studies were conducted in rats and dogs following oral and i.v. administration.

Approximate LD₅₀ values for intravenously administered granisetron were 17 and 25 mg/kg for male and female mice respectively. The LD₅₀ values for male and female rats were 14 and 16 mg/kg, respectively. Convulsions were observed in both mice and rats prior to death and in some surviving animals for a short period of time after dosing. Adverse clinical signs in surviving animals also included subdued behavior and tail erection in both species, and paddling of limbs, hunched posture and increased respiration rate in mice and abnormal gait and twitching in rats.

In repeat dose toxicity studies, granisetron was administered intravenously to rats at 0.03, 0.3, 3 and 9 mg/kg/day for one month and at 0.1, 0.5 and 6 mg/kg/day for 3 months, and in dogs at 0.03, 0.3 and 3 mg/kg/day for 1 month and at 0.1, 0.5 and 3 mg/kg/day doses for 3 months. A proportion of control and high dose animals were maintained for 2 and 4 week withdrawal periods after completion of the 1-month and 3-month dosing periods, respectively.

Physical signs recorded at the high dose included occasional convulsions in both species, and low incidences of vomiting in dogs. Mild, sporadic deviations in GOT and GPT activities were observed in dogs in the 1-month and 3-month toxicity studies, mainly affecting the high dose males. Fat deposition of diffuse or peri-portal type was observed in the liver of a majority of high dose females in the 3-month rat study; this change was not observed at the end of the 4-week recovery period. There were no toxicologically significant effects on body weight, food or water consumption, ophthalmoscopy, electrocardiogram, hematology, urinalysis, organ weights or macroscopic pathology. The no observed effects levels (NOAEL) for the 1- and 3-month toxicity studies were considered to be 3 and 0.5 mg/kg/day for rats and 0.3 and 0.5 mg/kg/day for dogs, respectively.

Toxicological Study of Granisetron Hydrochloride (Second Report) – Oral Repeated-Dose Toxicity in Rats. Abdi M, Hakansson S, Kelvin A and Toseland CD. *Jpn Pharmacol Ther* 1995, 23 (5); 1237-1247.

Sprague-Dawley rats (10 animals/sex/group, 5-6 weeks of age, body weights 101-125 g for the 1-month study; 20 animals/sex/group, 4-5 weeks of age, body weights 76-100 g for the 6-month study) were used in the studies. For the 1-month study, the doses were 0.25, 25 and 125 mg/kg/day, and for the 6-month study, the doses were 0.25, 5 and 100 mg/kg/day. There were recovery groups for the control and the high dose groups. The animals were subjected to observation of general condition, measurement of body weight, food and water consumption and ophthalmic examinations. Clinical chemistry, hematology and urinalysis were conducted in week 4 of administration and in week 2 of the recovery period for the 1-month study, and in weeks 5, 12 and 25 of dosing (for urinalysis, weeks 4, 11 and 24) and at the end of the 6-week recovery period for the 6-month toxicity study. At the end of the dosing and recovery periods, the animals were sacrificed, complete necropsies performed, and weights of the kidneys, liver, heart, lungs, spleen, adrenals, brain, testes, ovaries, pituitary, uterus and thymus were recorded. Histopathological examinations were conducted for a standard list of organs/tissues.

In the 1-month toxicity study, there were mortalities at the 250 mg/kg/day dose, and clinical signs such as reduced activity, ataxia, tremor, salivation, stained coat, rough coat and other changes were observed at this dose. The 250 mg/kg/day dose was reduced to 125 mg/kg/day from day 7. Decreased body weight gain was observed at the high dose. At the end of the dosing period, hematological (elevation of platelet, total leucocyte, neutrophil and lymphocyte count) and clinical chemistry (slight elevation of ALP and GTP) changes were observed in males receiving the high dose. High dose males and females had increased liver weight compared to controls. On histopathological examination, about half of the males and females of the high dose group exhibited stronger focal hepatitis as compared to controls; 5 males and two females from this group had higher liver glycogen levels. Both changes were reversible at the end of the recovery period.

In the 6-month study, salivation, rough coat and stained coat were noted at the high dose. Increased ALP levels were observed in males and females of the high dose group at all points of measurement. Slight elevation of bilirubin levels was observed in females of the high dose group. Hepatomegaly and increased liver weights were observed at the high dose, and histopathologically, eosinophilic or basophilic change of hepatocytes and increased hepatocyte glycogen levels were observed in males and females of the high dose group. Thus, the NOAEL was estimated as 25 mg/kg/day.

Toxicological Study of Granisetron Hydrochloride (Third Report) – Oral Repeated-Dose Toxicity in Dogs. Abdi M, Hakansson S, Kelvin A and Toseland CD. Jpn Pharmacol Ther 1995, 23 (5); 1249.

One and six month repeat dose oral toxicity studies with granisetron were conducted in beagle dogs. Eight to 9 month-old dogs were used for the 1-month study, and for the 9-month study, the animals were about 6 months old. For the main study groups, 3 animals/sex were used and for the recovery groups (control and the high dose groups, 2 and 6 weeks recovery, respectively), 2 animals/sex were used. The doses used were 0.25, 2.0 and 10 mg/kg/day for the 1-month study, and 0.25, 1.5 and 10 mg/kg/day for the 6-month study. For the 1-month study, the initial high dose of 20 mg/kg/day was reduced to 15 mg/kg/day on Day 2 and to 10 mg/kg/day on Day 5 because of deterioration of general condition.

No mortalities were observed in any group in the 1-month study. At the 20 mg/kg/day dose, convulsions were observed after the first dose. On Day 5, muscular tremor/spasm, reduced activity, protrusion of the nictitating membrane, conjunctival erythema, mydriasis, lacrimation and blepharoptosis were observed. Three females of the high dose group showed slight elevations of hemoglobin, hematocrit and RBC levels at the end of the treatment period.

In the 6-month study, High dose males and females showed protrusion of the nictitating membranes on Days 1-8 of administration. Transient clonic spasm was observed in one male on day 61 of dosing. Two males of the high dose group exhibited elevation of hemoglobin, RBC and hematocrit values. No significant effects on body weight, food consumption, Ophthalmology, ECG, Urinalysis and gross or histopathology parameters were observed in any group, either in the 1-month or 6-month studies.

2.6.6.1 Overall toxicology summary

Two-week bridging toxicology studies comparing granisetron patches with i.v. and orally administered granisetron HCl have been conducted in rats and dogs. Application of granisetron patches produced increased severity of edema at the application sites compared to placebo patches. In rats, lymphocytic infiltration in the heart was observed in groups receiving the patch, and oral or i.v. granisetron, and interstitial nephritis in the kidneys was observed in groups receiving the patch and the i.v. dose. In dogs, fatty infiltration in the liver and increased ALT levels were observed in groups receiving all three dosage forms. Thus, sustained exposure of granisetron to rats and dogs for 2 weeks through application of granisetron patch or continuous i.v. administration of granisetron hydrochloride showed similar toxicity profiles to granisetron administered orally once daily. No new target organs of toxicity were identified following application of the patch in rats and dogs.

2.6.6.3 Genetic toxicology

The following published study on the genotoxicity of granisetron was submitted.

Toxicological Study of Granisetron Hydrochloride – Mutagenicity Study. Mitchell IG, Rees RW, Carlton JB, Nishioka Y, Ishii R. The Clinical Report 1990, 24(13): 261.

The genotoxic potential of granisetron was examined in five different assays – the unscheduled DNA synthesis (UDS) assay in HeLa cells, the bacterial reverse mutation assay (Ames test), the gene mutation assay in mouse lymphoma L5178Y cells, the human lymphocyte chromosome aberration assay and the mouse micronucleus assay.

In the UDS assay in HeLa cells, a reproducible and statistically significant increase in the incidence was observed in the absence of microsomal activation (1000 and 2000 µg/mL concentrations). In the presence of metabolic activation, no significant increases in the UDS were observed at any doses. In the Ames test, no significant increases in mutant colonies were observed at any concentrations in the absence or presence of metabolic activation. Granisetron was negative in the mouse lymphoma cell chromosome aberration assay. However, in the human lymphocytes chromosome aberration assay, it was positive in the absence of metabolic activation, and negative in the presence of metabolic activation. The *in vivo* mouse micronucleus assay was negative.

Thus, granisetron was positive in the UDS assay in HeLa cells and the human lymphocyte chromosomal aberration assay in the absence of metabolic activation.

Genetic toxicology summary:

The genotoxic potential of granisetron was examined in five assays – the unscheduled DNA synthesis (UDS) assay in HeLa cells, the bacterial reverse mutation assay (Ames test), the gene mutation assay in mouse lymphoma L5178Y cells, the human lymphocyte chromosome aberration assay and the mouse micronucleus assay. Granisetron was not mutagenic in an *in vitro* Ames test and the mouse lymphoma cell forward mutation assay, and the *in vivo* mouse micronucleus test. It, however, produced a significant increase in UDS in HeLa cells *in vitro* and a significant increased incidence of cells with polyploidy in an *in vitro* human lymphocyte chromosomal aberration test

2.6.6.5 Carcinogenicity

No studies were submitted.

2.6.6.6 Reproductive and developmental toxicology

The sponsor submitted the following published studies in which the reproductive toxicity of granisetron was examined in rats and rabbits.

Toxicity study of Granisetron Hydrochloride – Intravenous Administration Study During Organogenesis in Rats and Rabbits. Baldwin JA, Davidson EJ, Goodwin J, Pritchard AL, Ridings JE, Nishioka Y, Iwabuchi M. The Clinical Report 1990, 24:423

Teratogenicity studies in rats and rabbits were conducted following intravenous administration granisetron. In pregnant female rats, granisetron was administered at i.v. doses of 0.3, 3 and 9 mg/kg/day on gestation days 6 through 15. In rabbits, 0.3, 1 and 3 mg/kg/day doses were administered on gestation days 6 through 18.

Single isolated incidences of tremor and convulsions were observed mainly in rat dams at 3 and 9 mg/kg/day, with one animal being killed in extremis and one death. Among rabbits at 1 and 3 mg/kg, single occurrences of transient increase in breathing rate were observed at each group. Reduced food consumption and a transient slight reduction in body weight were also observed at these doses. Abortions occurred in one rabbit each in the 1 and 3 mg/kg/day groups on gestation days 24 or 25. Autopsy did not show any relationship between abortion and the drug treatment. No treatment related effects on the number of corpora lutea, weight of gravid uterus, number of live fetuses, number of dead fetuses, number of resorptions, pre- or post-implantation loss were observed. No treatment related effects of granisetron were observed on the frequency or type of malformations, anomalies of fetal internal organs, or skeletal variations in rat or rabbit fetuses. Thus, granisetron was not teratogenic in rats and rabbits at i.v. doses up to 9 and 3 mg/kg/day, respectively.

Toxicology Study of Granisetron Hydrochloride – Fertility and General Reproductive Performance Study and Perinatal and Lactation Period Study in Subcutaneously Treated Rats. Baldwin JA, Davidson EJ, Goodwin J, Pritchard AL, Ridings JE, Nishioka Y, Iwabuchi M. The Clinical Report 1990, 24:435.

A fertility and general reproductive performance study (Segment I) and a perinatal and lactation period dosing study (Segment III) were conducted in rats following subcutaneous administration of granisetron. The doses used were 0.1, 0.5 and 6 mg/kg/day in both studies.

In the Segment I study in rats, males in the 6 mg/kg/day group exhibited mild suppression of weight gain throughout the dosing period. However, the body weights of F0 females remained unaffected, and no effect was noted on the general condition, food consumption, or reproductive performance of F0 animals. On examination of the F1 animals, no changes were noted in intrauterine or post-natal development, although the weight of the epididymides was lower than control in the 6 mg/kg/day group. However, no effects on the reproductive function of F1 animals were observed. The growth and reproductive performance of F0 females remained unaffected. In case F2 animals, a slight reduction of the body weight of live fetuses of the 6 mg/kg group was noted. However, the body weights of the neonates on the day after birth remained unaffected.

In the Segment III study, piloerection was noted in several dams of the 6 mg/kg/day group on days 17-18 of pregnancy, and one dam each of the 0.5 and 6 mg/kg/day groups on the day after delivery. In one dam of the 6 mg/kg/day group, the general condition deteriorated immediately after delivery, and all of its neonates died. The dam was sacrificed 3 days after delivery, and autopsy revealed no evidence of the mammary gland development. On analysis of the growth of neonates, the survival rate until weaning was slightly decreased in the 6 mg/kg/day group. This could be due to death of all

neonates from one dam in this group. The general condition, body weight, and physical and functional development of neonates were not affected by the treatment.

Thus, in the s.c. Segment I reproductive toxicity study in rats, granisetron had no significant effects on the fertility and general reproductive performance. In the Segment III pre- and post-natal toxicity study, post-natal growth was not affected at doses up to 0.5 mg/kg. The mean mortality was slightly increased at a dose level of 6 mg/kg.

Toxicological Study of Granisetron Hydrochloride – Reproductive Toxicity in Orally Treated Rats. Baldwin JA, Davidson EJ, Goodwin J, Pritchard AL, Ohta M, Yasuda E, Nishioka Y. Jpn Pharmacol Ther. 1993, 21 (6):115.

Granisetron was orally administered to rats in a fertility and general reproductive performance study (Segment I), an organogenesis study (Segment II) and a peri- and post-natal toxicity study (Segment III). The doses were set at 0.25, 5 and 100 mg/kg/day for Segments I and III, and 0.25, 25 and 125 mg/kg/day for the Segment II study.

In the Segment I study, a dam in the high dose group died on day 19 post partum. Enlarged liver was observed in three F0 males of the high dose group. There was a reduction of gestation body weight gain of high dose females, and there was a significant increase in food and water consumption. There were no treatment-related changes in physical signs or reproductive performance of F0 animals, or organogenesis, development and reproductive performance of the next generation.

In the Segment II study, the body weight gain of the high dose group was slightly reduced. The food consumption of the high dose group was increased. No treatment related effects were observed on the reproductive performance of dams or the development of offspring.

In the Segment III study, two dams of the high dose group died respectively on gestation day 21 and 22, respectively. Subsequent to the deterioration of physical signs, one dam of the mid dose group, and two dams of the high dose group lost their litters. There were no treatment-related effects on the body weight, water or food consumption and reproductive performance of dams, or development of offspring.

Reproductive and Developmental Toxicology Summary:

Granisetron at subcutaneous doses up to 6 mg/kg/day (36 mg/m²/day) and oral doses up to 100 mg/kg/day (600 mg/m²/day) had no effect on fertility and reproductive performance of male and female rats. Teratogenicity studies with granisetron hydrochloride have been conducted in pregnant rats at intravenous doses up to 9 mg/kg/day (54 mg/m²/day) and oral doses up to 125 mg/kg/day (750 mg/kg/day). Teratogenicity studies have been conducted in pregnant rabbits at intravenous doses up to 3 mg/kg/day (36 mg/m²/day) and at oral doses up to 32 mg/kg/day (384 mg/m²/day). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to granisetron. It was not teratogenic in rats and rabbits.

In a Segment III reproductive toxicity study in rats, there were no treatment-related effects on the body weight, water or food consumption and reproductive performance of dams, or development of offspring at oral doses up to 125 mg/kg/day.

Labeling Recommendations: None.

2.6.6.7 Local tolerance

Skin Sensitisation Test of Granisetron Base Laminate in Guinea Pigs

Key Study Findings: granisetron base laminate did not produce skin irritation or sensitization

Study # 19136/05

Vol. 13

Conducting Laboratory and Location: _____

b(4)

Date of Study Initiation: June 30, 2005 (report dated November 30, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: lot # C017GRATDS/SNR800646 (granisetron base patches); purity of drug was not stated

Methods: Male Dunkin-Hartley guinea pigs (age 46 days, 188-281 g) were used. The study groups were treated with dermal application of a placebo laminate (patch) or granisetron base laminate (10 and 20 guinea pigs, respectively). The granisetron patch contained 0.65 mg granisetron base/cm². The inactive matrix included an adhesive, the components of which were not stated. Both the placebo and granisetron patches were 6 cm². The site of application was shaven prior to each administration. In the induction phase of the study, the test and control articles were applied to the left flank region of guinea pigs on day 0. The exposure duration was 3.5 days. This procedure was repeated on days 7 and 14. Skin reactions were evaluated immediately after the end of each exposure (see grading scale below). The challenge phase was initiated on day 28, two weeks after the last application in the induction phase. Both the placebo laminate and granisetron laminate groups were challenged by application of granisetron laminate to the right flank. The exposure time was 6 hr. At 21 hr after removal of patches (day 29), the challenge area was cleaned. Three hours later, an evaluation of erythema and swelling at the application site was performed. Skin reactions were graded based on the following scale.

Grading Scale for Evaluation of Skin Reactions	Grade
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

A second evaluation was performed at 48 hr after patch removal. The positive control group was treated using dermal application of 2% aqueous p-phenylene diamene dihydrochloride solution (20 guinea pigs). The test procedures for the positive control article were similar to those used for granisetron laminate, except for the use of a 6-hr exposure period for the induction treatments. The positive control article was applied using a patch held in place with an occlusive dressing.

Results: In a preliminary test using two animals, the granisetron base laminate did not produce irritation after a 6-hr exposure. In the main study, each animal in the placebo patch and granisetron patch groups had scores of 0 (i.e. no irritation) at all time-points in the induction and challenge phases. All animals in the positive control group had skin reaction scores of 1 or 2 during the challenge phase.

Conclusions: Under the study conditions, granisetron base laminate did not produce skin irritation or sensitization.

Other Studies:**Study Title: Granisetron Base: Evaluation of in vitro phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay.**

Study No.: 2776/1

Conducting laboratory: _____

b(4)

Methods: Granisetron base (batch #0000035001, 98.7% purity) was assessed for phototoxicity to Balb/c 3T3 fibroblast cells using the Neutral Red uptake assay. Balb/c 3T3 fibroblast cells, seeded into 96 well microtiter plates (1×10^5 cells/mL), were treated with a range of concentrations of granisetron (0.3160 to 1000 $\mu\text{g/mL}$) and the positive control, chlorpromazine. Phosphate buffered saline (PBS) was used as the negative control. Final treatment concentrations of PBS were prepared for treatments in the presence and absence of UV-A. After incubations of 45 hours, the wells were washed with PBS and then 100 μL of vehicle, test article or positive control solutions were added to the appropriate wells. All plates were incubated at $37 \pm 1^\circ\text{C}$ in the dark for 60 minutes in a humidified chamber. On completion of the incubation, one plate for each test substance and negative control and one plate containing the positive control were irradiated using the UV-A light source to achieve a UVA dose of 5 J/cm^2 . The remaining plates were kept at room temperature in the dark for the same period.

Following treatment, test solutions were removed, cells were washed with PBS and supplemented DMEM was added to each well. The plates were then incubated at $37 \pm 1^\circ\text{C}$ in the dark for 20 ± 2 hours in a humidified chamber. At the end of incubation, the cells were briefly examined microscopically for signs of cytotoxicity. Immediately following visual assessment, the cells were washed and 100 μL of Neutral Red solution (50 $\mu\text{g/mL}$) was added to each well. The cells were incubated for approximately 3 hours, and at the end of incubation, Neutral Red solution was removed and the cells were washed.

Optical densities (OD) of each well were read on a _____ plate reader at a wavelength of 540 nm. Neutral Red absorbances were expressed in terms of absolute optical density (OD_{540}). Whenever possible, the IC_{50} (concentration inducing a 50% inhibition of Neutral Red uptake) was calculated in the presence and absence of UV-A light.

b(4)

If suitable concentration-response profiles were obtained for the test substance in the presence and absence of UV-A light, a Photo-Irritation Factor (PIF) was calculated.

$\text{PIF} = \text{IC}_{50}$ in the absence of UV-A / IC_{50} in the presence of UV-A

Acceptance Criteria: The assay was considered valid if the following criteria were met:

- a. Irradiated vehicle controls showed a viability of approximately 80% of the non-irradiated vehicle control
- b. OD_{540} in the untreated irradiated controls > 0.4
- c. The positive controls showed a clearly cytotoxic response in the presence of UV-A light, compared to the response in the absence of UV-A light, such that the PIF for the positive control was > 6 .

The test substance was considered phototoxic if PIF values of >5 were obtained, and was considered negative if the PIF values were <2.

Results:

Toxicity: There was evidence of concentration-dependent cytotoxicity at the two highest concentrations of chlorpromazine (100 and 1000 µg/ml) in the absence of UV-A and at 1.0 and 100 µg/mL in the presence of UV-A. There was evidence of cytotoxicity at the highest concentration of granisetron (1000 µg/mL) in the absence and presence of UV-A. No precipitation was observed at any concentration tested.

Phototoxicity: Chlorpromazine induced a positive response with a PIF value of 47.296. In the untreated controls, OD₅₄₀ value was >0.4.

Treatment of cultures with the highest concentration of granisetron (1000 µg/mL) base resulted in a decrease in cell survival, both in the presence and absence of UV-A light. The cell survival at the highest concentration tested was less than 50%. There were no significant differences in neutral red uptake in the presence of UV-A when compared with values in the absence of UV-A light.

Results of the IC₅₀ and PIF calculations are given below:

Test article	IC ₅₀ absence of UV-A (µg/mL)	IC ₅₀ presence of UV-A (µg/mL)	PIF Value
Granisetron Base	864.840	519.582	1.664
Chlorpromazine	41.242	0.872	47.296**

** PIF ≥ 6, therefore positive control response was acceptable.

Thus, under the conditions of the study, granisetron base was not phototoxic in this *in vitro* assay at concentrations up to 1000 µg/mL.

Study Title: Granisetron Base: Induction of chromosome aberrations in Chinese hamster ovary (CHO) cells in the presence of ultra violet light.

Key findings: Granisetron was positive in the Chinese hamster ovary (CHO) cell chromosome aberration assay when exposed to UV irradiation. It was negative in the absence of UV irradiation.

Study no.: 2776/2

Conducting laboratory and location: _____

b(4)

Date of study initiation: November 20, 2006

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Granisetron base, Lot # 0000035001; purity 98.7%.

Methods

Strains/species/cell line: Chinese hamster ovary (CHO) cells were used for the in vitro chromosome aberration assay.

Doses used in definitive study: The concentrations of granisetron base used in the definitive study were, 10, 50, 200, 300, 350, 380, 420, 460, 500, 540, 580 and 620 µg/mL.

Basis of dose selection: The granisetron concentrations for the main chromosome aberration assay were selected based on the phototoxicity range-finding experiment. In the range-finding experiment, no effect on the osmolarity or pH was observed in the presence or absence of UV irradiation. The test article did not show any evidence of phototoxicity in the range-finding experiment.

Negative controls: DMSO was used as the negative control.

Positive controls: 8-methoxypsoralen (8-MOP) was used with and without exposure to the chosen dose of UV light in order to confirm that photoactivation is required to induce photoclastogenicity. In the absence of UV light 4-Nitroquinone-1-oxide (NQO) was used as a positive control.

Incubation and sampling times: Quadruplicate cultures were treated with the vehicle and duplicate cultures were treated with the test article at appropriate concentrations (0.05 mL). Additional sets of duplicate cultures were treated with 0.05 mL of positive controls. The final culture volume was 5 mL. After incubation ($37 \pm 1^{\circ}\text{C}$) in the dark for 15 minutes, the flasks were exposed to the required dose of UV light or remained non-irradiated. Treatment and harvest times were staggered to ensure all flasks could be irradiated within 3 hours of the addition of vehicle, test article or positive control. Post-irradiation, all flasks were irradiated for at least 2 hours prior to removal of treatment media.

Three hours after addition of test or control article, treatment media was removed, cells were washed twice, and the cultures were then incubated for 17 hours before harvesting. Approximately 1.5 hours prior to harvest, colchicine was added (1 µg/mL final concentration) to arrest metaphase. Slides were prepared, cells stained in Giemsa, and examined under a microscope for chromosomal aberrations.

Where possible, 100 metaphases from each sample were analyzed for chromosome aberrations. Aberrant cells in each culture were categorized as follows: cells with structural aberrations including gaps, cells with structural aberrations excluding gaps, and polyploidy, endoreduplicated or hyperdiploid cells.

Results

Study validity: The study was considered valid if,

1. The binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures,
2. The proportion of cells with structural aberrations (excluding gaps) in negative control cultures (non-irradiated) fell within the historical negative control range,
3. At least 160 cells out of an intended 200 were suitable for analysis at each concentration,
4. The positive control chemicals induced statistically significant increases in the proportion of cells with structural aberrations.

Based on the above-mentioned criteria, the study was considered valid.

The study was considered positive if there was a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) at one or more concentrations. The proportion of cells with structural aberrations at each concentration exceeded the historical control range, significant increases in aberrations were observed in the presence of UV but not in the absence, and cells with chromosomal aberrations occur at lower concentrations in the presence of UV or with significantly higher frequencies than the total of aberration frequencies observed in the irradiated vehicle control plus the aberration frequency in the non-irradiated sample at that concentration.

Study outcome: In the range-finding study, cytotoxicity was observed at 129.6 µg/mL and higher concentrations in the presence of UV light at 350 mJ/cm². Phototoxicity data is summarized in the Tables below.

UV 350mJ/cm²

Concentration (µg/mL)	Replicate	Cell Counts	Total Cells/ flask x10 ⁶	Concentration Mean x10 ⁶	Cytotoxicity based on cell counts (%)	Population Doubling	Cytotoxicity based on population doubling (%)*																																																																																											
Vehicle	A	310600	3.11	3.264	0	1.386	0																																																																																											
	B	342200	3.42					3.628	A	382800	3.83	3.828	0	1.616	0	6.047	A	352400	3.52	3.524	0	1.497	0	10.08	A	333800	3.34	3.338	0	1.419	0	16.80	A	317600	3.18	3.176	3	1.347	3	27.99	A	389400	3.89	3.894	0	1.641	0	46.66	A	340200	3.40	3.402	0	1.446	0	77.76	A	338400	3.38	3.384	0	1.439	0	129.6	A	278400	2.78	2.784	15	1.157	17	216.0	A	260800	2.61	2.608	20	1.063	23	360.0	A	238200	2.38	2.382	27	0.932	33	600.0	A	129200	1.29	1.292	60	0.049	96	1000	A	22600
3.628	A	382800	3.83	3.828	0	1.616	0																																																																																											
6.047	A	352400	3.52	3.524	0	1.497	0																																																																																											
10.08	A	333800	3.34	3.338	0	1.419	0																																																																																											
16.80	A	317600	3.18	3.176	3	1.347	3																																																																																											
27.99	A	389400	3.89	3.894	0	1.641	0																																																																																											
46.66	A	340200	3.40	3.402	0	1.446	0																																																																																											
77.76	A	338400	3.38	3.384	0	1.439	0																																																																																											
129.6	A	278400	2.78	2.784	15	1.157	17																																																																																											
216.0	A	260800	2.61	2.608	20	1.063	23																																																																																											
360.0	A	238200	2.38	2.382	27	0.932	33																																																																																											
600.0	A	129200	1.29	1.292	60	0.049	96																																																																																											
1000	A	22600	0.23	0.226	93	-2.466	100																																																																																											

* Population doublings (PD) and relative toxicity are calculated for each concentration as follows:

$$PD = [\log(N \div X_0)] \div \log 2$$

Where, N = mean final cell count/culture at each concentration

X₀ = starting (baseline) count

A and B refers to the number of cultures treated (two [A, B] for vehicle controls and one [A] for test article)

Cytotoxicity was also observed at 77.76 µg/mL and higher concentrations in the presence of UV light at 700 mJ/cm².

UV 700mJ/cm²

Concentration (µg/mL)	Replicate	Cell Counts	Total Cells / flask x10 ⁶	Concentration Mean x10 ⁶	Cytotoxicity based on cell counts (%)	Population Doubling	Cytotoxicity based on population doubling (%)*
Vehicle	A	319200	3.19	3.193	0	1.355	0
	B	319400	3.19				
3.628	A	332400	3.32	3.324	0	1.413	0
6.047	A	309000	3.09	3.090	3	1.307	3
10.08	A	288000	2.88	2.880	10	1.206	11
16.80	A	295200	2.95	2.952	8	1.241	8
27.99	A	291000	2.91	2.910	9	1.221	10
46.66	A	294000	2.94	2.940	8	1.236	9
77.76	A	264400	2.64	2.644	17	1.083	20
129.6	A	212600	2.13	2.126	33	0.768	43
216.0	A	241600	2.42	2.416	24	0.952	30
360.0	A	227600	2.28	2.276	29	0.866	36
600.0	A	119200	1.19	1.192	63	-0.067	100
1000	A	22400	0.22	0.224	93	-2.479	100

* Population doublings (PD) and relative toxicity are calculated for each concentration as follows:

$$PD = [\log(N \div X_0)] \div \log 2$$

Where, N = mean final cell count/culture at each concentration

X₀ = starting (baseline) count

A and B refers to the number of cultures treated (two [A, B] for vehicle controls and one [A] for test article)

As there were no marked differences in toxicity following treatment at the two UV doses (as illustrated above), there was no evidence of phototoxicity. Therefore, one dose of UV light (700 mJ/cm²) was used in the main experiment treatments.

Chromosomal Aberration:

Structural aberration: Treatment of the cells with granisetron base in the absence of UV irradiation did not cause a significant increase in the frequencies of cells with structural chromosome aberrations as compared to concurrent vehicle control. The positive control caused a significant increase in the frequencies of cells with chromosome aberrations. The data for non-irradiated samples are summarized in the Table below.

Table 1
Non irradiated, -S-9, Experiment 1

Treatment ($\mu\text{g/mL}$)	Replicate	Cells Score d	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	Cytotoxicity %
Vehicle	A	100	1	0		
	B	100	1	1		
	Totals	200	2	1		0
200.0	A	100	0	0		
	B	100	0	0		
	Totals	200	0	0	NS	22
350.0	A	100	1	1		
	B	100	1	0		
	Totals	200	2	1	NS	46
380.0	A	100	1	1		
	B	100	0	0		
	Totals	200	1	1	NS	44
8-MOP, 1.0	A	100	2	0		
	B	100	2	2		
	Totals	200	4	2	NS	-
NQO, 0.30	A	100	46	46		
	B	100	37	36		
	Totals	200	83	82	$p \leq 0.001$	-

Binomial Dispersion Test $\chi^2 = 5.04$, Not Significant

§ Statistical significance (Appendix 5)

Numbers highlighted exceed historical negative control range (Appendix 7)

* Cytotoxicity based on population doubling (PD) calculated for each concentration as follows:

$$\text{PD} = [\log(N + X_0)] \div \log 2$$

Where, N = mean final cell count/culture at each concentration

X₀ = starting (baseline) count

Treatment of the cells with granisetron base in the presence of UV irradiation resulted in statistically significant, concentration-related increase in the frequencies of cells with structural

chromosome aberrations, and these increases were higher than the historical control range at 200 and 300 µg/mL concentrations. The data for UV-irradiated samples are summarized in the Table below.

Table 2
UV 700 mJ/cm², -S-9, Experiment 1

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	Cytotoxicity %
Vehicle	A	100	3	2		
	B	100	3	3		
	Totals	200	6	5		
50.00	A	100	5	4		
	B	100	2	1		
	Totals	200	7	5	NS	16
200.0	A	100	11	10		
	B	100	21	17		
	Totals	200	32	27	p ≤ 0.001	28
300.0	A	100	32	32		
	B	100	35	35		
	Totals	200	67	67	p ≤ 0.001	50
8-MOP,1.0	A	100	98	98		
	B	100	97	97		
	Totals	200	195	195	p ≤ 0.001	

Binomial Dispersion Test $\chi^2 = 4.35$, Not Significant

§ Statistical significance (Appendix 5)

Numbers highlighted exceed historical negative control range (Appendix 7)

NS = Not Significant

* Cytotoxicity based on population doubling (PD) calculated for each concentration as follows:

$$PD = [\log(N \div X_0)] \div \log 2$$

Where, N = mean final cell count/culture at each concentration

X₀ = starting (baseline) count

The numbers and types of aberrations are shown in the Table below.

Table 4
UV 700 mJ/cm², -S-9, Experiment 1

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Vehicle	A	100	2	2	0	0	1	0	5	3
	B	100	0	1	2	0	1	0	4	4
	Total	200	2	3	2	0	2	0	9	7
50.00	A	100	1	2	2	1	1	0	7	6
	B	100	1	0	0	1	0	0	2	1
	Total	200	2	2	2	2	1	0	9	7
200.0	A	100	1	2	0	1	10	0	14	13
	B	100	6	6	1	3	14	2	32	26
	Total	200	7	8	1	4	24	2	46	39
300.0	A	100	1	5	1	7	37	0	51	50
	B	100	2	11	3	5	40	3	64	62
	Total	200	3	16	4	12	77	3	115	112
8-MOP,1.00	A	100	0	8	1	19	34	78	140	140
	B	100	5	14	0	15	52	65	151	146
	Total	200	5	22	1	34	86	143	291	286

* Total cells examined for structural aberrations
Totals given for each culture may differ from values given in Appendix 1 if cells are observed which have more than one aberration

Numerical aberration: Frequencies of cells with numerical aberrations were within historical negative control ranges for all granisetron concentrations both in the absence and presence of UV irradiation.

Thus, granisetron base induced increases in the frequency of structural chromosome aberrations in Chinese hamster ovary cells in the presence of UV light, as a maximum UVA dose level of 700 mJ/cm² in the absence of metabolic activation.

Study Title: Photosensitization Test of Granisetron Transdermal Patch after Dermal Application

Key Findings: Under the condition of the experiment, Granisetron Transdermal Patch did not show any photosensitizing effects.

Study Report No.: 21022/06

Conducting Laboratory: _____

b(4)

Date of study initiation: March 06, 2007

Drug: Granisetron Transdermal Patch (52.0 cm², sterile package), containing 34.3 mg/unit granisetron; batch no. 35072. Placebo patches used were of the same size without any drug substance.

Methods: Twenty-five (25) female guinea pigs (age, 31 days; wt., 269-330 g) were randomized into 5 groups (5 animals/group), and each group received the following treatments;

Group	Test item	UV irradiation
1	Untreated control	yes
2	Placebo patch	yes
3	Granisetron Transdermal patch	yes
4	Positive control 2,2'-thio-bis(4,6-dichlorophenol) ³	yes
5	Irritation control: Granisetron Transdermal patch	no

Stage 1 (Induction): The neck region (approximately 10 cm²) of the animals was shaved for application of the patches. Granisetron and placebo patches were cut into pieces of 10 cm² and placed on the shaven skin of the animals for 72 hours. The animals of the positive control group (Group 4) were treated with 1 mL of 5% 2, 2'-thio-bis-(4,6-dichlorophenol)/animal (dissolved in ethanol), applied to gauze patch and placed on the shaved skin of the animals. All patches were covered with a plastic foil and secured with an adhesive bandage. After 72 hours, the patches were removed and the animals were exposed to irradiation using a mercury lamp. The doses of UV irradiation were 10 J/cm² for UV-A and 0.1 J/cm² for UV-B at wave lengths predominantly between 320 – 400 nm. The exact irradiation time (sec) was calculated as follows:

$$\text{Irradiation time (sec)} = 10 \text{ J/cm}^2 / x \text{ W/cm}^2$$

$$1/\text{cm}^2 = v \text{ W/cm}^2 \cdot v = \text{measured UV-A dose in W/cm}^2$$

Test day	4	7	14
UV-A intensity (mW/cm ²)	14.5	14.7	14.4
UV-B intensity (mW/cm ²)	0.1	0.1	0.1
Irradiation time (min)	11.5	11.3	11.5

The induction procedure – 72 hours patch application plus irradiation, was repeated twice starting on test days 4 and 11.

Stage 2 (challenge): After 10 days of the last irradiation, both flank regions of all animals were depilated (approx. 7.5 cm x 5.0 cm). On the following day, the test item patch was applied to the left flank and the placebo patches on the right flank of animals of group 3 and 5. The animals of the placebo control group (Group 2) received the Granisetron Transdermal Patch, applied to the left flank, and placebo patch, applied to the right flank. For the positive control group, 2 mL of 1% 2, 2'-thio-bis-(4,6-dichlorophenol)/animal were applied to gauze patch and placed on the shaved skin of the animals' left flank. No placebo patches were applied to the right flank. The patches were covered and immediately after a 72-hour exposure, UV radiation was applied to the application sites at the following intensities:

Test day	28
UV-A intensity (mW/cm ²)	17.4
UV-B intensity (mW/cm ²)	0.2
Irradiation time (min)	9.6

Skin Evaluation and Scoring: Twenty-four (24) hours after the end of each treatment period, skin reactions were evaluated. During the challenge period, skin reactions were also examined at 48 and 72 hours. The evaluation was based on the following scheme:

Erythema and Eschar Formation	Grade
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible:	4

Oedema Formation	Grade
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approx. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible:	4

The animals were also observed for mortalities (daily), clinical signs (daily) and body weights (prior to and at the end of treatment).

Results: Under the test condition, Granisetron Transdermal Patch did not show any photosensitizing effects in female guinea pigs. The animals of the positive control group showed photosensitizing effect in the form of a severe erythema (beet redness) to slight eschar formation (injuries in depth) 24 to 72 hours after UV exposure. There were no mortalities, and no effects on body weights or clinical signs were observed.

2.6.6.8 Special toxicology studies

None

2.6.6.9 Discussion and Conclusions

The sponsor submitted the NDA according to 505(b)(2) of the Federal Food, Drug and Cosmetic Act, and as such is relying on the Agency's previous findings of safety and effectiveness of the Reference Listed Drugs. The Reference Listed Drugs for this product are the three dosage forms of Kytril (granisetron HCl) approved under NDA 20-239 (Injectable), NDA 20-305 (Tablets) and NDA 21-238 (Oral Solution). However, since this is a new dosage form with no previous experience, the Division recommended that "Preclinical Bridging Studies Comparing the Transdermal (Using the Proposed Patch Formulation) to the Intravenous Route of Administration will need to be Performed in Multiple Species Because the Route, Dosage Form and Duration are Different from What has been Approved". The sponsor conducted 2-week bridging toxicity studies in rats and dogs comparing the patches with the i.v. and oral formulations.

In addition, published pharmacology, PK and toxicology studies with granisetron are provided in the NDA submission. The sponsor also conducted studies to examine the irritation and photosensitization potentials of the patch, and photogenotoxicity potential of granisetron. Toxicology studies with the patch in rats and dogs did not identify any new target organs of toxicity, and the toxicological profiles were similar for the patch, and i.v. and oral formulations. However, there were irritations at the site of application in both species. Thus, the new patch formulation does not raise any serious concerns about the adverse effects of the new dosage form of the drug.

2.6.6.10 Tables and Figures

Tables and Figures are incorporated in appropriate sections of the review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Granisetron is a selective 5-hydroxytryptamine-3 (5-HT₃) receptor antagonist with little or no affinity for other serotonin receptors, including 5-HT₁, 5-HT_{1A}, 5-HT_{1B/C}, 5-HT₂, alpha₁, alpha₂ or beta-adrenoreceptors, dopamine D₂, histamine-H₁, benzodiazepine, picrotoxin or opioid receptors. Serotonin receptors of the 5-HT₃ type are located peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. Stimulation of 5-HT₃ receptors in these areas induces vomiting. Animal studies indicate that granisetron, by binding to 5-HT₃,

receptors, blocks serotonin stimulation and subsequent vomiting after emetogenic stimuli such as cisplatin. Granisetron is currently approved as tablets, oral solution and injectable dosage forms for prevention of nausea and vomiting associated with initial and repeat courses of emetogenic chemotherapy, and nausea and vomiting associated with radiation. The sponsor developed a patch formulation of granisetron and submitted NDA 22-198 According to FDC Act 505 (b)(2). The indication is for the prevention of chemotherapy-induced nausea and vomiting. The sponsor conducted 2-week bridging toxicity studies in rats and dogs comparing the patches with the i.v. and oral formulations. The skin sensitization potential of the patch was examined in guinea pigs, and the in vitro phototoxicity of granisetron was examined on Balb/c3T3 fibroblasts. The photogenotoxic potential of granisetron was examined in the CHO cell chromosome aberration assay, and the photosensitization potential of the patch was examined in guinea pigs. In addition, published pharmacology, PK and toxicology studies with granisetron were provided in the NDA submission.

Following two consecutive applications for 7 days of the granisetron patch (6%) to rats, the maximum plasma concentration was reached in 24 hours. The AUC was higher than that following i.v. administration of a 9 mg/kg/day dose; however, the C_{max} was lower than that following the i.v. administration. The exposure levels in female rats were higher than that in males. Similar to rats, in dogs granisetron patch group had higher mean daily AUC compared to that following a 3 mg/kg/day i.v. dose. The C_{max} values for the granisetron patch group were similar to that of the i.v. dose group. No apparent sex differences in the exposure levels were observed between male and female dogs. Following application of the patches, the maximum plasma concentration was reached in about 19 hours in males and 48 hours in females.

Two-week bridging toxicology studies comparing granisetron patches with i.v. and orally administered granisetron HCl was conducted in rats and dogs. Application of granisetron patches produced increased severity of edema at the application sites compared to placebo patches. In rats, lymphocytic infiltration in the heart was observed in groups receiving the patch, and oral or i.v. granisetron, and interstitial nephritis in the kidneys was observed in groups receiving the patch and the i.v. dose. In dogs, fatty infiltration in the liver and increased ALT levels were observed in groups receiving all three dosage forms. Thus, sustained exposure of granisetron to rats and dogs for 2 weeks through application of granisetron patch or continuous i.v. administration of granisetron hydrochloride showed similar toxicity profiles to granisetron administered orally once daily. No new target organs of toxicity were identified following application of the patch in rats and dogs.

Conclusions:

Thus continuous exposure of granisetron for 2 weeks through application of granisetron patch or continuous i.v. administration of granisetron hydrochloride showed similar toxicity profiles to granisetron administered orally once daily. No new target organs of toxicity were identified following application of the patch in rats and dogs. Thus, from a nonclinical standpoint the granisetron patch does not appear to have any serious safety concerns.

Unresolved toxicology issues (if any): None

Recommendations: From a nonclinical standpoint, approval of the NDA application is recommended.

Suggested labeling: See the labeling recommendations in the Executive Summary section of the review.

Sushanta Chakder, Ph. D.
Pharmacologist, HFD-180

Date

cc.

NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Chakder

HFD-102/Dr Jacobs

HFD- 048/Dr. Viswanathan

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APPENDIX/ATTACHMENTS:

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

/s/

Sushanta Chakder
6/11/2008 05:30:24 PM
PHARMACOLOGIST