Methods

Groups of **5 male** CD-1 mice (**body weight**: 27 to 34 g) received a single dose of 100 or 200 mg/kg of GW433908A (**dose volume:** 5 or 10 ml/kg) intravenously on Day 1. The mice were observed for 14 days thereafter. On Day 15 (prior to necropsy), mice were euthanized for post mortem examination. All mice were examined macroscopically.

Results

Clinical signs:	One of five animals given 100 mg/kg GW433908A exhibited partially closed eyes beginning 19 minutes after dosing, which last about 2 hours.
Body weights:	There was no effect of test article treatment on body weight.
Gross pathology:	No treatment-related macroscopic findings occurred.

25. Study M40431 – GW433908A: Single-dose intravenous toxicity study in CD-1 mice (Report No. RD1998/02552/00)

GW study No.: M40431; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 16 February 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/194/2; Formulation: GW433908A solution in 0.9% sodium chloride solution for injection

Key study findings: The intravenous **NOEL** of GW433908A in mice was established to be **136mg/kg**. This is equivalent to a human dose of approximately **11mg/kg/day** based on body surface area. Myocardial fiber degeneration, necrosis, and myocardial inflammation were seen in animals given 347mg/kg and 500mg/kg within 24 and 48 hours after dosing. Hepatocellular hypertrophy and reduced hepatic glycogen were found in mice at 347mg/kg. These lesions were not observed 14 days after the treatment.

Methods

Groups of three CD-1 mice (body weight for males: 27.3-33.9 g; for females: 21.1-25.7 g; Age: 7 to 8 weeks) were intravenously given GW433908A at dose levels of 136, 217, 347 or 500mg/kg (dose volume: 5 ml/kg), respectively (Stage A study). Groups of six males and six females received intravenously 347mg/kg of GW433908A or 0.9% sodium chloride solution, respectively (Stage B study). Animals were daily observed for signs of ill health. Body weights were recorded twice weekly. At terminal-kill necropsy, a complete gross examination was carried out on each animal and lesions were recorded. Tissues from unscheduled and scheduled deaths were microscopically examined.

Results

Clinical signs:	Decreases in activity, labored breathing, low body carriage, rough coat, gasping, tremors, coolness to touch, splayed hindlegs, sensitive to touch and loose feces were noted in mice ≥217mg/kg.
Body weights:	Decreases in body weight gain in males (6%) and females (4%) at 347mg/kg were noted.
Gross pathology:	No treatment-related macroscopic findings occurred.
Histopathology:	Myocardial fiber degeneration, necrosis, and myocardial inflammation were seen in animals given 347mg/kg and 500mg/kg within 24 and 48 hours after dosing. Hepatocellular hypertrophy and reduced hepatic glycogen were found in mice at 347mg/kg. These lesions were not observed 14 days after the treatment.

Comments

Note that the myocardial changes have not been previously reported in the toxicology studies with amprenavir or GW433908G in mice (Ref.: GW Report No.: RD 1996/00752/00; RD 1999/00017/00).

26. GW433908A: Acute oral toxicity study in rats (Report No. RD1998/00777/00)

GW study No.: R40368; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 20 March 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A solution in reverse-osmosis treated water

Methods

A group of 5 male Han Wistar rats (body weight: 283 to 327 g; age: 9-10 weeks) received a single dose of 2000mg/kg/day of GW433908A (dose volume: 10 ml/kg) by oral gavage and observed for clinical signs daily for 14 days thereafter. Body weights were record once weekly. At necropsy, all rats were euthanized and examined macroscopically.

Results

Clinical signs: One rat exhibited salivation 30 minutes after dosing.
Body weights: There was no effect of test article treatment on body weight.
Gross pathology: There were no dose-related macroscopic findings.

27. GW433908G: A single-dose oral toxicity study in Han Wistar rats (Report No. RD1999/00018/00)

GW study No.: R40425; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 26 January 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/31/1; Formulation: GW433908G in 0.5% (w/w) hydroxypropylmethylcellulose in 0.1% (w/w) Tween 80

Key findings: The oral **NOEL** of GW433908G in male rats was established to be ≥2986mg/kg. This is equivalent to a human dose of approximately ≥**496mg/kg** based on body surface area.

Methods

Groups of six male and female Han Wistar rats (body weight for males: 213 to 284 g; for females: 153-194 g; age: 9-10 weeks) received a single dose of 2986mg/kg/day of GW433908G (dose volume: 10 ml/kg) by oral gavage. The rats were observed for clinical signs daily for 14 days. Body weights were measured. At necropsy, rats were euthanized and examined macroscopically.

Results

Clinical signs and mortality: No treatment-related abnormal clinical signs were observed.
Body weights: There was no effect of test article treatment on body weight.
Gross pathology: There were no dose-related macroscopic findings.

28. GW433908A: Acute intravenous toxicity study in Han Wistar rats (Report No. RD1998/00656/00)

GW study No.: R40371; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 20 March 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A solution in reverse-osmosis treated water

Key findings: The NOEL of GW433908A was established to be ≥100 mg/kg in rats. This is equivalent to a human dose of approximately ≥15.8mg/kg/day based on body surface area.

Methods

Groups of 5 male Han Wistar rats (body weight: 253 to 315 g) received a single intravenous dose of 100 or 200 mg/kg of GW433908A (dose volume: 5 or 10 ml/kg), respectively, and were observed for clinical signs daily for 14 days. Body weights were measured immediately prior to dosing, and 14 days after

dosing. At necropsy, rats were euthanized and examined macroscopically. Tissues from all rats were collected and preserved in 10% neutral buffered formalin except eyes, optic nerves, testes, and epididymides that were preserved in Bouin's fluid. All tissues were examined microscopically by a pathologist.

Results

Clinical signs:	Three of four animals given 200mg/kg GW433908A exhibited vocalization and intermittent jumping motion after dosing.
Body weights:	There was no test article-related body weight changes.
Gross pathology:	Dilation of the left kidney and multifocal red coloration of the prostate were seen in one male rat at 100mg/kg. Tubular basophilia, unilateral hydronephrosis, and serosal hemorrhage of the prostate were observed, which were related to injection trauma. In addition, alopecia was noted in two males given 100mg/kg of GW433908A.

29. GW433908A: Single-dose intravenous toxicity study in Han Wistar rats (Report No. RD1998/02551/00)

GW study No.: R40432; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 16 February 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/194/2; Formulation: GW433908A solution in 0.9% sodium chloride solution for injection

Key findings: The intravenous NOEL of GW433908A for this study was not established. Myocardial fiber degeneration and necrosis, and diffuse periportal hepatocellular vacuolation and reduced hepatic glycogen were observed in rats at 347mg/kg, which were reversible on Day 14.

Methods

Groups of three Han Wistar rats (body weight for males: 232.8-347.5 g; for females: 158.9-226.7 g; age: 9 to 10 weeks) were intravenously given GW433908A at dose levels of 136, 217, 347, or 500mg/kg (dose volume: 5 ml/kg), respectively. Groups of six males and six females received intravenously 347mg/kg of GW433908A, or 0.9% sodium chloride solution, respectively. Animals were observed daily for signs of ill health. Body weights were recorded twice weekly. On necropsy, a complete gross examination was carried out on each animal and lesions were recorded. All tissues from unscheduled and scheduled deaths were microscopically examined.

Results

Clinical signs and mortality:	Ataxia, decreased activity, labored breathing, chewing movement, low body carriage, rough coat, gasping, tremors, red material around the eyes, shaking of the head, and coolness to touch were noted in rats at ≥136mg/kg. One female rat died at 347mg/kg. Three males and three females died at 500mg/kg.
Body weights:	A decrease (11%) in body weight gain in males was noted at 347mg/kg.
Gross pathology:	No treatment-related macroscopic findings occurred.
Histopathology:	Myocardial fiber degeneration and necrosis, and diffuse periportal hepatocellular vacuolation and reduced hepatic glycogen were observed in rats at 347mg/kg, which were reversible on Day 14.

Comments

The intravenous **NOEL** of GW433908A for this study was not established. Note that the myocardial changes have not been reported in previous toxicology studies with amprenavir and GW433908G in rats (Ref.: GW Report No.: RD 1996/00584/00; GW Report No.: RD 1999/00018/00).

Repeated dose toxicity studies in Rats:

30. GW433908A: A 2-Week oral toxicity study in male Han Wistar rats (Report No. RD1998/00711/00)

GW study No.: R40364; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 24 April 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908G solution in reverse-osmosis treated water

This study evaluated the oral toxicity and toxicokinetics of GW433908A when administered to male rats for 14 days.

Methods

Groups of 7 male Han Wistar rats (Glx:Han:WifBR; body weight: 225 - 374 g; age: 67 to 74 days) to evaluate toxicity and groups of 3 (control) or 12 male rats (treated) to evaluate toxicokinetics were dosed twice daily at daily doses of 0, 50, 190, or 750mg/kg/day of GW433908 by oral gavage, for 14 days. Each animal was given a detailed clinical examination once during the pretreatment period and prior to necropsy and observed four times daily for signs of ill health. Body weights and food consumption were recorded weekly. Ophthalmoscopic examinations were performed on all rats pre- and after dosing. Blood samples were collected at terminal necropsy for clinical chemistry and hematology analysis. Urine samples were collected and analyzed after overnight fasting. All animals were killed at the end of the treatment period. A complete gross examination was carried out on each animal and organ weight (adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids) and lesions were recorded. A spectrum of tissues from all animals was taken and preserved in 10% neutral buffered formalin except eyes and optic nerves, which were preserved in Bouin's's solution (Table 1). Histopathologic evaluation was performed on all prepared tissues from control and high dose animals by a pathologist.

Results

Clinical signs:	Salivation was noted on Day 10 in 4 rats given 750mg/kg/day.
Body weights and food consumption:	No body weight and food consumption changes related to the test article were noted.
Ophthalmoscopy:	No ocular changes related to the test article were recorded.
Hematology:	There were no treatment-related effects on cell parameters.
Clinical chemistry and urine analysis:	Serum triglyceride concentration was reduced at **all** doses. More concentrated urine, reflected in increases in urinary creatine (1.8-fold), Na (7.5-fold), K (1.6-fold) and Cl (5-fold) concentrations and an decrease in urine volume (1.7-fold), were observed in males at 750mg/kg/day.
Gross pathology:	Increases in absolute liver weight (12%) and relative liver to body weight (9%) were noted in males at 750mg/kg/day.
Histopathology:	Hepatocellular hypertrophy and hepatocellular basophilia were seen in all rats. Focal myocardial degeneration was seen in rats at 50mg/kg/day and 750mg/kg/day (Table 1).

Comments

The **NOEL** for this study was < 50 **mg/kg/day**. This is equivalent to a **human** dose of approximately < **8 mg/kg/day** based on body surface area. Focal myocardial degeneration was observed in rats at 50mg/kg/day and at 750mg/kg/day.

Toxicokinetics:

Methods

Blood samples were taken at 0, 1, 2, 6 (prior to the second dosing), 7, 8, 10, and 24 hours after the first dosing (controls bled at 7 hours postdose only) on Day 1 and Day 14 for toxicokinetic evaluation. Plasma concentrations of GW433908A and amprenavir were determined, using an [redacted] method.

Results

Both GW433908A, the prodrug of amprenavir, and amprenavir were detected in plasma in this study. Plasma concentrations of amprenavir were determined in all dose groups. Plasma concentrations of GW433908A, however, were determined only for the high-dose group. Amprenavir was present in higher levels than GW433908A in the plasma evaluated (Table 2). Average adjusted values for C_{max} and AUC_{∞} (GW433908A and amprenavir) were shown in Table 2. Note that C_{max} of GW433908A to amprenavir ranged from 0.1% and 5% on Day 1 and Day 14, respectively.

Comments

These results provide evidence of exposure to amprenavir and GW433908A after oral dosing of GW433908A. Because of possible effects of alkaline phosphatase on the hydrolysis of GW433908A in the gut, it would be desirable to perform an enzymatic study to demonstrate the kinetic properties of GW433908A or GW433908G with regard to this enzyme in rats.

Table 1. A 2-week oral toxicity study in male Han Wistar rats

Tissue	GW433908A (0mg/kg/day)	GW433908A (50mg/kg/day)	GW433908A (190mg/kg/day)	GW433908A (750mg/kg/day)	Comments
Adrenal	X	X	X	X	
Aorta	X	X	X	X	X: collected and examined
Brain	X.	X	X	X	
Cecum	X	X	X	X	XX: collected but not examined
Colon	X	X	X	X	
Duodenum	X	X	X	X	
Epididymides	X	X	X	XX	
Esophagus	X	X	X	X.	
Eye & optic nerves	X	X	X	X	
Femur/joint	X	X	X	X	
Harderian glands	X	X	X	X	
Heart	X	X* (1/7)	X	X* (2/7)	X*: focal myocardial degeneration
Ileum	X	X	X	X	
Jejunum	X	X	X	X	
Kidneys	X	X	X	X	
Liver	X	X* (7/7)	X* (7/7)	X* (7/7)	X*: slight hepatocellular hypertrophy and increased hepatocellular basophilia
Lungs (with bronchi)	X	X	X	X	
Mandibular lymph node	X	X	X	X	
Mesenteric lymph node	X	X	X	X	
Pancreas	X	X	X	X	
Peripheral nerve (sciatic)	X	X	X	X	
Pituitary	X	X	X	X	
Prostate	X	X	X	X	
rectum	X	X	X	X	
Salivary gland	X	X	X	X	
Seminal vesicles	X	X	X	X	
Skeletal muscle	X	X	X	X	
Skin (mammary glands)	X	X	X	X	
Spinal cord cervical	X	X	X	X	
Spinal cord lumbar	X	X	X	X	
Spinal cord thoracic	X	X	X	X	
Spleen	X	X	X	X	
Sternum (bone marrow)	X	X	X	X	
Stomach	X	X	X	X	
Testes	X	X	X	X	
Thymus	X	X	X	X	
Thyroids (parathyroids)	X	X	X	X	
Tongue	X	X	X	X	
Trachea	X	X	X	X	
Urinary bladder	X	X	X	X	
Head	X	X	X	X	
Femoral bone marrow smear	X	X	X	X	

Figures in brackets represent the number of animals from which this tissue was examined.

Table 2. Toxicokinetics parameters of GW433908A and amprenavir (141W94) in male Han Wistar rats after oral administration of GW433908G during a 14-day toxicity study

Dose (mg/kg/day)	Day 1				Day 14			
	C_{max} (µg/ml		AUC_{-} (µg*h/ml)		C_{max} (µg/ml		AUC_{-} (µg*h/ml)	
	I	II	I	II	I	II	I	II
50	0.6	--	3.8	--	0.7	--	4.1	--
190	4.2	--	26.0	--	2.1	--	11.5	--
750	7.3	0.08	105	0.4	3.8	0.2	50.2	0.6

I. = amprenavir (141W94); II = GW433908A

31. GW433908G: A 4-week oral gavage toxicity study in Han Wistar rats (RD1998/02573/00)

GW study No.: R40427; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 10 November 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A [redacted], Drug Lot: R4283/31/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethylcellulose in 0.1% (w/w) Tween 80

The study assessed the oral toxicity of GW433908A when administered to male rats twice daily for 4-weeks with a 27-day untreated recovery period.

Methods

Groups of 12 or 21 male or female Han Wistar rats (Glx:Han:WifBR; body weight for males: 150 - 366 g, for females: 100 -250; age: 9 to 10 weeks at the start of dosing) received total daily dose of 0 (vehicle), 149, 478, 1493 or 2240mg/kg/day (dose volume: 10 ml/kg/day) by oral gavage administered twice daily for four weeks. Groups of twelve rats to evaluate toxicity were euthanized for post mortem examination and the remainder (9 rats) maintained untreated for 27 days to study the regression or progression of any treatment-related abnormalities. Each animal was given a detailed clinical examination once during the pretreatment period and prior to necropsy. The animals were observed four times daily for signs of ill health during the study period. Body weights and food consumption were measured once daily from Day 1 to Day 59. Ophthalmoscopic examinations were performed on all pre-treated animals and treated animals. Blood samples were collected at terminal necropsy for clinical chemistry and hematology analysis. Urine samples were collected and analyzed after overnight fasting. At necropsy, a complete gross examination was carried out on each animal and organ weight (adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids) and lesions were recorded. Histopathologic evaluation was performed on all prepared tissues from all animals by a pathologist (Table 3).

Results

Clinical signs. Treatment-related salivation was observed in all animals of both sexes at ≥ 478mg/kg/day during the treatment period.
Body weights and food consumption. There were decreases in body weights in males (11%) and females (17%) at 2240mg/kg/day.
Ophthalmoscopy. There were no ocular changes related to test article.
Hematology. There were no treatment-related effects on cell parameters.
Clinical chemistry and urine analysis: Triglyceride concentration was reduced at all doses in males (28% - 60%) and in females (17% to 56%), which was dose-related and reversible at the end of the recovery period (Day 57). Bile acids were increased in females at 2240mg/kg/day (3-fold), which is treatment-related and reversible. There was a treatment-related, reversible, increase in urine volume in males given ≥478mg/kg/day (1.1 to 2-fold) and females given 2249mg/kg/day (1.5-fold). Correspondingly, decreases in K (-30%), Na (-25%), Cl (-30%) and Cr (-34%) were observed in males at 2240mg/kg, but not at lower doses.
Gross pathology: Males given ≥149mg/kg/day exhibited increases in absolute liver weight (males: 13-26%; female: 10-54% fold) and relative liver to body weight (9%) were noted in males at 750mg/kg/day (male: 9%) liver weights on Day 31.
Histopathology: Dose-related diffuse hepatocytomegaly, multifocal and diffuse follicular cell hypertrophy in both sexes were observed on Day 31, which were not observed at the end of recovery period (Day 57).

Comments

The **NOAEL** for this study was 149**mg/kg/day**. This is equivalent to a **human** dose of approximately 24 **mg/kg/day** based on body surface area. GW433908 caused decreases in triglyceride levels, and increases in liver weights and levels of bile acids, other liver function indicators such as AST, ALT, alkaline phosphatase, total bilirubin and total protein, however, were not changed. Toxicology relevance of these findings remains to be settled. Note that no focal myocardial degeneration was observed in this study.

Table 3. 4-Week oral gavage toxicity study in Han Wistar rats

Tissue No. Examined (Terminal/recovery)	GW433908G 0mg/kg/day (n =12)	GW433908G 149mg/kg/day (n =12)	GW433908G 478mg/kg/day (n =12)	GW433908G 1493mg/kg/day (n =12)	GW433908G 2240mg/kg/day (n =12)	Comments
Adrenal	X	X	X	X	X	X: collected and examined
Aorta	X	X	X	X	X	
Brain	X.	X.	X.	X	X	
Cecum	X	X	X	X	X	
Colon	X	X	X	X	X	
Duodenum	X	X	X	X	XX	
Epididymides	X	X	X	X		
Esophagus	X	X	X	X	X	
Eye & optic nerves	X	X	X	X	X	
Femur/joint	X	X	X	X.	X	
Harderian glands	X	X	X	X	X	
Heart	X	X	X	X	X	
Ileum	X	X	X	X	X	
Jejunum	X	X	X	X	X	
Kidneys	X	X	X	X	X	
Liver	X (M:0; F: 0)	X* (M:9; F:10)	X* (M:12; F:12)	X* (M:12; F:12)	X* (M:12; F:12)	X*: diffuse hepatocyto megaly (Day 31)
Lungs (with bronchi)	X	X	X	X	X	
Mandibular lymph node	X	X	X	X	X	
Mesenteric lymph node	X	X	X	X	X	
Pancreas	X	X	X	X	X	
Peripheral nerve (sciatic)	X	X	X	X	X	
Pituitary	X	X	X	X	X	
Prostate	X	X	X	X	X	
Rectum	X	X	X	X	X	
Salivary gland	X	X	X	X	X	
Seminal vesicles	X	X	X	X	X	
Skeletal muscle	X	X	X	X	X	
Skin (mammary glands)	X	X	X	X	X	
Spinal cord cervical	X	X	X	X	X	
Spinal cord lumbar	X	X	X	X	X	
Spinal cord thoracic	X	X	X	X	X	
Spleen	X	X	X	X	X	
Sternum (bone marrow)	X	X	X	X	X	
Stomach	X	X	X	X	X	
Testes	X	X	X	X	X	
Thymus	X	X	X	X	X	
Thyroids (parathyroids)	X(M:1; F: 0)	X*(M:3; F: 0)	X*(M:10; F: 5)	X*(M:7; F: 7)	X*(M:9; F: 10)	X*: multifocal or diffuse follicular hypertrophy
Tongue	X	X	X	X	X	
Trachea	X	X	X	X	X	
Urinary bladder	X	X	X	X	X	
Head	X	X	X	X	X	
Femoral bone marrow smear	X	X	X	X	X	

Figures in brackets represent the incidence of abnormal findings from animals in which this tissue was examined.
M: male rats; F: female rats.

Toxicokinetics:

Methods

Satellite groups of 4 (control) or 12 rats (treated) of each sex received daily doses of 0 (vehicle), 149, 478, 1493 or 2240mg/kg/day of GW433908G by oral gavage administered twice daily 6 hours apart for 14 days. Blood samples were obtained from these animals on Day 1 and 23 to evaluate toxicokinetics. Blood samples were taken at 0 (predose), 1, 2, 6 (prior to the second dosing), 7, 8, 10, and 24 hours after the first daily dosing (blood samples from control rats were taken 7 hours after the first daily dose) on Day 1

and Day 23. Plasma concentrations of GW433908A were determined using an [redacted] method. Toxicokinetic parameters, C_{max} and AUC_{∞} were calculated.

Results

Both GW433908G, the calcium salt of the phosphate prodrug of amprenavir, and amprenavir were detected in plasma in this study. Exposure to GW433908G varied in a non-dose-dependent manner in male and females on Day 1, but increased dose- dependently on Day 23. Exposure to amprenavir increased dose-dependently in male and females on Day 1 and Day 23. Amprenavir was present in higher levels than GW433908G in the plasma evaluated (Table 4).

Comments

These results provide evidence of exposure to amprenavir and GW433908A after oral dosing of GW433908A. Exposure to amprenavir increased dose-dependently in male and females on Day 1 and Day 23. Amprenavir was present in higher levels than GW433908G in the plasma evaluated.

Table 4. Toxicokinetics parameters of GW433908G and amprenavir (141W94) in Han Wistar rats after oral administration of GW433908G during a 4-week toxicity study

Dose mg/kg/day	Day 1								Day 23							
	C_{max} (µg/ml)				AUC_{∞} (µg*h/ml)				C_{max} (µg/ml)				AUC_{∞} (µg*h/ml)			
	I		II		I		II		I	II			II			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
149	3.0	2.4	2.1	0.4	32	31	15	3.2	1.6	1.7	0.1	0.01	20	23	0.5	0.1
478	5.6	7.1	0.3	0.1	62	83	2.8	1.0	3.5	4.3	0.6	0.1	34	50	1.6	0.6
1493	9.6	11.1	0.1	0.5	224	133	1.6	1.8	5.4	4.9	0.3	0.3	47	68	0.8	1.2
2240	11.3	10.4	0.2	0.1	184	234	1.4	1.8	4.5	4.3	4.9	5.4	53	81	1.2	1.6

I = amprenavir (141W94); II = GW433908G

32. GW433908G: Six month oral gavage toxicity study in Han Wistar rats (Report No. RD1998/02858/01)

Study No.: R40417 [redacted] Study No.: 6169-6233; Conducting facility: [redacted], Date Initiation: 7 December 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/31/1 and R4283/33/1; Formulation: GW433908G suspension (14.9, 47.8. 149.3 and 224 mg/ml) in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

- Liver was identified as the major target organ for toxicity with GW433908 in this study. Multinucleated hepatocytes were seen on microscopic examination at ≥149 mg/kg/day GW433908 (≥100 mg/kg/day APV dose equivalence). This study showed a lower incidence of individual necrotic hepatocytes. A No Observed Adverse Effect Level (NOAEL) was not determined for males in this study. The No Observed Adverse Effect Level (NOAEL) for females was 149 mg/kg/day GW433908 (100 mg/kg/day APV dose equivalence) based on the absence of multinucleated hepatocytes at this dose level.
- The AUC to APV at the end of the study was similar in female rats but approximately 1.9-fold less in male rats compared to previous rat studies with APV (Re: Reports TTEP/96/0017/00 and RD1966/00584/00).

Method

Dosing:

Species/Strain:	Rat/Han Wistar; Tac:Glx:WifBR
#/sex/group or time point (main study):	12 rats/sex/group
Satellite groups used for recovery:	8 rats/sex/group
Satellite groups used for toxicokinetic:	4 (control group) or 12 rats/sex/group (treatment)
Age:	approximately 10 weeks
Body Weight:	Male = 260 to 344 g; Female = 162 to 225 g

Doses in administration units:	0, 149, 478, 1493 and 2240 mg/kg/day GW433908G, equivalent to 0 (vehicle), 320, 1000 and 1500 mg/kg/day amprenavir, respectively
Rout, dosing frequency and dose volume:	Oral (gavage); dosed twice daily, 6 hours apart; 5 mL/kg/dose (10 mL/kg/day)

Observations and times:

Clinical examiniation:	Once pretreatment and once prior to necropsy
Body weights:	Once daily pretreatment and once daily during the treatment period
Food consumption:	Once daily pretreatment and once daily during treatment period
Hematology:	At Weeks 5, 14, 27, and at the end of recovery period
Clinical chemistry:	At Weeks 5, 14, 27, and at the end of recovery period
Urinalysis:	At Weeks 5, 14, 27, and at the end of recovery period
Gross pathology (main):	At the end of the treatment period (terminal)
Gross pathology (recovery):	at the end of the recovery period (recovery)
Organs weighed:	Adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids
Histopathology:	A spectrum of tissues from all animals were taken, preserved, and examined by a pathologist (Appendix Table 1).
Toxicokinetics:	For toxicokinetic evaluations, blood samples from rats in the control group (4/sex/group) and the test article treatment groups (12/sex/group) were collected on Days 1, 92 and 179 of the study period and plasma APV and GW433908G were analyzed.
Other:	For recovery evaluation, rats (8/sex/goup) in the control, the highest dose GW433908G (2240 mg/kg/day) and the intermediate dose group GW433908G (1493 mg/kg/day) were maintained untreated for an additional 30 days to study the regression or progression of any treatment-related abnormalities

The doses used in this study were selected based upon the results of 2-week and 1-month rat studies (Re: RD 1998/00711/00, & RD 1998/02573/00). Note that the 6-hour interval was selected to aid technical efficiency and was not based on the kinetics of amprenavir or GW433908G.

Results

Mortality: Three rats died or sacrificed over the course of the study. One female at 478 mg/kg/day GW433908 (320 mg/kg/day APV dose equivalence) died on Day 139 due to urolithiasis of the kidney and urinary bladder, which was considered not related to treatment by the sponsor. One control male rat and one male in the 1493 mg/kg/day GW433908 group (1000 mg/kg/day APV dose equivalence) was sacrificed in moribund condition on Days 139 and 93, respectively. These two rats were designated as toxicokinetic animals and were not necropsied. The cause of moribund condition or death was not determined.

Clinical signs: Treatment-related excessive salivation was observed in rats at doses of ≥478 mg/kg/day GW433908 (320 mg/kg/day APV dose equivalence) one hour post-dose.

Body weight: No treatment-related changes in body weights in males and females were seen.

Food consumption: Female rats at ≥1493 mg/kg/day GW433908 (≥1000 mg/kg/day APV dose equivalence) had mild increase (5-15%) in food consumption, beginning at Weeks 5 and 8, respectively. Consumption returned to control levels during the recovery period.

Hematology: Treatment-related alterations in hematology occurred at all doses (Table 1-1).

Clinical chemistry: Treatment-related changes in clinical chemistry parameters were seen in rats at all doses. Treatment-related increases in liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamate dehydrogenases (GDH) were seen in male rats at ≥149 mg/kg/day GW433908, which were associated with the microscopic findings observed in the liver (Table 1-2).At Week 27, minimal increases (9-15%) in serum inorganic phosphorus were seen in rats at 478 mg/kg/day GW433908

Urinalysis: Increased urine volume with decreased specific gravity and increased creatine, potassium and sodium excretion were seen in rats at all doses (Table 1-3). These changes were consistent over

time, but did not show a clear relationship to dose. These changes were considered inconclusive evidence of an adverse toxicological effect due to the small magnitude of the differences.
Gross pathology: Treatment-related increases in absolute liver weight (males: 11-44%; female: 15-69%) and in relative liver to body weight (males: 10-46%; female: 16-61%) were noted in rats given ≥149mg/kg/day, which were reversible (Table 1-4).
Histopathology: Treatment-related microscopic findings were limited to the liver. Minimal to moderate hepatocellular hypertrophy, minimal necrosis of individual hepatocytes, increased pigment in hepatocytes and Kupffer cells, and minimal to moderate hepatocellular vacuolation were seen in male rats at ≥149 mg/kg/day GW433908 (≥100 mg/kg/day APV dose equivalence) and in female rats at ≥478 mg/kg/day GW433908 (≥320 mg/kg/day APV dose equivalence). At the end of the recovery period, microscopic hepatic findings included minimal multinucleated hepatocytes, increased pigment in hepatocytes and Kupffer cells and minimal to slight hepatocellular vacuolation (Table 1-4).

Toxicokinetics:
Toxicokinetic parameters for APV and GW433908 from the 6-month toxicity study in rats are in general agreement with parameter estimates from a previous 4-week oral toxicity study with GW433908G in rats. Toxicokinetic data demonstrated that systemic exposure to amprenavir and GW433908G was achieved and that estimates of GW433908 and amprenavir C_{max} and AUC generally increased with increasing dose in a less than dose-proportional manner on Days 1, 92 and 179. In general, exposure ratios (GW433908 to APV) were less than 0.02. Mean C_{max} and total systemic exposure in all dose groups (except males receiving the 100mg/kg/day APV equivalent dose) decreased from Day 1 to Day 179, consistent with auto-induction (Table 1-5).

Toxicology Summary

- A No Observable Adverse Effect Level (NOAEL) could not be determined in male rats in the 6 month study.
- The impurities are the [redacted]. The impurities present in batches of GW433908G used in the study are considered to be toxicologically qualified since animals in the intermediate and high dose groups received total daily doses in excess of potential clinical exposure.
- Findings from repeat dose studies in rats with GW433908G of 6-month duration were generally consistent with the toxicological profile of amprenavir. GW433908G was well tolerated when administered to rats at doses up to 2240 mg/kg/day (1500 mg/kg/day amprenavir dose equivalence) for 6 months. Excessive salivation was the only clinical sign related to treatment and the liver was the major target organ for toxicity. Liver and liver-related changes observed at all doses comprised increased liver weights, increases in the activity of liver-related serum enzymes and a number of microscopic liver changes including hepatocellular hypertrophy. Most changes generally showed evidence of partial or total recovery upon withdrawal of treatment.

Toxicology conclusions

- In 6 month repeat dose toxicity studies with GW433908G in rats, findings were generally consistent with the toxicological profile of APV and previous 2 week and 1 month studies with GW433908 (A or G salt form) where adverse effects comprised gastrointestinal disturbances and liver changes.
- Terminal exposure to APV at the high dose level in the rat study with GW433908 was approximately 0.9 to 1.7 times the exposure previously obtained in humans following a dose of 1200 mg APV + 200 mg ritonavir once daily.
- Terminal exposure to GW433908 at the high dose level in the rat study with GW433908 was approximately 15.4 to 25.9 times the exposure previously obtained in humans following dosing with GW433908G equivalent to 1200 mg BID APV.
- Toxicities seen in rats at the highest dose were consistent with those seen previously for APV and were generally reversible. It is considered that this study provides assurance of the safety of GW433908G in the proposed clinical trials at doses equivalent to 1200 mg BID APV, 1200 mg APV + 200 mg ritonavir once daily and 600 mg APV + 100 mg ritonavir BID (Tables 1-6a and 1-6b).

Table 1-1 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats – Hematology

	Male					Female				
APV Base Equivalent Dose (mg/kg/day)	0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)	0	149	478	1493	2240	0	149	478	1493	2240
Hematology	Control	% Change from control				Control	% Change from control			
HG, g/dL										
Week 5	15.0	1	-1	-3	-2	14.7	0	-2	-5*	-5*
Week 14	15.2	1	-3	-5*	-5*	14.6	-1	-4*	-8*	-8*
Week 27	16.0	-1	04*	-4*	-4*	15.9	-1	-2	-8*	-7*
Recovery	16.0	--	--	-3	-1	16.0	--	--	-4*	-4*
MCV, fL										
Week 5	54.6	1	0	0	0	57.3	2	1	0	-1
Week 14	52.8	1	-2	-2	-2	57.0	0	-2	-4*	-5*
Week 27	50.6	-1	-5*	-4*	-5*	55.0	-2	-4*	-6*	-7*
Recovery	51.3	--	--	-6*	-4*	55.8	--	--	-5*	-6*
MCH, pg										
Week 5	18.3	1	-2	-2	-3	19.2	1	-2	-4*	-4*
Week 14	17.5	1	-3	-3*	-4*	19.0	-1	-4*	-7*	-8*
Week 27	17.8	-1	-5*	-6*	-6*	19.5	-1	-5*	-9*	-10*
Recovery	17.7	--	--	-6*	-3	19.5	--	--	-6*	-5*
MCHC, %										
Week 5	33.5	0	-1	-1	-2	33.6	-1	-3*	-4*	-4*
Week 14	33.2	0	-1	-1*	-2*	33.4	-1	-2*	-3*	-4*
Week 27	35.2	0	-1	-1	-1	35.5	1	-1	-3*	-3*
Recovery	34.6	--	--	0	1	34.9	--	--	0	-1
Hct, %										
Week 5	44.7	0	1	-1	0	43.7	1	1	-2	-1
Week 14	45.6	2	-2	-4*	-3*	43.7	0	-2	-5*	-3*
Week 27	45.3	0	-3	-3	-2	44.8	-2	-1	-4*	-4*
Recovery	46.4	--	--	-2	-2	46.0	--	--	-4	-5*
Platelet, x103/μL										
Week 5	885	2	7	8	10*	826	11*	12*	15*	15*
Week 14	898	5	16*	21*	20*	842	8	9	17*	22*
Week 27	892	4	21*	21*	16*	801	12	13*	16*	25*
Recovery	887	--	--	12	12	741	--	--	16*	17

HG: hemoglobin; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; Hct: hematocrit;
* P≤0.05

Table 1-2 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats – Clinical Chemistry

	Male					Female				
APV Base Equivalent Dose (mg/kg/day)	0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)	0	149	478	1493	2240	0	149	478	1493	2240
Clinical Chemistry	Control	% Change from control				Control	% Change from control			
Total protein, g/dL										
Week 5	7.0	1	3	3	3*	7.6	4*	7*	-8*	7*
Week 14	7.3	3	4	4	5*	8.0	6*	6*	10*	8*
Week 27	7.3	4	3	5	5*	8.0	4	5*	10*	8*
Recovery	7.5	--	--	4	1	8.1	--	--	5	2
Albumin, g/dL										
Week 5	4.8	2	2	4*	4*	5.4	7*	7*	9*	7*
Week 14	4.9	2	2	4	4*	5.7	5*	7*	9*	7*
Week 27	5.0	2	2	4*	6*	5.8	5	9*	9*	7*
Recovery	5.1	--	--	4	4	5.7	--	--	9	5
Cholesterol, mg/dL										
Week 5	60	-15	10	0	2	46	28*	57*	57*	46*
Week 14	69	-16	4	3	-1	57	37*	40*	49*	47*
Week 27	73	-11	11	1	-1	68	32*	25	46*	35*
Recovery	75	--	--	5	3	67	--	--	3	-22
HDL, mg/dL										
Week 5	49	-18	10	6	6	39	33*	62*	67*	49*
Week 14	56	-20	5	9	5	48	40*	46*	56*	56*
Week 27	60	-10	17	12	5	58	36*	24	43*	43*
Recovery	65	--	--	6	-2	74	--	--	-11	-12
LDL, mg/dL										
Week 5	2	0	0	0	0	1	0	200	200*	300*
Week 14	2	0	0	0	0	1	300*	300*	400*	300*
Week 27	2	0	100	0	0	3	67	167*	100	67
Recovery	0	--	--	0	--	0	--	--	--	0
Triglycerides, mg/dL										
Week 5	54	4	4	-33*	-24	34	-12	-12	-35*	-38*
Week 14	64	-6	-19	-38*	-38*	42	-10	-14*	-36*	-38*
Week 27	80	-24	-39*	-45*	-46*	41	-7	-27*	-37*	-46*
Recovery	98	--	--	-22	-31	56	--	--	-20	-29
AST, IU/L										
Week 5	96	-2	-7	-13*	-15*	90	-14*	-18*	-19*	-22*
Week 14	91	3	11	16	13	85	-8	-14	-19*	-9*
Week 27	84	39*	45*	18*	25*	74	-3	-4	-8	-5
Recovery	90	--	--	44*	53*	99	--	--	26	41*
ALT, IU/L										
Week 5	29	-3	-7	-7	-14	23	-4	4	0	0
Week 14	34	44	71*	68*	44*	28	-4	-11	-7	14
Week 27	33	182	227*	103*	88*	28	4	4	0	7
Recovery	31	--	--	256*	165*	34	--	--	62	132*
GDH, IU/L										
Week 5	8	63	-25	0	-13	9	0	-33	44	-11
Week 14	10	0	80	300*	130	8	-13	-50	0	0
Week 27	9	322*	433*	300*	189*	10	0	-30	-40	-30
Recovery	9	--	--	0	233	11	--	--	282*	173*
γ-GT, IU/L										
Week 5	1	-100	0	-5	100	0	--	--	--	--
Week 14	1	-100	100	3	500*	1	0	100	500*	700*
Week 27	2	0	100*	13*	350*	2	0	100*	350*	500*
Recovery	0	--	--	2	--	2	--	--	0	0

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GDH: glutamate dehydrogenase; GT: glutamyltransferase; P≤0.05

Table 1- 3 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats – Urinalysis

	Male					Female				
APV Base Equivalent Dose (mg/kg/day)	0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)	0	149	478	1493	2240	0	149	478	1493	2240
Urinalysis	Control	% Change From Control				Control	% Change From Control			
Urine volume, mL										
Week 5	15.6	49	56	72*	74*	21.4	-54	-1	75*	68*
Week 14	7.4	151	319	185*	359*	24	10	-30	118*	128*
Week 27	47.4	5	*	-13	-8	29.4	46	159	102*	190*
Recovery	12.6	--	-11	-25	-28	19.3	--	--	144	161
Urine specific gravity										
Week 5	1.018	0	0	-1*	-1	1.011	0	0	0	0
Week 14	1.027	-1	-1	-1*	-1	1.013	0	0	0	-1
Week 27	1.013	0	0	0	0	1.012	0	-1	-1*	-1
Recovery	1.018	--	--	0	1	1.015	--	--	0	0
Creatinine, mg excretion										
Week 5	6.57	-1	23*	22*	10	3.60	-9	12	38*	33*
Week 14	6.30	24	33*	35*	34*	2.97	-4	38*	40*	38*
Week 27	8.55	7	-4	14	7	3.71	-1	16	49*	19
Recovery	6.86	--	--	7	3	2.99	--	--	-7	9
Na^+, mmol excreation										
Week 5	0.56	0	30	30	46*	0.30	-7	47*	90*	93*
Week 14	0.40	40*	45*	60*	68*	0.31	0	45	58*	74*
Week 27	0.39	28	15	54*	74*	0.26	42	27	104*	50
Recovery	0.24	--	--	13	0	0.13	--	--	38	54
K^+, mmol excreation										
Week 5	1.10	-3	32*	18	32*	0.61	-11	16	51*	54*
Week 14	0.75	43*	52*	59*	68*	0.57	-4	18	42	61*
Week 27	1.14	4	7	29	23	0.60	18	35	63*	67*
Recovery	0.74	--	--	-3	5	0.42	--	--	5	36

*: P≤0.05

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2323 **Table 1-4 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats – Gross**
2324 **Pathology and Histopathology**

	Male					Female				
APV Base Equivalent Dose (mg/kg/day)	0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)	0	149	478	1493	2240	0	149	478	1493	2240
Organ Weights	Control (g)	% Change From Control				Control (g)	% Change From Control			
Relative liver to body weight (%)										
Terminal	2.177	11*	21*	37*	44*	2.601	14*	34*	74*	69*
Recovery	2.279	--	--	-2	-3	2.448	--	--	16*	17*
Absolute liver weight (%)										
Terminal	10.849	10	23*	35*	46*	6.837	16	33*	68*	61*
Recovery	11.649	--	--	-4	-4	6.551	--	--	11	15
Microscopic findings	Incidence of Findings									
No. Examined (terminal)	12	12	11	12	12	12	12	11	12	12
Liver (hepatocyte):										
Hypertrophy										
Minimal	3	9	9	11	7	7	8	7	10	3
Slight	0	0	0	0	5	0	0	0	0	5
Moderate	0	0	0	0	0	0	0	0	0	4
Multinucleate										
Minimal	0	9	5	9	9	0	0	0	4	6
Slight	0	1	4	1	0	0	0	1	0	0
In situ, Necrosis										
Minimal	0	0	0	1	0	0	0	0	1	3
Increased pigment										
Minimal	0	0	0	4	4	0	0	0	6	7
Slight	0	0	0	0	0	0	0	0	1	0
Vacuolation										
Minimal	6	11	10	10	8	0	0	0	1	3
Slight	0	0	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	3	0	0	0	0	1
Liver (Kupffer cells):										
Minimal	0	3	6	8	6	0	0	0	3	5
No. Examined (recovery):	8	0	0	8	8	8	0	0	8	8
Liver (hepatocyte):										
Hypertrophy:										
Minimal	6	--	--	5	6	2	--	--	8	2
Multinucleate:										
Minimal	0	--	--	7	6	0	--	--	1	0
Increased pigment:										
Minimal	0	--	--	6	1	1	--	--	8	5
Slight	0	--	--	1	0	0	--	--	0	0
Vacuolation:										
Minimal	3	--	--	2	3	1	--	--	0	0
Slight	0	--	--	0	1	0	--	--	0	0
Liver (hepatocyte):										
Increased pigment										
Minimal	0	--	--	8	8	1	--	--	5	8
Slight	0	--	--	0	0	0	--	--	1	0

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Table 1-5 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats - Toxicokinetics

		Male					Female				
APV Base Equivalent Dose (mg/kg/day)		0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)		0	149	478	1493	2240	0	149	478	1493	2240
No. of Animals: TK		4	12	12	12	12	4	12	12	12	12
GW433908G											
AUC_{0-} (µg•h/mL)	Day 1	-	0.052	0.156	1.575	0.616	-	0.034	0.299	4.719	1.256
AUC_{0-24} (µg•h/mL)	Day 92	-	0.124	0.367	1.435	2.597	-	0.130	0.368	1.681	2.421
	Day 179	-	0.030	0.229	0.930	1.079	-	0.270	3.267	1.001	1.815
GW433908G											
C_{max} (µg/mL)	Day 1	-	0.021	0.036	0.050	0.047	-	0.010	0.132	0.069	0.124
	Day 92	-	0.022	0.045	0.152	0.035	-	0.029	0.047	0.233	0.322
	Day 179	-	0.015	0.032	0.102	0.012	-	0.102	0.381	0.184	0.143
APV											
AUC_{0-} (µg•h/mL)	Day 1	-	13	84	154	243	-	33	74	237	253
AUC_{0-24} (µg•h/mL)	Day 92	-	16	48	58	63	-	19	52	77	93
	Day 179	-	20	46	57	55	-	23	54	62	107
APV											
C_{max} (µg/mL)	Day 1	-	3	8	10	9	-	3	9	13	10
	Day 92	-	2	4	6	6	-	2	5	7	7
	Day 179	-	3	5	5	5	-	2	5	7	8

Table 1-6a Exposure Ratio of APV in Rats Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G or Amprenavir (APV) and Ritonavir (RTV)

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC_{0-24h} [b] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)	Ratio of Rat to Human AUC Following APV/RTV administration (APV20001)
Rat 6 month RD1998/02858/01	149 (100)	M	2.90	19.8	0.6	0.3
		F	2.31	22.7	0.6	0.4
	478 (320)	M	5.09	46.2	1.3	0.7
		F	5.23	54.3	1.5	0.8
	1493(1000)	M	5.33	57.0	1.6	0.9
		F	7.28	62.3	1.7	1.0
	2240 (1500)	M	5.28	54.9	1.5	0.9
		F	7.68	107	3.0	1.7
Human GW433908G study (APV20001)	(48[c])	M+F	5.30	35.8[d]	--	--
Human APV/RTV study (APV20001)	(48[e])	M+F	7.17	64.4[f]	--	--

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: End of study data. Arithmetic mean values are quoted for rat data
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: Based on multiple dose following administration of GW433908, i.e., AUC_{0-12h} (17.89 µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours
e.: 1200 mg QD APV in a 50 Kg person
f.: Based on multiple dose following administration of 1200 mg APV + 200 mg RTV QD

Table 1-6b Exposure Ratio of GW433908 in Rats Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC_{0-24h} [b] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)
Rat 6 month RD1998/02858/01	149 (100)	M	0.015	0.030	0.4
		F	0.102	0.271	0.39
	478 (320)	M	0.032	0.229	3.3
		F	0.381	3.267	46.7
	1493(1000)	M	0.102	0.930	13.3
		F	0.184	0.100	14.3
	2240 (1500)	M	0.117	0.108	15.4
		F	0.143	0.182	25.9
Human GW433908G study (APV20001)	(48[c])	M+F	0.030	0.070[d]	–

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: End of study data. Arithmetic mean values are quoted for rat data
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: AUC_{last} value since GW433908 levels were very low and were not present past a few hours. Based on multiple dose following administration of GW433908, i.e., AUC_{last} (35 ng•hr/mL), multiplied by 2 to obtain exposure for 24 hours

Repeat dose studies – neonatal and juvenile rats

33. GW433908G: Two week oral gavage pilot toxicity study in neonatal and juvenile Han Wistar rats (Report No. RD2000/02506/00)

GW study No.: R40877; study No.: 96920; Conducting facility: [redacted] Date Initiation: 15 January 2001; GLP Compliance: No (x); Drug Lot: F005430; Formulation: GW433908G solution in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) and 0.1% (w/w) Tween 80 in reverse osmosis-treated water

Methods

Groups of 25 to 28 neonatal Han Wistar rat pups/sex (Tac:Glx:W1, Approximately 4 days old, 5.2-12 g; [redacted]) were orally treated with the vehicle and GW433908G by gavage using a plastic gavage tube at doses of 0/0 (vehicle), 61, 184, 553 and 1105 mg/kg/day (dose frequency: twice daily, at least 6 hours apart; dose volume: 10 mL/kg/day) for 15 consecutive days (from Day 4 to 18 *post partum*). The number of pups assigned to each study group was as follows.

GW433908G	0	61	184	553	1105
GW433908X equivalents[1]	0	50	150	450	900
APV equivalents (mg/kg/day)[2]	0	43	130	389	777
Male pups/group	25	25	25	25	28
Main	11	10	10	20	21
Toxicokinetic	14	15	15	5	7
Female pups/group	25	25	25	25	27
Main	11	10	11	19	21
Toxicokinetic	14	15	14	6	6

[1]Conversion factor for GW433908G to GW433908X is 1.228; [2]Conversion factor for GW433908X to APV is 1.1588

Note that the dose levels of 50, 150, 450 and 900 mg GW433908X/kg/day selected for this study were based on preliminary results from a previous pilot neonatal/juvenile toxicity study in rats (Report RD 1999/02344/00; IND 58627, SN122). Dose levels of 5-160 mg/kg/day for 30 days in that study did not produce significant changes in body weights, clinical pathology parameters or pathological findings.

All pups were examined twice daily for mortality and signs of ill health. Each pup was given a detailed

examination once prior to the initiation of treatment and daily during the treatment period. Individual body weights were measured daily. All animals were subjected to a gross necropsy on Day 19 *post partum*, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, parathyroid,** and **uterus**. The following tissues were collected and examined histologically by a pathologist (Appendix Table 1).

Results

Clinical signs and mortality: All pups at 900 mg GW433908X/kg/day and 18 of 20 male and 15 of 20 female pups at 450 mg GW433908X/kg/day were found dead on Day 5 *post partum*. Of the surviving female pups at 450 mg/kg/day, one pup was found dead on Day 6 *post partum*, one was found dead on Day 7 *post partum*. Weakness with decreased activity, labored breathing, decreased respiratory rate, hypersensitivity and hyper-reactivity were seen in pups at 450 mg/kg/day or greater on Days 4 to 6 *post partum*. One female pup at 150 mg/kg/day was found dead on Day 13 *post partum*. Salivation was seen in this animal on Days 5 and 7 *post partum*. A prominent backbone with several subcutaneous masses in the axillary, thoracic and lumbar regions were seen in one pup at 50 mg/kg/day, which was considered to be associated to the gavage procedure.

Body weights and food consumption: No mean body weight changes were seen in pups at 50 and 150 mg/kg/day. Decreases in mean body weights were seen in pups at 450 mg/kg/day during the treatment period (Table 1).

Hematology: An increase in RBC, hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and absolute reticulocyte (RETIC) and a decrease in red cell distribution width (RDW) were seen in two female and one male pups at 450 mg/kg/day (Table 2).

Table 1. Changes in body weights in neonatal rats with 14-days oral administration of GW433908G

mg/kg/day	Male					Female				
GW433908G	0	61	184	553	1105	0	61	184	553	1105
GW433908X	0	50	150	450	900	0	50	150	450	900
equivalents	0	43	130	389	777	0	43	130	389	777
APV equivalent										
Body weight (g)										
Post partum Day 4	9.4	9.3	9.9	9.7	8.4	8.9	8.9	9.4	10.4	8.0
Post partum Day 5	11.1	11.0	11.5	9.0	--	10.5	10.5	11.0	9.5	--
Post partum Day 6	12.7	12.8	13.2	10.1	--	12.0	12.2	12.6	11.4	--
Post partum Day 7	14.6	14.6	15.0	11.8	--	13.9	14.0	14.3	12.6	--
Post partum Day 14	29.9	27.8	30.6	21.0	--	28.9	28.7	29.5	21.2	--
Post partum Day 19	42.1	39.3	42.7	30.4	--	40.2	39.4	41.1	30.5	--

Table 2. Changes in hematological parameters in neonatal rats with 15-days oral administration of GW433908G

mg/kg/day	Male					Female				
GW433908G	0	61	184	553	1105	0	61	184	553	1105
GW433908X	0	50	150	450	900	0	50	150	450	900
equivalents	0	43	130	389	777	0	43	130	389	777
APV equivalent										
Hematology										
Post partum Day 19										
RBC ($10^6/\mu L$)	4.1	4.0	3.98	4.58	--	4.1	4.2	4.1	4.82	--
Hemoglobin (g/dL)	7.4	7.4	7.3	9.3	-	7.6	8.0	7.5	9.5	--
Ht (%)	25.6	25.3	25	33.2	--	26	26.9	26	32.4	--
RETIC ($10^9/\mu L$)	330	360	.356	663	--	361	441	411	606	--

Clinical chemistry: Increases in cholesterol values in male pups at 150 and 450 mg/kg/day and in female pups at 450 mg/kg/day were seen. An increase in phosphorus levels was seen in male pups at 50 mg/kg/day or greater (Table 3). However, the toxicological significance of this finding is uncertain based on limited microscopic findings. No test article-related changes were observed.

Histopathology. No treatment-related macroscopic pathological changes associated with the administration of GW433908X were seen. Test article-related increases in mean relative liver weights

(relative to body weight) were seen in pups at 150 mg/kg/day or greater. Note that minimal to slight hepatocellular vacuolation was seen in the liver of all surviving pups at 450 mg/kg/day (6/6). An increase in adrenal gland weights relative to body weight was seen in male pups at 450 mg/kg/day (Table 4). Due to the lack of histological examination of the adrenal glands, the significance of this finding is uncertain.

Comments

The NOAEL for two weeks of exposure in neonatal rats was considered to be 150 mgGW433908X/kg/day. Mean relative liver weights (relative to body weight) were increased for 150 and 450 mg/kg/day GW433908X-treated groups, and minimal to slight hepatocellular vacuolation was seen in pups at 450 mg/kg/day.

Table 3. Changes in clinical chemistry in neonatal rats with 15-days oral administration of GW433908G

mg/kg/day	Male					Female				
GW433908G	0	61	184	553	1105	0	61	184	553	1105
GW433908X equivalents	0	50	150	450	900	0	50	150	450	900
APV equivalent	0	43	130	389	777	0	43	130	389	777
Clinical chemistry *Post partum* Day 19										
Cholesterol (mg/dL)	115	136	148	254	--	130	134	160	254	--
Phosporus (mg/dL)	11.0	12.7	12.7	13.2	--	12.3	12.8	12.4	12.6	--

Table 4. Changes in organ weights in neonatal rats with 15-days oral administration of GW433908G

mg/kg/day	Male					Female				
GW433908G	0	61	184	553	1105	0	61	184	553	1105
GW433908X equivalents	0	50	150	450	900	0	50	150	450	900
APV equivalent	0	43	130	389	777	0	43	130	389	777
Organ weights										
Liver										
Absolute (g)	1.47	1.49	1.67	1.22	--	1.44	1.47	1.65	1.32	--
Relative (%)	3.48	3.77	3.88*	4.54*	--	3.58	3.71	4.00*	4.36*	--
Spleen										
Absolute (g)	0.18	0.19	0.15	0.10	--	0.18	0.16	0.17	0.11	--
Relative (%)	0.42	0.52	0.36	0.38	--	0.45	0.39	0.41	0.35	--
Adrenal gland										
Absolute (g)	0.012	0.012	0.01	0.01	--	0.01	0.01	0.01	0.01	--
Relative (%)	0.030	0.030	0.03	0.04	--	0.03	0.04	0.03	0.04	--

*P≤0.05

Toxicokinetics

Methods

An additional 6 to 15 rats/sex/group in the control group and four drug-treated satellite groups were used for plasma drug level determinations. Animals were treated with 0/0 (vehicle), 61, 184, 553 and 1105 mg GW433908G/kg/day (dose frequency: twice daily, at least 6 hours apart; dose volume: 10 mL/kg/day) for 15 consecutive days (from Day 4 to 18 *post partum*). Blood samples (0.5 mL/sample) were collected on Day 4 *post partum* from the *vena cava* of selected pups (5 pups/sex/group for the 0, 61, 184, and 553 mg/kg/day groups, and 7 males and 6 females for the 1105 mg/kg/day group) at 7 hours past the 1st dose of the day (approximately one hour after the 2nd dose of the day). Due to mortality, samples were collected on Day 18 *post partum* from 9/sex in the vehicle control group, 10/sex in the 61 mg/kg/day group, 10 males and 9 females in the 184 mg/kg/day group and 1 female in the 553 mg/kg/day group. Plasma levels of GW433908X and APV were measured by an [redacted] method at [redacted]

Results

Plasma concentrations for GW433908X and APV on Days 4 and 18 *post partum* are summarized in Table 5.

Table 5. Plasma concentration of GW433908X and APV in neonatal rats after 15 days oral administration of GW433908G

GW433908G (mg/kg/day)*	Plasma GW433908X Concentration (ng/mL)		Plasma APV Concentration (µg/mL)	
	Day 4 *post partum*	Day 18 *post partum*	Day 4 *post partum*	Day 18 *post partum*

	M	F	M	F	M	F	M	F
61	28.5	41.0	5.86	5.46	1.79	3.46	1.27	1.20
184	38.2	35.9	26.4	19.4	6.96	3.81	4.19	4.33
553	1567	1590**	--	86.2*	22.6	29.2**	--	12.3*
1105	978	1399	--	--	22.9	8.27	--	--

* n = 2; **. n = 1

Comments

Mean plasma concentration of APV at 7 hours on Day 4 generally increased in a greater than dose-proportional manner in males in the 184 and 553 mg GW433908G /kg/day. Note that plasma APV levels in the neonatal Han Wistar rats appeared to be 2-3 fold higher than in the adult rats. In a previous one-month oral toxicity study in adult rats, the APV C_{max} was found to be 3.5-4.3 µg/ml in rats at 478 mg GW433908G/kg/day (Re.: RD1998/02573/00; Study report No.: R40427). By contrast, the APV $C_{7\ hour}$ was 4.13-4.33 µg/ml in neonatal rats at 184mg GW433908G/kg/day.

34. GW433908G: Oral gavage pilot toxicity study in neonatal rats (Report No. RD1999/02344/00)

GW; study No.: R40576; Contract study No.: 650-085; Conducting facility: [redacted]
Date Initiation: 11 December 1999; GLP Compliance: Yes (x); Drug Lot: R4283/34/1; Formulation: GW433908G solution in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) and 0.1% (w/w) Tween 80 in reverse osmosis-treated water

Methods

Groups of 10 male and 10 female neonatal Han Wistar rats/sex (Tac:Glx:WIfBR, Approximately 5 days old, 6.9-13.6 g) were orally treated with the vehicle and GW433908G by gavage using a plastic gavage tube at doses of 0 (vehicle), 5, 10 , 20, 40, 80 or 160 mg/kg/day (dose frequency: twice daily, at least 6 hours apart; dose volume: 10 mL/kg/day) for 15 consecutive days (from Day 4 to 18 *post partum*).A further 10 animals /sex were included in each group for toxicokinetic analysis.

GW433908G	0	5	10	20	40	80	160
APV equivalents (mg/kg/day)[2]	0	3.3	6.7	13.4	26.8	53.5	107
Rats/sex/group							
Main	10	10	10	10	10	10	10
Toxicokinetic	10	10	10	10	10	10	10

[1]Conversion factor for GW433908G to GW433908X is 1.228; [2]Conversion factor for GW433908X to APV is 1.1588

Note that the dose levels of 5, 10, 20, 40, 80 or 160mg GW433908G/kg/day selected were based on the results from juvenile toxicity studies carried out with APV (Report RD1997/02121/00; NDA 21-007) when doses of 250 mg/kg/day and above caused a greater incidence of pup mortality as early in the study as Dose Day 4. All pups were examined twice daily for mortality and signs of ill health. Each pup was given a detailed examination once prior to the initiation of treatment and daily during the treatment period. Individual body weights were measured daily. All animals were subjected to a gross necropsy on Day 35 *post partum*, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, parathyroid**, and **uterus**. The following tissues were collected and examined histologically by a pathologist (Appendix Table 1).

Results

Clinical signs and mortality: No increase in frequency or severity of clinical signs was seen in rats at 5, 10, 20, 40, 80 or 160mg GW433908G/kg/day. Mortality was slightly higher in female pups at 160 mg/kg/day, with 2 females found dead during the study, one on Day 19 and one on Day 20 of dosing.
Body weights and food consumption: No mean body weight changes were seen in rats at 5, 10, 20, 40, 80 or 160mg GW433908G/kg/day.
Hematology: No treatment-related hematological parameters were seen in rats at 5, 10, 20, 40, 80 or 160mg GW433908G/kg/day.
Clinical chemistry: No treatment-related changes in clinical chemistry were seen in rats at 5, 10, 20, 40, 80 or 160mg GW433908G/kg/day.

Organ weights and Histopathology: Red discoloration of lungs was seen at necropsy in the decedent animals. Heart weights were increased in males at 160 mg/kg/day compared to controls, but there were no correlating microscopic findings (Table 1).

Comments

The NOAEL for this study in neonatal rats was considered to be 80 mgGW433908G/kg/day. Slightly increased mortality in females and increased heart weights were seen in males at 160 mg/kg/day.

Table 1. Changes in organ weights in neonatal rats with 30-days oral administration of GW433908G

mg/kg/day	Male						
GW433908G	0	5	10	20	40	80	160
APV equivalent	0	3.3	6.7	13.4	26.8	53.5	107
Organ weights in Males							
Heart							
Absolute (g)	0.6	0.6	0.6	0.7	0.6	0.7	0.8*
Relative to body (%x10)	5.9	5.6	5.9	6.4	5.8	6.2	7.7*

*P≤0.05

Toxicokinetics

Methods

An additional 10 rats/sex/group in the control group and drug-treated satellite groups were used for plasma drug level determinations. Blood samples (0.5 mL/sample) were collected on Days 6 and 30 post dose at 7 hours past the 1st dose of the day (approximately one-hour after the 2nd dose of the day). Plasma levels of GW433908X and APV were measured by an —— method.

Results

Plasma concentrations for GW433908X and APV on Days 6 and 30 post dose are summarized in Table 2.

Table 2. Plasma concentration of GW433908X and APV in neonatal rats after 30 days oral administration of GW433908G

GW433908G (mg/kg/day)*	Plasma GW433908X Concentration (ng/mL)				Plasma APV Concentration (µg/mL)			
	Day 6		Day 30		Day 6		Day 30	
	M	F	M	F	M	F	M	F
5	--	--	1.5	--	0.2	0.2	0.05	0.1
10	--	--	--	--	0.4	0.3	0.07	0.2
20	10.5	--	7.2	3.5	0.6	0.99	0.12	1.0
40	222	20.7	3.2	--	2.4	2.2	1.0	1.0
80	86	2076	12.0	5.9	6.8	5.0	1.1	1.8
160	96	1114	28.1	13.6	8.2	6.6	4.6	3.6

* n = 2; **. n = 1

Comments

Note that plasma APV levels in the neonatal Han Wistar rats appeared to be 2-3 fold higher than those in adult rats. In a previous one-month oral toxicity study, the APV C_{max} was found to be 3.5-4.3 µg/ml in adult rats at 478 mg GW433908G/kg/day (Re.: RD1998/02573/00; Study Report No.: R40427). By contrast, the APV $C_{7\ hour}$ on Day 30 was 3.6-4.6 µg/ml in neonatal rats at 160 mg GW433908G/kg/day.

Note that the exposure of APV and GW433908X were not measured in this study because of the difficulties of blood sampling in neonatal animals.

Note that the approved treatment regimen with APV capsules in pediatric patients (4 to 12 years old) is 20 mg/kg BID, where the treatment regimen with APV oral solution (4 years and older) is 17 mg/kg/day TID. In general, these treatment regimens are well tolerated with no additional adverse effects seen. Plasma levels in children at 20 mg/kg APV BID

Comment

Note that during the development of APV results from repeat dose studies showed a high mortality in neonatal/juvenile rats. The mortality was probably related to the effects of the vehicle that contained high concentrations of propylene glycol (PG) and polyethylene glycol 400 (PEG400). Thus, formulations with no PEG400 and significantly less PG than those for APV has been developed and used in the clinical trials by the sponsor. Thus, the results of the juvenile rat studies did not preclude the clinical administration of GW433908G in combination with NRTIs with or without ritonavir.

Table 3. Exposure Ratio of GW433908G in Juvenile Rats and Humans

Study Type Report No. (Study No.)	GW433908G Dosage [Amprenavir equivalents] (mg/kg/day)	Sex	C_{max}*		Ratio of Animal to Human Exposure (C_{max}) ^	
			APV (µg/mL)	GW433908X (ng/mL)	APV	GW433908X
Non-GLP Juvenile Rat RD2000/02506/00 (R40877)	61 [43]	M	1.27	5.86	0.2	0.3
		F	1.20	5.46	0.2	0.2
	184 [130]	M	4.19	26.4	0.5	1.1
		F	4.33	19.4	0.6	0.8
	553 [389] #	M	22.6	1567	2.9	68
		F	29.2	1590	3.7	69
	1105 [777] #	M	22.9	978	2.9	43
		F	8.72	1399	1.1	61
GLP Juvenile Rat RD1999/02344/00 (R40576)	5 [3.3]	M	0.05	1.48	<0.1	<0.1
		F	0.08	BQL	<0.1	BQL
	10 [6.7]	M	0.07	BQL	<0.1	BQL
		F	0.16	BQL	<0.1	BQL
	20 [13.4]	M	0.12	7.18	<0.1	0.3
		F	1.02	3.53	0.1	0.2
	40 [26.8]	M	0.97	3.22	0.1	0.2
		F	0.96	BQL	0.1	BQL
	80 [53.5]	M	1.07	12.0	0.1	0.5
		F	1.81	5.85	0.2	0.3
	160 [107]	M	4.62	28.1	0.6	1.2
		F	3.64	13.6	0.5	0.6
Adult GW433908X BID (APV20001)	55.8 [48†]	M+F	5.15	23	-	-
Adult APV/RTV QD (APV20001)	[24]‡	M+F	7.85	-	-	-
Paediatric APV BID (PROB2004)	[40]	M+F	6.4	-	-	-

Key:
* = Final day data for animal studies unless indicated, human data is arithmetic mean $C_{max,ss}$.
^ = Most stringent assessment used for APV comparison, i.e. Adult APV/RTV QD
# = Day 1 data due to high mortality during the study.
† = 1395 mg GW433908G BID (1200 mg APV dose equivalence BID) in a 50 kg person.
‡ = 1200 mg APV: 200 mg Ritonavir once daily.
RTV = Ritonavir.
BQL = Below Quantification Limit.

36. GW433908G: Thirteen-week oral gavage toxicity study in neonatal and juvenile wistar Han Wistar (Report No.RD2002/00045/00)

GW Study Number R40860; Study Number [redacted]. 98109; Testing Facility: [redacted] Date Initiation: 24 July 2001; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: F017604; Formulation: GW433908G suspension (14.9, 47.8. 149.3 and 224 mg/ml) in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

- Liver was identified as the target organ for toxicity with GW433908G in this study. Increases in hepatic weight (absolute and relative) and slight elevation of AST and ALT were seen in males at 300 mg/kg/day at the end of treatment and recovery periods. There was no histopathological correlation of the heatic weight changes seen. A No Observed Adverse Effect Level (NOAEL) in the neonatal and juvenile female rat was considered to be 175 mg/kg/day.
- Treatment related histopathological changes were seen in the kidneys of male rats from all dose levels consisted of hyaline droplet accumulation in the cortical tubule epithelial cells, which were not seen after the 4-week recovery period.

Methods

Dosing:

Species/Strain:	Rat/Wistar Hannover
#/sex/group or time point (main study):	20 rats/sex/group
Satellite groups used for recovery:	10 rats/sex/group
Satellite groups used for toxicokinetic:	
Toxicokinetic Day 10 Postpartum	13-16 (control group) or 13-14 rats/sex/group (treatment)

Toxicokinetic Day 94 Postpartum	10 (control group) or 6-15 rats/sex/group (treatment)
Age:	approximately 4 days old (Day 0 postpartum = Day of completion of littering)
Body Weight:	Male = 6.1 – 11.9 g; Female = 5.3 – 11.4 g
Doses in administration units:	0, 100, 175 and 300 mg/kg/day GW433908G, equivalent to 0 (vehicle), 71, 124 and 213 mg/kg/day amprenavir, respectively
Rout, dosing frequency and dose volume:	Oral (gavage); dosed twice daily, 6 hours apart; 5 mL/kg/dose (10 mL/kg/day)
Duration of Treatment:	At least 91 days
Duration of Recovery:	4 weeks

Observations and times:

Clinical examiniation: Once pretreatment and once prior to necropsy
Body weights: Once daily pretreatment and once daily during the treatment period
Food consumption: Once daily pretreatment and once daily during treatment period
Hematology: On treatment Days 95 and 123, respectively
Clinical chemistry: On treatment Days 95 and 123, respectively
Urinalysis: On treatment Days 95 and 123, respectively
Gross pathology (main): At the end of the treatment period (terminal)
Gross pathology (recovery): at the end of the recovery period (recovery)
Organs weighed: Adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids
Histopathology: A spectrum of tissues from all animals were taken, preserved, and examined by a pathologist (Appendix Table 1).
Toxicokinetics: For toxicokinetic evaluations, blood samples from rats in the control group and the test article treatment groups (n= 8 to 16 males/group and 6 to 15 female/group) were collected 7 hours after the first daily doe on Days 10 and 94 of the study period and plasma APV and GW433908G were analyzed.
Other: For recovery evaluation, rats (10/sex/goup) in the control, the test article treatment group were maintained untreated for an additional 4 weeks to study the regression or progression of any treatment-related abnormalities

Results

Mortality and clinal signs: Fourty-seven rats died or sacrificed over the course of the study (Table 1). Most unscheduled deaths had macroscopic or microscopic evidence of dosing error. All unscheduled deaths occurred during the first two weeks of dosing, with the exception of one male found dead on Day 30. In the few animals where a cause of death could not be established, there was no evidence of any treatment-related effect on the level of mortality.
Body weight: Slightly lower pup weights during the first 4 weeks of treatment were seen.
Food consumption: An increased incidence of suspected empty stomachs (decreased amount of milk or no milk) in pups dosed at 300 mg/kg/day during the first 2-3 days of treatment. No treatment-related food comsumption changes were seen in pups during the post weaning phase.
Ophthalmological findings: No treatment-related ophthalmological changes were seen in pups during the treatment period.
Hematology: No toxicologically significant changes in hematology parameters were seen in pups.
Clinical chemistry: No treatment-related changes in clinical chemistry parameters were seen in pups at all doses. Treatment-related increases in liver enzymes including aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were seen in male rats at ≥300 mg/kg/day GW433908G, which were associated with increased hepatic weights (absolute and relative) (Table 1-2).
Urinalysis: No treatment-related changes in urinary parameters were seen in pups at all doses.
Gross pathology: Treatment-related increases in absolute liver weight (males: %; female: %) and in relative liver to body weight (males: %; female: %) were noted in rats given ≥300mg/kg/day, which were reversible (Table 1-4).
Histopathology: No treatment-related microscopic findings were seen in rats at all doses. Treatment related histopathological changes were seen in the kidneys of male rats from all dose levels consisted of

hyaline droplet accumulation in the cortical tubule epithelial cells, which were not seen after the 4-week recovery period. This finding are considered to be of limited toxicological relevance to humans, and was likely due to male rat specific metabolism of α_{2u} globulin (Goldstein and Schnellmann, 1996). (Table 1-4).

Toxicokinetics

Toxicokinetic parameters for APV and GW433908 from the 6-month toxicity study in rats are in general agreement with parameter estimates from a previous 4-week oral toxicity study with GW433908G in rats. Toxicokinetic data demonstrated that systemic exposure to amprenavir and GW433908G was achieved and that estimates of GW433908 and amprenavir C_{max} and AUC generally increased with increasing dose in a less than dose-proportional manner on Days 1, 92 and 179. In general, exposure ratios (GW433908 to APV) were less than 0.02. Mean C_{max} and total systemic exposure in all dose groups (except males receiving the 100mg/kg/day APV equivalent dose) decreased from Day 1 to Day 179, consistent with auto-induction (Table 1-5).

Toxicology summary

The NOAEL in neonatal and juvenile rats is considered to be 175 mg/kg/day. Liver and liver-related changes observed at ≥300 mg/kg/day comprised increased liver weights, and increases in the activity of liver-related serum enzymes. Most changes generally showed evidence of partial or total recovery upon withdrawal of treatment. Treatment related histopathological changes were seen in the kidneys of male rats from all dose levels consisted of hyaline droplet accumulation in the cortical tubule epithelial cells, which were not seen after the 4-week recovery period. This finding are considered to be of limited toxicological relevance to humans, and was likely due to male rat specific metabolism of α_{2u} globulin (Goldstein and Schnellmann, 1996).

Toxicology conclusions

The NOAEL in neonatal and juvenile rats is considered to be 175 mg/kg/day.

Table 1-1 GW433908G: 13-weeks Oral Gavage Toxicity Study in Han Wistar Rats – Unscheduled Deaths and Changes in Body Weights

	Male				Female			
GW433908 (mg/kg/day)	0	100	175	300	0	100	175	300
APV Base Equivalent Dose (mg/kg/day)	0	71	124	213	0	71	124	213
Number of Animals								
Main	20	20	20	20	20	20	20	20
Recovery	10	10	10	10	10	10	10	10
TK Day 10 postpartum	16	14	14	14	13	13	13	14
TK Day 94 postpartum	10	13	13	8	10	15	13	6
# of Unscheduled Deaths (missing pups)	6 (3)	2 (1)	2 (1)	10 (2)	9 (1)	2 (0)	3 (1)	13 (1)
Pup body weight (g)	Group litter means				Group litter means			
# of litters	16	15	15	16	16	15	15	16
Day 4 postpartum (pretreatment)	9.1	8.7	9.3	9.3	8.8	8.4	8.9	8.8
Day 14 postpartum	31.7	30.7	31.5	30.3	31.6	30.2	30.7	28.8
Day 21 postpartum	49.6	48.6	49.8	48.0	48.5	47.2	48.1	45.8
Post weaning body weight (g)	Group litter means				Group litter means			
# of rats	40	43	44	38	40	45	43	36
Day 22postpartum	50.8	50.3	51.3	48.9	50.4	48.3	49.5	47.3*
Day 24 postpartum	60.2	59.1	60.8	57.6	58.4	56.8	58.2	54.8*
Day 26 postpartum	70.8	70.4	71.9	67.9	67.6	66.2	67.4	63.8*
Day 27 postpartum	75.6	75.7	77.3	73.4	71.9	71.0	72.4	68.3*

- $P \leq 0.05$

Table 1-2 GW433908G: A 13-Week Oral Gavage Toxicity Study in Neonatal and Juvenile Han Wistar Rats – Clinical Chemistry (Days 95 and 123)

	Male				Female			
GW433908 (mg/kg/day)	0	100	175	300	0	100	175	300
APV Base Equivalent Dose (mg/kg/day)	0	71	124	213	0	71	124	213
Clinical Chemistry	Group means							

AST, IU/L								
Day 95	69.6	82.2*	72.2	81.4	79.6	69.7	66.9	59.9*
Day123 (recovery)	74.0	84.3	83.9	94.0*	74.6	68.2	117.2	75.0
ALT, IU/L								
Day 95	38.7	43.8	39.3	51.5*	39.2	35.5	34.2	31.4
Day123 (recovery)	38.0	49.0	44.3	71.7*	34.3	34.5	41.5	38.0

AST: aspartate aminotransferase; ALT: alanine aminotransferase; P≤0.05

Table 1-3 GW433908G: A 13-Week Oral Gavage Toxicity Study in Neonatal and Juvenile Han Wistar Rats – Gross Pathology and Histopathology

	Male					Female				
GW433908 (mg/kg/day)	**0**	**100**	**175**	**300**		**0**	**100**	**175**	**300**	
APV Base Equivalent Dose (mg/kg/day)	**0**	**71**	**124**	**213**		**0**	**71**	**124**	**213**	
Organ Weights	**Control (g)**	**% Change From Control**				**Control (g)**	**% Change From Control**			
Relative liver to body weight (%)										
Terminal	**2.177**	**11***	**21***	**37***	**44***	**2.601**	**14***	**34***	**74***	**69***
Recovery	**2.279**	**--**	**--**	**-2**	**-3**	**2.448**	**--**	**--**	**16***	**17***
Absolute liver weight (%)										
Terminal	**10.849**	**10**	**23***	**35***	**46***	**6.837**	**16**	**33***	**68***	**61***
Recovery	**11.649**	**--**	**--**	**-4**	**-4**	**6.551**	**--**	**--**	**11**	**15**
Microscopic findings	**Incidence of Findings**									
No. Examined (terminal)	**12**	**12**	**11**	**12**	**12**	**12**	**12**	**11**	**12**	**12**
Liver (hepatocyte):										
Hypertrophy										
Minimal	**3**	**9**	**9**	**11**	**7**	**7**	**8**	**7**	**10**	**3**
Slight	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**	**5**
Moderate	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**
Multinucleate										
Minimal	**0**	**9**	**5**	**9**	**9**	**0**	**0**	**0**	**4**	**6**
Slight	**0**	**1**	**4**	**1**	**0**	**0**	**0**	**1**	**0**	**0**
In situ, Necrosis										
Minimal	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**3**
Increased pigment										
Minimal	**0**	**0**	**0**	**4**	**4**	**0**	**0**	**0**	**6**	**7**
Slight	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
Vacuolation										
Minimal	**6**	**11**	**10**	**10**	**8**	**0**	**0**	**0**	**1**	**3**
Slight	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
Moderate	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**1**
Liver (Kupffer cells):										
Minimal	**0**	**3**	**6**	**8**	**6**	**0**	**0**	**0**	**3**	**5**
No. Examined (recovery):	**8**	**0**	**0**	**8**	**8**	**8**	**0**	**0**	**8**	**8**
Liver (hepatocyte):										
Hypertrophy:										
Minimal	**6**	**--**	**--**	**5**	**6**	**2**	**--**	**--**	**8**	**2**
Multinucleate:										
Minimal	**0**	**--**	**--**	**7**	**6**	**0**	**--**	**--**	**1**	**0**
Increased pigment:										
Minimal	**0**	**--**	**--**	**6**	**1**	**1**	**--**	**--**	**8**	**5**
Slight	**0**	**--**	**--**	**1**	**0**	**0**	**--**	**--**	**0**	**0**
Vacuolation:										
Minimal	**3**	**--**	**--**	**2**	**3**	**1**	**--**	**--**	**0**	**0**
Slight	**0**	**--**	**--**	**0**	**1**	**0**	**--**	**--**	**0**	**0**
Liver (hepatocyte):										
Increased pigment										
Minimal	**0**	**--**	**--**	**8**	**8**	**1**	**--**	**--**	**5**	**8**
Slight	**0**	**--**	**--**	**0**	**0**	**0**	**--**	**--**	**1**	**0**

Table 1-5 GW433908G: 6-Month Oral Gavage Toxicity Study in Han Wistar Rats - Toxicokinetics

		Male					Female				
APV Base Equivalent Dose (mg/kg/day)		0	100	320	1000	1500	0	100	320	1000	1500
GW433908 (mg/kg/day)		0	149	478	1493	2240	0	149	478	1493	2240
No. of Animals: TK		4	12	12	12	12	4	12	12	12	12
GW433908G											
$AUC_{0-\infty}$ (µg•h/mL)	Day 1	-	0.052	0.156	1.575	0.616	-	0.034	0.299	4.719	1.256
AUC_{0-24} (µg•h/mL)	Day 92	-	0.124	0.367	1.435	2.597	-	0.130	0.368	1.681	2.421
	Day 179	-	0.030	0.229	0.930	1.079	-	0.270	3.267	1.001	1.815
GW433908G											
C_{max} (µg/mL)	Day 1	-	0.021	0.036	0.050	0.047	-	0.010	0.132	0.069	0.124
	Day 92	-	0.022	0.045	0.152	0.035	-	0.029	0.047	0.233	0.322
	Day 179	-	0.015	0.032	0.102	0.012	-	0.102	0.381	0.184	0.143
APV											
$AUC_{0-\infty}$ (µg•h/mL)	Day 1	-	13	84	154	243	-	33	74	237	253
AUC_{0-24} (µg•h/mL)	Day 92	-	16	48	58	63	-	19	52	77	93
	Day 179	-	20	46	57	55	-	23	54	62	107
APV											
C_{max} (µg/mL)	Day 1	-	3	8	10	9	-	3	9	13	10
	Day 92	-	2	4	6	6	-	2	5	7	7
	Day 179	-	3	5	5	5	-	2	5	7	8

Table 1-6a Exposure Ratio of APV in Rats Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G or Amprenavir (APV) and Ritonavir (RTV)

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC_{0-24h} [b] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)	Ratio of Rat to Human AUC Following APV/RTV administration (APV20001)
Rat 6 month RD1998/02858/01	149 (100)	M	2.90	19.8	0.6	0.3
		F	2.31	22.7	0.6	0.4
	478 (320)	M	5.09	46.2	1.3	0.7
		F	5.23	54.3	1.5	0.8
	1493(1000)	M	5.33	57.0	1.6	0.9
		F	7.28	62.3	1.7	1.0
	2240 (1500)	M	5.28	54.9	1.5	0.9
		F	7.68	107	3.0	1.7
Human GW433908G study (APV20001)	(48[c])	M+F	5.30	35.8[d]	--	--
Human APV/RTV study (APV20001)	(48[e])	M+F	7.17	64.4[f]	--	--

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: End of study data. Arithmetic mean values are quoted for rat data
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: Based on multiple dose following administration of GW433908, i.e., AUC_{0-12h} (17.89 µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours
e.: 1200 mg QD APV in a 50 Kg person
f.: Based on multiple dose following administration of 1200 mg APV + 200 mg RTV QD

Table 1-6b Exposure Ratio of GW433908 in Rats Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC_{0-24h} [b] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)
Rat 6 month RD1998/02858/01	149 (100)	M	0.015	0.030	0.4
		F	0.102	0.271	0.39
	478 (320)	M	0.032	0.229	3.3
		F	0.381	3.267	46.7
	1493(1000)	M	0.102	0.930	13.3
		F	0.184	0.100	14.3
	2240 (1500)	M	0.117	0.108	15.4
		F	0.143	0.182	25.9
Human GW433908G study (APV20001)	(48[c])	M+F	0.030	0.070[d]	--

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: End of study data. Arithmetic mean values are quoted for rat data
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: AUC_{last} value since GW433908 levels were very low and were not present past a few hours. Based on multiple dose following administration of GW433908, i.e., AUC_{last} (35 ng•hr/mL), multiplied by 2 to obtain exposure for 24 hours
Data Collected: toxicokinetic, clinical observation, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weight, macroscopic and microscopic pathology

36. GW433908A: 14-Day oral gavage toxicity study in beagle dogs (GW report No.: RD1998/00487/00)

GW study No.: D40350; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 18 March 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908G solution in reverse-osmosis treated water

Methods

Four groups of 3 male Beagle dogs (body weight: 11-14 kg; age: approximately 11 months at the start of dosing) received daily doses of 0 (vehicle), 50, 130, or 350mg/kg/day GW433908A by oral gavage twice daily approximately 6 hours apart for 14 days. Each animal was given a detailed clinical examination once during the pretreatment period and prior to necropsy. The animals were observed four times daily for signs of ill health. Body weights and food consumption were recorded once weekly. Ophthalmoscopic examinations were performed on all animals before and after dosing. Electrocardiogram was measured in all animals pre- and after the first daily dose. Blood samples were collected at terminal necropsy for clinical chemistry and hematology analysis. Urine samples were collected and analyzed after overnight fasting. At necropsy, a complete gross examination was carried out on each animal and organ weight (adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids) and lesions were recorded. A spectrum of tissues from all animals were taken and preserved, and examined by a pathologist (Appendix Table 2).

Results

Clinical signs: Salivation was noted at ≥130mg/kg/day. Vomiting and loose feces were noted in the ≥130mg/kg/day group.

Body weights and food consumption: There was a decrease in body weights in the 350mg/kg/day group on Day 8 (10%), and 15 (16%). A moderate decrease in food consumption (50%-75%) was noted in two dogs given 350mg/kg/day during the second week of treatment.

Ophthalmoscopy: No treatment related ocular changes were noted.

ECG: One dog dosed with 350mg/kg/day exhibited frequent, ventricular complexes.
Hematology: A decrease in reticulocyte percent (64%) and number (65%), a moderate decrease (40%) in WBC (due to 22%-66% decreases in neutrophils, lymphocytes, and eosinophils), minimal increases (3-9%) in RBC, hematocrit, hemoglobin and MCHC values along with a minimal decrease in MCV values (3%) were noted on Day 14 in the 350mg/kg/day group.
Clinical chemistry and urine analysis: Decrease in serum sodium (3%), potassium, chloride, and phosphate (13-22%) along with slight increases in total protein (11%), and albumin (16%) were noted in dogs at 350mg/kg/day. Decreases (68%-95%) in the urine electrolytes (potassium, sodium and chloride) and in the total fractional excretion were noted in the 350mg/kg/day group (Table 1).
Gross pathology: Male dogs given 350mg/kg/day exhibited an increase (22%) in liver weights. Decreases in absolute prostate weights were seen in two or three animals treated with 130mg/kg/day (32%) and 350mg/kg/day (39-49%).
Histopathology: Tubular basophilia, hepatocellular hypertrophy and focal centrilobular vacuolation, and oligospermia and tubular cell debris in the epididymis were seen in the 350mg/kg/day group. Sertoli cells vacuolation and the presence of Sertoli cell-only tubules were recorded in testes of treated. Immaturity of the prostate gland was noted in all animals from the 350mg/kg/day group and in one dog from the 130mg/kg/day group. Coronary arteritis and prominent C-cell complexes in the thyroid glands were seen in the 350mg/kg/day group (Table 2).

Comments
The **NOEL** for this study was 50mg/kg/day. This is equivalent to a human dose of approximately 28mg/kg/day based on body surface area. ECG changes (ventricular complexes) were seen in dogs at 350mg/kg/day, which might be attributed to the hypokalemia secondary to the effect of the test materials. Decreases in electrolyte values (due to repeated vomiting and hemoconcentration) are consistent with the toxicological profile of GW433908. Decreased fractional excretion of urinary electrolytes was considered as a response to the decreases of serum electrolyte levels. Pathological changes including tubular basophilia associated with mononuclear infiltrate and tubular mineralization, hepatocellular hypertrophy and focal centrilobular vacuolation of hepatocytes are consistent with the toxicological profile of GW433908.

Toxicokinetics

Methods
Blood samples were taken at 0 (predose), 1, 2, 6 (prior to the second dosing), 7, 8, 10, and 24 hours after the first dosing (controls bled at 7 hours postdose only) on Day 1 and Day 13. Plasma concentrations of GW433908A and amprenavir were determined, under the GLP conditions, using an [redacted] method. Toxicokinetic parameters, C_{max} and AUC_{∞} were calculated for the full dosing period.

Results
Both GW433908A, the prodrug of amprenavir, and amprenavir were detected in plasma in this study. Plasma concentrations of amprenavir were determined in all dose groups. Plasma concentrations of GW433908A, however, were determined only for the 350mg/kg group. Amprenavir, was present in higher levels than GW433908A in the plasma evaluated. Average adjusted values for C_{max} and AUC_{∞} (GW433908A and amprenavir) were shown in Table 3.

Comments
These data provide evidence of exposure to amprenavir and GW433908A after oral dosing of GW433908A. At the NOEL (50mg/kg/day), amprenavir AUC was 19 µg*h/ml.

Table 1. Fractional excretion of urine electrolytes in dogs with oral administration of GW433908A twice daily for 14 days

On Day -6 GW433908A (350mg/kg/day)	Potassium (%)	Sodium (%)	Chloride (%)
No. 10	13.60	0.20	0.68
No. 11	15.40	0.25	0.93

No. 12	13.20	0.27	0.93
On Day 10 GW433908A (350mg/kg/day)	Potassium (%)	Sodium (%)	Chloride (%)
No. 10	9.61	0.02	0.05
No. 11	4.51	0.01	0.03
No. 12	6.45	0.14	0.04

Table 2. 14-Day oral gavage toxicity study in beagle dogs with GW433908A

Tissue	0 mg/kg/day	50 mg/kg/day	130 mg/kg/day	350 mg/kg/day	Comments
Adrenals	X	X	X	X	X: collected and
Aorta	X	X	X	X	examined
Brain	X.	X.	X.	X	XX: prepared but
Rib bone marrow smear	XX	XX	XX	XX	not examined
Cecum	X	X	X	X	
Colon	X	X	X	X	
Duodenum	X	X	X	X	
Epididymides	X	X	X	X.	
Esophagus	X	X	X	X	
Eye & optic nerves	X	X	X	X	
Femur head	X	X	X	X	
Gall bladder	X	X	X	X	
Harderian glands	X	X	X	X	
Heart	X	X	X	X* (1/3)	X*:coronay arteritis
Ileum	X	X	X	X	
Jejunum	X	X	X	X	X*:tubular
Kidneys	X	X	X	X*(1/3)	basophilia
Liver	X	X	X	X*(1/3)	X*:hepatocellular
Lungs (with bronchi)	X	X	X	X	hypertrophy and
Mandibular lymph node	X	X	X	X	centrilobular
Mesenteric lymph node	X	X	X	X	vacuolation
Pancreas	X	X	X	X	
Peripheral nerve (sciatic)	X	X	X	X	
Pituitary	X	X	X	X	
Prostate	X	X	X* (1/3)	X* (3/3)	X*: immaturity of
rectum	X	X	X	X	the prostate gland
Salivary gland	X	X	X	X	
Seminal vesicles	X	X	X	X	
Skeletal muscle	X	X	X	X	
Skin (mammary glands)	X	X	X	X	
Spinal cord cervical	X	X	X	X	
Spinal cord lumbar	X	X	X	X	
Spinal cord thoracic	X	X	X	X	
Spleen	X	X	X	X	
Sternum (bone marrow)	X	X	X	X	
Stomach	X	X	X	X	X*: Sertoli cell
Testes	X	X*(1/3)	X*(1/3)	X***(1/3)	vaculation
Thymus	X	X	X	X	X***: Oligospermia
Thyroids (parathyroids)	X	X	X	X	Tubular cell debris
Tongue	X	X	X	X	
Trachea	X	X	X	X	
Urinary bladder	X	X	X	X	

Figures in brackets represent the number of animals from which this tissue was examined.

Table 3. Toxicokinetics parameters of GW433908A and amprenavir (141W94) in male beagle dogs after oral administration of GW433908G during a 14-day toxicity study

Dose *(mg/kg/day*	Day 1				Day 13			
	C_{max} *(µg/ml)*		AUC_{∞} *(µg*h/ml)*		C_{max} *(µg/ml)*		AUC_{∞} *(µg*h/ml)*	
	I	II	I	II	I	II	I	II
50	**5**	**--**	**28**	**--**	**3**	**--**	**19**	**--**
130	**6**	**--**	**53**	**--**	**4**	**--**	**30**	**--**
350	**14**	**0.22**	**95**	**0.29**	**21**	**0.77**	**226**	**1.46**

I. = amprenavir (141W94); II = GW433908A

37. GW433908G: One-month oral gavage toxicity study in beagle dog (GW report No.: RD1998/02605/00)

GW study No.: D40436; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 15 December 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/31/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethyl-cellulose in 0.1% (w/w) Tween 80

Methods

Five groups of 4 to 6 male or female beagle dogs (body weight for males: 8.6-11.6 kg, for females: 8.1-9.3; age: approximately 15 months at the start of dosing) received total daily dose of 0 (vehicle), 75, 194, 523 or 747mg/kg/day (dose volume: 10 ml/kg/day) by oral gavage administered twice daily for 33 days. Four dogs per group to evaluate toxicity were euthanized for post mortem examination and the remainder (2 dogs) maintained untreated for an additional 20 days to study the regression or progression of any treatment-related abnormalities. Each animal was given a detailed clinical examination once during the pretreatment period and prior to necropsy. Animals were observed four times daily for signs of ill health during the study period. Body weights and food consumption were measured once daily. Ophthalmoscopic examinations were performed on all pre-treated animals and treated animals. Electrocardiogram was measured in all animals pretreatment and at the end of the treatment period. Blood samples were collected at terminal necropsy for clinical chemistry and hematology analysis. Urine samples were collected and analyzed after overnight fasting. Four dogs per group were necropsied at the end of the treatment period and the remainder maintained untreated for additional 20 days to study the regression or progression of any treatment-related abnormalities. A complete gross examination was carried out on each animal and organ weight (adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids) and lesions were recorded. A spectrum of tissues from all animals were taken, preserved, and examined by a pathologist (Table 1).

Results

Clinical signs and mortality: Salivation was observed in dogs given ≥75mg/kg/day. A dose-related loose, watery feces was noted in dogs given ≥ 194mg/kg/day. Slight to moderate dehydration was noted in males (≥ 194mg/kg/day) and in females (≥ 523mg/kg/day). Note that two male dogs were sacrificed: one on Day 9 (unconfirmed dosing accident, 75mg/kg/day) and one on Day 20 (confirmed dosing accident, 523mg/kg/day).

Body weights and food consumption: There were decreases in body weights and food consumption in males and females at ≥ 523mg/kg/day mg/kg/day (Table 2).

Ophthalmoscopy: There were no ocular changes related to test article.

ECG: Relative increases in QT interval (not exceeded normal limits) were observed in animals given ≥194mg/kg/day. Three dogs given 523mg/kg/day and four dogs given 747mg/kg/day showed increases in U wave amplitude in precordial leads and one female dog had ventricular begeminy. An increase in the numbers of leads with T wave notching was found in animals given ≥194mg/kg/day.

Hematology: There were no treatment-related effects on cell parameters.

Clinical chemistry and urine analysis: Slight sodium and chloride increases (2-4%) and potassium decreases (3-13%) were observed in both sexes (≥ 523mg/kg/day). Decreases in urine pH (in female: 10%; in male: 24%), specificity (in male: 2%) and urinal potassium (in female: 37%; in male: 54%) were noted in the males and females treated with 747mg/kg/day. Increases in serum alkaline phosphatase in males (105%-184%), and in females (124%-192%) given ≥ 194mg/kg/day were not dose-related.

Gross pathology: Treatment-related increases in absolute liver weights (males: 9-31%; female: 5-27%) were noted in dogs given ≥75mg/kg/day, which was reversible. Decreases in absolute thymus weights (males: 7-29%; female: 30-61%) in dogs at ≥75mg mg/kg/day, which, however, were irreversible.

Histopathology: No microscopic findings were noted in liver to explain the liver weight changes. Thymic atrophy, however, was found in animals with decreased thymus weights. Decreases in bone marrow cellularity were observed in males at 747mg/kg/day and females at ≥523mg/kg/day (Table 1).

Comments

The **NOAEL** for this study should be 75 mg/kg/day and equivalent to a human dose of approximately 38 mg/kg/day based on body surface area. Higher doses (≥190mg/kg/day) caused more clinical signs including dehydration, body weight and food consumption reductions, increases in liver weight, and increase in alkaline phosphatase as well as ECG changes. The ECG changes were associated with

decreased serum potassium levels that accompanied diarrhea and vomiting, which are typical of hypokalemic effects on the ECG including the coupled ventricular extrasystoles in a female dog at 523mg/kg/day dose.

Note that no microscopic findings were noted in liver to explain the liver weight changes caused by GW433908G. Thymic atrophy, however, were found in animals with decreased thymus weights, which was considered as a secondary, stress-related response to treatment with GW433908G.

Table 1. Histopathology of beagle dogs in the one-month oral gavage toxicity study

Tissue No. Examined (Terminal/recovery)	GW433908G (0mg/kg/day) (M:4; F: 4)	GW433908G (75mg/kg/day) (M: 3; F: 4)	GW433908G (194mg/kg/day) (M:4, F:4)	GW433908G (523mg/kg/day) (M:3, F:4)	GW433908G (747mg/kg/day) (M:4, F:4)	Comments
Adrenals	X	X	X	X	X	X: collected and examined
Aorta	X	X	X	X	X	
Brain	X.	X.	X.	X	X	
Cecum	X	X	X	X	X	
Colon	X	X	X	X	X	
Duodenum	X	X	X	X	X	
Epididymides	X	X	X	X	X	
Esophagus	X	X	X	X.	X.	
Eye & optic nerves	X	X	X	X	X	
Femur/joint	X* (M:1,F:0)	X*(M:2,F: 1)	X*(M:2, F:1)	X*(M: 0, F:2)	X*(M:3; F:2))	
Gall bladder	X	X	X	X	X	
Harderian glands	X	X	X	X	X	
Heart	X	X	X	X	X	
Ileum	X	X	X	X	X	
Jejunum	X	X	X	X	X	
Kidneys	X	X	X	X	X	
Liver	X	X	X	X	X	
Lungs (with bronchi)	X	X	X	X	X	
Mandibular lymph node	X	X	X	X	X	
Mesenteric lymph node	X	X	X	X	X	
Pancreas	X	X	X	X	X	
Peripheral nerve (sciatic)	X	X	X	X	X	
Pituitary	X	X	X	X	X	
Prostate	X	X	X	X	X	
rectum	X	X	X	X	X	
Salivary gland	X	X	X	X	X	
Seminal vesicles	X	X	X	X	X	
Skeletal muscle	X	X	X	X	X	
Skin (mammary glands)	X	X	X	X	X	
Spinal cord cervical	X	X	X	X	X	
Spinal cord lumbar	X	X	X	X	X	
Spinal cord thoracic	X	X	X	X	X	
Slpeen	X	X	X	X	X	
Sternum (bone marrow)	X	X	X	X	X	
Stomach	X	X	X	X	X	
Testes	X	X	X	X	X	
Thymus	X*(M: 0, F:1)	X*(M:2, F,:3)	X*(M:2; F: 3)	X*(M:3, F:4)	X*(M:4, F:4)	X*: thymic atrophy(Day3 3)
Thyroids (parathyroids)	X(M:0; F: 0)	X*(M:1; F: 0)	X*(M:1; F: 3)	X*(M:1; F: 2)	X*(M:1; F:1)	
Tongue	X	X	X	X	X	
Trachea	X	X	X	X	X	X*: diffuse follicular hypertrophy (Day33)
Urinary bladder	X	X		X	X	
Head						
Femoral bone marrow smear	XX XX	XX XX	XX XX	XX XX	XX XX	

***Figures in brackets represent the incidence of abnormal findings from animals in which this tissue was examined.**
M: male dogs; F: female dogs.

Table 2. Body weight changes in dogs with oral administration of GW433908A (twice daily for one month with a 20-day recovery period)

Daily dose (mg/kg/day)	0	75	194	523	747	0	75	194	523	747
Number of animals										
Main	6	4	4	6	6	6	4	4	6	6
Recovery	2	–	–	2	2	2	–	–	2	2
Body weight	(% change from control)					(% change from control)				
Day 33 (End of treatment)	–	-2	-8	-13	-9	–	-3	0	-14	-12
Day 56 (End of recovery)	–	0	0	-7	-2	–	0	0	-3	2

Body weight gain	(% change from control)					(% change from control)				
Day 33 (Day 1, 2-34, 37)	--	-41	-57	-164	-210	--	-80	-29	-581	-487
Day 56 (Day 37-56)	--	0	0	78	100	--	0	0	435	159

Toxicokinetics

Methods

Four groups of 4 to 6 male and female beagle dogs received daily doses of 0 (vehicle), 75, 194, 523 or 747mg/kg/day of GW433908G by oral gavage administered twice daily for 33 days. Blood samples were obtained from these animals on Day 1 and 28 to evaluate toxicokinetics. Blood samples were taken at 0 (predose), 1, 2, 6 (prior to the second dosing), 7, 8, 10, and 24 hours after the first daily dosing (blood samples from control rats were taken 7 hours after the first daily dose). Plasma concentrations of GW433908A were determined, under GLP conditions, using an [redacted] method. Toxicokinetic parameters, C_{max} and AUC_{∞} were calculated for the full dosing period.

Results

GW433908G and amprenavir C_{max} increased in a dose-dependent, but not dose-proportional manner, on day 1 and day 28. Estimates of GW433908/amprenavir t_{max} (1/1-2 hours) and $t_{1/2}$ (0.5-2/2-3 hours) were similar on Day 1 to Day 28. Estimates of GW433908G and amprenavir AUC ratio varied in a non-dose-dependent manner on Day 1, and were less than 1% in all doses. Average estimates of GW433908G C_{max}, AUC, and AUC ratio tended to decrease from Day 1 to Day 28 in the two low dose groups, and increased from Day 1 to Day 28 in the two high dose groups (Table 3).

Table 3. Toxicokinetics parameters of GW433908G and amprenavir (141W94) in beagle dogs after oral administration of GW433908G (twice daily, oral, one month administration)

Dose mg/kg/day	Day 1								Day 28							
	C_{max} (µg/ml)				AUC_{∞} (µg*h/ml)				C_{max} (µg/ml)				AUC_{∞} (µg*h/ml)			
	I		II		I		II		I		II		I		II	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
75	5.4	5.2	0.01	0.02	27.5	32.5	0.02	0.02	5.5	5.5	0.01	0.01	29.8	35	0.01	0.01
194	10.4	10.7	0.02	0.2	86.4	67.7	0.04	0.4	10.4	12.2	0.05	0.05	68.2	56.7	0.05	0.07
523	15.4	15.1	0.1	0.1	145	128	0.6	0.2	18.4	24.5	0.3	0.7	95.6	238	0.6	2.6
747	15.5	16.5	0.2	0.5	133	128	0.4	1.4	31.5	23.4	0.8	0.9	208	156	1.6	2.2

I = amprenavir (141W94); II = GW433908G

38. GW433908G: Nine-Month Oral Toxicity Study in Beagle Dogs (GW report No.: RD1998/02861/01)

Study No.: D40418: [redacted] Study No.: 6169-235; Conducting facility: [redacted] Date Initiation: 21 December 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/32/1, R4283/33/1 and R4283/34/1; Formulation: GW433908G suspension (7.5, 19.5 and 33.7 mg/mL) in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study finding: The no-observed-adverse-effect level (NOAEL) was < 75 mg/kg/day for male and female dogs. Clinical observations (vomiting and abnormal feces), elevated alkaline phosphatase and increased hepatocellular pigment were noted at this dose. The results from the current study are qualitatively similar to those from previous dog studies with 141W94. Liver was identified as the major target organ for toxicity with GW433908G as in previous 141W94 dog toxicity studies. The major differences are that this study showed no statistically significant differences in liver weights and no microscopic evidence of hepatocellular hypertrophy. Lymphocytic depletion (synonymous with atrophy) in the thymus was observed in some of the unscheduled deaths in the study, which was considered a secondary, stress-related response to the treatment with GW433908G. Thymic atrophy was observed in the one-month study at both the scheduled and unscheduled necropsies with GW433908G (Report RD1998/02605/00). GW433908G induced effects were reversible at all dose levels except for the hepatocellular pigment.

Methods

Dosing:

Species/Strain: Dog/beagle
#/sex/group (main study): 6 dogs/sex/group (vehicle), 4 dogs/sex/group (75 and 195 mg/kg/day), 12 dogs/sex/group (337 mg/kg/day); satellite groups used for recovery: 2 dogs/sex/group (vehicle), 4 dogs/sex/group (337 mg/kg/day)
Age: approximately 14 to 16 months
Body Weight: Male = 8.2 to 11 kg; Female = 8 to 10.8 kg
Doses in administration units: 0, 75, 195 and 337 mg/kg/day GW433908G, equivalent to 0 (vehicle), 50, 130 and 225[1] mg/kg/day amprenavir, respectively ([1]Note that on Days 1 to 23 of the study, the high dose dogs were dosed with either 350 or 500 mg/kg/day APV dose equivalence, dosing was suspended on Day 24 and resumed on Day 29 with the two higher dose groups combined and the dose reduced to 225 mg/kg/day APV dose equivalence)
Rout, dosing frequency and dose volume: Oral (gavage); dosed twice daily, 6 hours apart; 5 mL/kg/dose (10 mL/kg/day)
Duration of treatment: at least 39 weeks, with a 4-week treatment-free recovery period

Observations and times:
Clinical examiniation: Once pretreatment and once prior to necropsy
Clinical signs: 4 times daily during the treatment period
Body weights: Once daily pretreatment and once daily during the treatment period
Food consumption: Once daily pretreatment and once daily during treatment period
Ophthalmic examination: Once daily pretreatment and during treatment period
ECG and respiration rate and rectal body temperature: Once daily pretreatment and during treatment period
Hematology: At Weeks 5, 14, 26, 39, and at the end of recovery period (Week 44)
Clinical chemistry: At Weeks 5, 14, 26, 39, and at the end of recovery period (Week 44)
Urinalysis: At Weeks 5, 14, 26, 39 and at the end of recovery period
Gross pathology (main): At the end of the treatment period (terminal)
Gross pathology (recovery): at the end of the recovery period (recovery)
Organs weighed: Adrenal glands, brain, heart, kidney, liver, lungs, pituitary, prostate, spleen, testes, thymus and thyroids
Histopathology: A spectrum of tissues from all animals were taken, preserved, and examined by a pathologist (Appendix Table 1).
Toxicokinetics: For toxicokinetic evaluation, blood samples were taken at 0 (predose), 1, 2, 6 (prior to the second dosing), 7, 8, 10, and 24 hours after the first daily dosing. Plasma concentrations of GW433908A and APV were determined, under GLP conditions, using an [redacted] method.
Other: For recovery evaluation, dogs (2 dogs/sex/group) in the control and the highest dose group (337 mg/kg/day GW433908G; 4 dogs/sex/group) were maintained untreated for an additional 30 days to study the regression or progression of any treatment-related abnormalities.

The doses used in this study were selected based upon the results of 2 week and 1-month dog studies (Re: RD 1998/00487/00, & RD 1998/02605/00), and the 12-month oral toxicity study with APV in dogs (Re: RD1997/01249/00). Note that the 6-hour interval was selected to aid technical efficiency and was not based on the kinetics of amprenavir or GW433908G.

Results

Mortality: Eleven dogs died or were sacrificed over the course of the study. Four females and one male at ≥350 mg/kg/day APV dose equivalence (≥525 mg/kg/day GW433908) died in the first 23 days of the study due to dose intolerance with nausea, anorexia and vomiting, which was considered treatment-related. Dosing at these levels was terminated after 24 days of dosing. The remaining deaths were seen in one control male, one male at 50 mg/kg/day APV equivalent dose (75 mg/kg/day GW433908) and one male and two females at 225 mg/kg/day APV equivalent dose (337 mg/kg/day GW433908). The cause of these deaths was aspiration of the dose preparation based on the clinical signs, macroscopic and microscopic findings, and was not related to the administration of GW433908.

Clinical signs: A dose dependent, treatment-related vomiting, abnormal feces and excessive salivation were observed in dogs at doses of ≥75 mg/kg/day GW433908 (≥50 mg/kg/day APV dose equivalence) during the study period.
Body weight: Mild, treatment-related decreases in body weights were seen in females at doses of ≥75 mg/kg/day GW433908 (≥50 mg/kg/day APV dose equivalence) from week 27 through week 35 (Table 2-1).
Food consumption: Meaningful food consumption data was not obtained due to nutritional supplementation given to the animals because of the excessive clinical signs.
Ophthalmic examination: No treatment-related changes were seen.
ECG and respiration rate and rectal body temperature: No treatment-related changes were seen.
Hematology: No treatment-related alterations in hematology were seen at all doses.
Clinical chemistry: Treatment-related increases in alkaline phosphatase were seen in one or more dogs at ≥75 mg/kg/day GW433908. Mild increases in ALT were seen in female dogs at 337 mg/kg/day GW433908 (Table 2-2). These changes were associated with an increased hepatocellular pigment accumulation compatible with lipofusin.
Urinalysis: No treatment-related alterations in urine volume, specific gravity, and creatine, potassium and sodium excretion were seen in dogs at all doses.
Gross pathology: There were no statistically significant differences in the liver weights and no microscopic evidence of hepatocellular hypertrophy noted.
Histopathology: Treatment-related microscopic findings were limited to the liver. Hepatocellular pigment was seen in dogs at all doses in both male and female dogs. At the end of the recovery period, this change was non-reversible. Lymphocytic depletion in the thymus was seen in some of the unscheduled deaths in this study, and was considered a secondary, stress-related response to the treatment with GW433908 (Table 2-3).

Toxicokinetics: Toxicokinetic parameters for APV and GW433908 from the 9-month toxicity study in dogs are in general agreement with parameter estimates from previous 1 month oral toxicity study with GW433908G in dogs. Toxicokinetic data demonstrated that systemic exposure to APV and GW433908G was achieved and that estimates of GW433908 and amprenavir C_{max} and AUC generally increased with increasing dose in a dose-proportional manner on Days 1, 95, 180 and 273. In general, exposure ratios (GW433908 to APV) were less than 0.005 (Table 2-4).

TOXICOLOGY SUMMARY

- A No Observed Adverse Effect Level (NOAEL) could not be determined in this study due to clinical observations (vomiting and abnormal feces), elevated alkaline phosphatase and increased hepatocellular pigment seen in both male and female dogs in the low dose group (75 mg/kg/day GW433908).
- The impurities are the . The impurities present in batches of GW433908G used in the study are considered to be toxicologically qualified since animals in the intermediate and high dose groups received total daily doses in excess of potential clinical exposure.
- Findings from repeat dose studies in dogs with GW433908G of 9-month duration were generally consistent with the toxicological profile of amprenavir. GW433908G was not well tolerated when administered to dogs at doses up to 337 mg/kg/day GW433908G (225 mg/kg/day amprenavir dose equivalence) for 9 months. Vomiting, abnormal feces and excessive salivation were the clinical signs related to treatment and the liver was the major target organ for toxicity. Treatment-related increases in alkaline phosphatase were seen in one or more dogs at ≥75 mg/kg/day GW433908. Mild increases in ALT were seen in female dogs at 337 mg/kg/day GW433908. These changes were associated with an increased hepatocellular pigment accumulation compatible with lipofusin, which is non-reversible.

TOXICOLOGY CONCLUSIONS

- In 9 month repeat dose toxicity studies with GW433908G in dogs, findings were generally consistent with the toxicological profile of APV and previous 1 month studies with GW433908 (A or G salt form) where adverse effects comprised gastrointestinal disturbances and liver changes. A No Observed Adverse Effect Level (NOAEL) could not be determined in this study due to clinical observations

(vomiting and abnormal feces), elevated alkaline phosphatase and increased hepatocellular pigment seen in both male and female dogs in the low dose group (75 mg/kg/day GW433908).

- Terminal exposure to APV at the high dose level (225 mg/kg/day) in the dog study with GW433908 was approximately 2.5 to 4.0 times the exposure previously obtained in human following a dose of 1200 mg APV + 200 mg ritonavir once daily.
- Terminal exposure to unbound APV at the low (50 mg/kg/day), intermediate (130 mg/kg/day) and high dose levels (225 mg/kg/day) in the dog study with GW433908G were approximately 0.7 to 1.0, 4.8 to 6.0 and 6.7 to 10.8 times, respectively, the estimated exposure to unbound APV obtained in human following a dose of 1200 mg APV + 200 mg ritonavir once daily.
- Terminal exposure to GW433908 at the high dose level in the dog study with GW433908 was approximately 7.9 to 23.3 times the exposure previously obtained in human following dosing with GW433908G equivalent to 1200 mg BID APV.
- It is considered that this study provides assurance of the safety of GW433908G in the proposed clinical trials at doses equivalent to 1200 mg BID APV, 1200 mg APV + 200 mg ritonavir once daily and 600 mg APV + 100 mg ritonavir BID (Tables 2-5a and 2-5b).

Table 2-1 GW433908G: 9-Month Oral Gavage Toxicity Study in Beagle Dogs– Body Weight and Body Weight Change

	Male				Female			
APV Base Equivalent Dose (mg/kg/day)	0	50	130	225	0	50	130	225
GW433908 (mg/kg/day)	0	75	195	337	0	75	195	337
Body Weight (kg)								
Week 2	9.7	9.5	9.6	9.6	9.0	8.9	9.0	8.3
Week 3	9.8	9.7	9.6	9.5	9.1	8.9	9.0	8.3
Week 4	9.7	9.8	9.5	9.2	8.9	8.8	9.1	8.2
Week 27	11.0	10.7	10.7	10.5	10.7	9.6	9.2	8.7*
Week 28	10.9	10.7	10.7	10.5	10.8	9.6	9.2	8.7*
Week 29	10.9	10.8	10.8	10.7	10.9	9.6	9.4	8.9*
Week 32	10.8	10.7	10.8	10.4	10.8	9.7	9.2	8.8
Week 33	10.8	10.7	10.8	10.4	10.8	9.6	9.2	8.7*
Week 34	10.9	10.8	10.9	10.6	11.0	9.7	9.3	8.7*
Week 35	11.0	10.6	10.9	10.6	10.8	9.7	9.3	9.0
Week 40	11.0	10.9	10.8	10.6	11.0	9.9	9.3	9.0
(Recovery) Week 44	11.8	–	–	10.4	12.3	–	–	–
Body Weight Change (kg)								
Week 1-4	0	0.1	-0.2	-0.6	-0.2	-0.2	-0.1	-0.4
Week 7-8	0	0.2	0	0.1	0.1	0	0.4	0
Week 8-9	0.1	0	0.1	0.1	0.2	0.1	0	0
Week 29-30	0	-0.2	0	-0.1	-0.2	0	-0.1	-0.1
Week 5-40	0.8	1.1	1.3	1.3	1.8	1.0	0.6	0.7
(Recovery) Week 40-44	0	–	–	0.2	0.3	–	–	0.7

- $P \leq 0.05$

Table 2-2 GW433908G: 9-Month Oral Gavage Toxicity Study in Beagle Dogs– Clinical Chemistry

	Male				Female			
APV Base Equivalent Dose (mg/kg/day)	0	50	130	225	0	50	130	225
GW433908 (mg/kg/day)	0	75	195	337	0	75	195	337
Clinical Chemistry	Control	% Change From Control			Control	% Change From Control		
ALT, IU/L								
Week 5	35	-3	-9	3	27	0	15	-7
Week14	37	11	014	3	27	0	44	56*
Week 26	42	-19	-21	-12	30	-7	13	40
Week 39	32	6	0	16	24	21	21	88
(Recovery) Week 44	30	–	–	10	24	–	–	4
Alkaline Phosphatase, IU/L								
Week 5	80	23	89	94	53	32	87	119*
Week14	66	82	48	192	55	38	142*	176*
Week 26	64	136	47	209	52	62	154*	187*
Week 39	57	146	56	272*	59	44	147*	188*
(Recovery) Week 44	65	–	–	18	46	–	–	52

- P≤0.05

Table 2-3 GW433908G: 9-Month Oral Gavage Toxicity Study in Beagle Dogs– Gross Pathology and Histopathology

	Male				Female			
APV Base Equivalent Dose (mg/kg/day)	0	50	130	225	0	50	130	225
GW433908 (mg/kg/day)	0	75	195	337	0	75	195	337
Pathology Findings:	Incidence of Findings							
Microscopic, Terminal No. of Animals Examined	3	3	4	8	4	4	3	4
Liver: Hepatocellular pigment Minimal Slight	0 0	3 0	2 1	7 0	0 0	2 0	2 0	3 1
Pathology Findings	Incidence of Findings							
Microscopic, Recovery No. of Animals Examined	2	-	-	2	2	-	-	2
Liver: Hepatocellular pigment Minimal	0	-	-	2	0	-	-	2

Table 2-4 GW433908G: 9-Month Oral Gavage Toxicity Study in Beagle Dogs – Toxicokinetics and Unscheduled Deaths

		Male				Female			
APV Base Equivalent Dose (mg/kg/day)		0	50	130	225	0	50	130	225
GW433908 (mg/kg/day)		0	75	195	337	0	75	195	337
No. of Animals:									
Main		6	4	4	12	6	4	4	12
Recovery		2	0	0	4	2	0	0	4
GW433908G									
AUC_t (µg•h/mL)	Day 1	-	0.038	0.062	0.627	-	0.039	0.060	0.335
$AUC_{0\text{-}24}$ (µg•h/mL)	Day 95	-	--	0.052	0.666	-	--	0.340	0.772
	Day 180	-	0.040	0.103	0.417	-	0.044	0.204	1.045
	Day 273	-	0.028	0.127	0.556	-	0.048	0.381	1.632
GW433908G									
C_{max} (µg/mL)	Day 1	-	0.012	0.029	0.301	-	0.013	0.019	0.134
	Day 95	-	--	0.020	0.281	-	--	0.172	0.285
	Day 180	-	0.075	0.105	0.317	-	0.056	0.129	0.838
	Day 273	-	0.058	0.114	0.375	-	0.041	0.292	0.850
APV									
AUC_t (µg•h/mL)	Day 1	-	24	64	86	-	30	63	129
	Day 95	-	28	95	153	-	30	169	195
	Day 180	-	30	98	139	-	35	147	214
	Day 273	-	23	113	139	-	31	143	257
APV									
C_{max} (µg/mL)	Day 1	-	5	12	12	-	6	8	13
	Day 95	-	4	12	17	-	5	19	20
	Day 180	-	6	13	17	-	7	17	21
	Day 273	-	4	14	18	-	5	21	25

Table 2-5a Exposure Ratio of APV in Dogs Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G or Amprenavir (APV) and Ritonavir (RTV)

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC $_{0-24h}$ [a] *µg•h/mL*	Ratio of Dog to Human AUC Following GW433908G administration (APV20001)	Ratio of Dog to Human AUC Following APV/RTV administration (APV20001)
Dog 9 month RD1998/02861/01	75	M	3.98	22.9	0.6	0.4
	(50)	F	4.55	30.6	0.9	0.5
	195	M	14.4	113	3.2	1.8
	(130)	F	20.5	143	4.0	2.2
	337[b]	M	17.9	159	4.4	2.5
	(225)	F	25.1	257	7.2	4.0
Human GW433908G study (APV20001)	(48[c])	M+F	5.30	35.8[d]	–	–
Human APV/RTV study (APV20001)	(48[e])	M+F	7.17	64.4[f]	–	–

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: On days 1-23, dogs were dosed with either 525 or 750 mg/kg/day GW433908G (equivalent to 350 and 500 mg/kg/day APV base). Due to severe intolerance, dosing was suspended on Day 24 and resumed on Day 29 with dose reduced to 337 mg/kg/day GW433908G (equivalent to 225 mg/kg/day APV base)
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: Based on multiple dose following administration of GW433908, i.e., AUC_{0-12h} (17.89 µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours
e.: 1200 mg QD APV in a 50 Kg person
f.: Based on multiple dose following administration of 1200 mg APV + 200 mg RTV QD

Table 2-5b Exposure Ratio of GW433908 in Dogs Following Repeat Dose Administration of GW433908G and in Human Following Administration of GW433908G

Study Type Report No.	Dose of GW433908G (APV base dose equivalence) *mg/kg/day*	Sex	Mean C_{max} [a] *µg/mL*	Mean AUC $_{0-24h}$ [a] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)
Dog 9 month RD1998/02858/01	75	M	0.058	0.028	0.4
	(50)	F	0.041	0.048	0.7
	195	M	0.114	0.127	1.8
	(130)	F	0.292	0.381	5.4
	337[b]	M	0.375	0.556	7.9
	(225)	F	0.850	1.632	23.3
Human GW433908G study (APV20001)	(48[c])	M+F	0.030	0.070[d]	–

a.: End of study data. Arithmetic mean values are quoted for rat data
b.: .: On days 1-23, dogs were dosed with either 525 or 750 mg/kg/day GW433908G (equivalent to 350 and 500 mg/kg/day APV base). Due to severe intolerance, dosing was suspended on Day 24 and resumed on Day 29 with dose reduced to 337 mg/kg/day GW433908G (equivalent to 225 mg/kg/day APV base)
c.: 1200 mg BID APV dose equivalence in a 50 kg person
d.: AUC_{last} value since GW433908 levels were very low and were not present past a few hours. Based on multiple dose following administration of GW433908, i.e., AUC_{last} (35 ng•hr/mL), multiplied by 2 to obtain exposure for 24 hours

Histopathology Inventory for Repeat Dose Toxicology Studies

Study	GW Study No.:R40860 Rat 13-Week Study	GW Study No.: R40417 Rat 6-Month Study	GW study No.: D40436 Dog 1-Month Study	GW Study No.: D40418 Dog 9-Month Study
Species	Han Wistar Rat	Han Wistar Rat	Beagle Dog	Beagle Dog
Adrenals	X*	X*	X*	X*
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X	X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X*	X*	X*	X*
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube	X	X	X	X
Gall bladder				
Gross lesions	X	X	X	X
Harderian gland	X	X	X	X
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland	X	X	X	X
Larynx	X	X	X	X
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes, cervical	X	X	X	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity	X	X	X	X
Optic nerves	X	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	X*	X*	X*	X*
Parathyroid	X*	X*	X*	X*
Peripheral nerve	X	X	X	X
Pharynx	X	X	X	X
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X	X	X	X
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X

Uterus	X	X	X	X
Vagina	X	X	X	X
Zymbal gland	X	X	X	X
Standard List	X	X	X	X

X, histopathology performed; *, organ weight obtained

3.4.4 Genetic Toxicology Studies

39. GW433908A: Salmonella-Escherichia coli/mammalian-microsome reverse mutation plate incorporation and pre-incubation assays

GW report No.: RD1998/000935/00; GW study No.: V40351; Conducting facility [redacted]
Date Initiation: 10 April 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1

The ability of GW433908A to induce reverse mutations at the histidine locus in the genome of Salmonella typhimurium tester strains (TA98, TA100, TA1535 and TA1537) and at the tryptophan locus in an Escherichia coli tester strain WP2 uvrA (PKM101) was evaluated both in the presence and absence of an exogenous metabolic activation system derived from Aroclor™ - induced rat liver (S9).

Methods
A dose range finding study was carried out using ten doses of test article from 114 to 5500 µg per plate. The mutagenicity assay was conducted in the presence and absence of S9 mix along with vehicle and positive controls using three plates per dose. The doses tested were 114, 343, 380, 1010, 1120, 3360, 3360, 5050 and 5500 µg per plate in both the presence and absence of S9 mix. Positive controls (TA98-2.5 µg/plate benzopyrene or 1.0 µg/plate 2-nitrofluorene; TA100-2.5 µg/plate aminoanthracene or 2.0 µg/plate sodium azide; TA1535-2.5 µg/plate aminoanthracene or 2.0 µg/plate sodium azide; TA1537-2.5 µg/plate aminoanthracene or 2.0 µg/plate ICR-191; WP2uvrA(PKM101)-5 µg/plate aminoanthracene or 2.0 µg/plate 4-nitroquinoline-N-oxide) were used in this study to produce appropriate mutagenic responses in the tested bacterial strains.

Results
GW433908 was not mutagenic in the Salmonella-Escherichia coli mammalian microsome plate incorporation plate incorporation and pre-incubation assays. No toxicity was observed in these assays at concentrations up to 4010 µg per plate. No increase in the number of revertants was observed with the Salmonella strains tested and with *E. Coli* strain *WP2uvra* (pKM101) in the presence and absence of S9 mix. No positive response was observed (at concentration up to 5050 µg/per plate) in the plate incorporation and up to 5500 µg/plate in the pre-incorporation assay, while positive controls produced appropriate mutagenic responses in all bacterial strains in the presence and absence of S9 metabolic activation.

40 GW433908A: L5178Y/TK mouse lymphoma in vitro mammalian cell mutagenesis Assay

GW report No.: RD1998/001213/00; GW study No.: V40376; Conducting facility [redacted] Date Initiation: 1 May 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1

The mutagenic potential of GW433908A was assessed in mammalian cells *in vitro* in the L5178Y mouse lymphoma assay.

Methods
In the initial chromosomal aberration assay, GW433908A, up to 5000 µg/ml, was tested for 4 hours in L5178Y mouse lymphoma cells in the presence and absence of Aroclor induced rat S9 and harvested 22 hours after the initiation of treatment for the analysis. In the confirmation assay, cells were treated for 24 hours without metabolic activation or 4 hours with Aroclor induced rat liver S9 and harvesting 22 hours after initiation of treatment for the analysis.

Results

There was no observable increase in mutation frequency at concentration up to 5000 µg/ml GW433908A in either the initial or confirmatory mutagenicity assay. GW433908A was not mutagenic *in vitro* in the mouse lymphoma assay, while the positive cotrols showed positive results.

41. GW433908G (batch number DNPIA/38/25/3): Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report No.RD1999/02761/00)

GW study No.: V40707; Conducting facility: [redacted] Date Initiation: 14 December 1999; GLP Compliance: Yes (X) No ( ); Drug reference No.: GW433908G containing elevated impurities ([redacted]), Drug Lot: DNPIA/38/25/3

The ability of GW433908G with impurities [redacted] to induce reverse mutations at the histidine locus in the genome of *Salmonella typhimurium* tester strains (TA98, TA100, TA1535 and TA1537) and at the tryptophan locus in an *Escherichia coli* tester strain WP2 *uvr*A (PKM101) was evaluated both in the presence and absence of an exogenous metabolic activation system derived from Aroclor™ - induced rat liver (S9).

Methods

A standard plate incorporation assay with TA98, TA100, TA1535, TA1537 and WP2 *uvr*A (PKM101) was carried out at dose levels of 33.3, 100, 333, 1000, 3330, and 5000 µg per plate both in the presence and absence of S9 metabolic activation along with vehicle (DMSO, CAS#67-68-5. [redacted] Lot A012097501) and positive controls (TA98-2.5 µg/plate benzopyrene or 1.0 µg/plate 2-nitrofluorene; TA100-2.5 µg/plate aminoanthracene or 2.0 µg/plate sodium azide; TA1535-2.5 µg/plate aminoanthracene or 2.0 µg/plate sodium azide; TA1537-2.5 µg/plate aminoanthracene or 2.0 µg/plate ICR-191; WP2uvrA(PKM101)-5 µg/plate aminoanthracene or 2.0 µg/plate 4-nitroquinoline-N-oxide) using three plates per dose. The confirmatory assay with TA1535 was carried out at dose levels of 33.3, 100, 333, 1000, 3330, and 5000 µg per plate both in the absence of S9 metabolic activation along with vehicle and positive controls using three plates per dose. Appropriate positive controls were used to produce a mutagenic response in all bacterial strains in the presence and absence of S9 metabolic activation.

Results

GW433908G. batch number DNPIA/38/25/3 with impurities [redacted] was not mutagenic in *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and in an *Escherichia coli* strain WP2 *uvr*A (PKM101) in the standard plate incorporation assay. No toxicity was observed in these assays at concentrations up to 5000 µg per plate. No increase in the mean number of revertants was observed with the *Salmonella* strains tested and with *E. Coli* strain *WP2uvra* (pKM101) in the presence and absence of S9 mix. No positive response was observed (at concentration up to 5000 µg per plate) in the plate incorporation assay, while positive controls showed positive responses in the assay.

Comments

GW433908G, batch number DNPIA/38/25/3 with impurities [redacted] was not mutagenic in either the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9) in the standard *Salmonella-Escherichia coli* mammalian microsome plate incorporation assay.

42. GW433908G (Batch number DNPIA/38/25/1): Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report No. RD1999/02762/00)

IND No.: 58627; Serial No.: 122; Vol. No.: 6 of 6; Page 215; GW report No.: RD1999/02762/00; GW study No.: V40708; Conducting facility: [redacted] Date Initiation: 11 January 2000; GLP Compliance: Yes (X); Drug reference No.: GW433908G containing elevated impurities [redacted] Drug Lot: DNPIA/38/25/1