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 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 absence of S9 metabolic activation along with vehicle and positive controls using three plates per dose. Positive controls produced an appropriate mutagenic response in all bacterial strains in the presence and absence of S9 metabolic activation.

Results

GW433908G, batch number DNPIA/38/25/2 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 TA100 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.

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.

43. GW433908G (Batch number DNPIA/38/25/2): Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report RD1999/02763/01)

GW study No.: V40706; Conducting facility: [redacted] Date Initiation: 11 January 2000; GLP Compliance: Yes (X); Drug reference No.: GW433908G containing elevated impurities [redacted], Drug Lot: DNPIA/38/25/2

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 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 absence of S9 metabolic activation along

with vehicle and positive controls using three plates per dose. Positive controls produced an appropriate mutagenic response in all bacterial strains in the presence and absence of S9 metabolic activation.

Results

GW433908G, batch number DNPIA/38/25/2 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.

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.

44. GW433908G: Micronucleus frequencies in bone marrow polychromatic erythrocytes from male han wistar rats following oral administration (Report No. RD1999/00412/00)

GW report No.: RD1999/00412/00; Study No.: R40476; Conducting facility: [redacted]
Date Initiation: 28 June 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80 in reverse osmosis water

Methods

To evaluate its clastogenic potential, GW433908G was tested in the bone marrow micronucleus assay. Drugs were administered to male Han Wistar rats (7 rats/group; age: 10 weeks; body weight: 177-330 g) by oral gavage at dosages of 0 (vehicle), 748, 1495, or 2990 mg/kg/day for one day. Doses were given on one day in two equal portions, the second portion was given six hours after the first. These doses gave APV dose equivalents of 500, 1000 and 2000 mg/kg. Positive control groups were treated with cyclophosphamide (20 mg/kg). Rats were sacrificed and bone marrow was harvested from the femur approximately 28 and 48 hours after the last dose and examined for micronucleus frequencies, polychromatic erythrocytes (PCEs) and PCE/NCE ratios (7 animals/timepoints; three slides/animal). A total of 1000 erythrocytes per animal were counted to determine the PCE:NCE ratio. A total of 2000 PCEs per animal were analysed to assess the incidence of micronucleated PCEs. A total of 2000 cells per animal were scored for micronuclei. Four satellite groups were used to provide blood for determination of plasma concentrations of GW433908G. Rats received an oral dose of 0 (vehicle), 748, 1495, and 2990 mg/kg. Blood samples were collected from the abdominal vena cava from satellite groups approximately 2 hours following the 2nd dosing. Plasma drug concentrations were measured by an HPLC method.

Results

No clastogenic effect was detected at any dose level with GW433908G 24 and 48 hours after the last dose. GW433908G did not produce more micronucleated bone marrow polychromatic erythrocytes (PCEs) than that occurring in vehicle controls, while negative and positive controls produced met the criteria for a valid assay and were consistent with laboratory historical data. Note that the positive control (20 mg/kg cyclophosphamide) was positive in the bone marrow micronucleus assay. Systemic exposure to GW433908X and APV was achieved in all animals treated with GW433908G. For GW433908X, the average plasma concentrations at 2 hours after the second dose were 9.6 ± 4.7, 40.6 ± 34.2 and 56.8 ± 26.8 ng/ml, for the 748, 1495 and 2990 mg/kg GW433908G dose groups, respectively. The average plasma concentrations for APV at 2 hours after the second dose were 8.2, 10.1 and 11.9 µg/ml, for the 748, 1495 and 2990 mg/kg GW433908G dose groups, respectively.

Conclusions

GW433908 was not clastogenic in the in vivo micronucleus assay in rats.

3.4.5. Carcinogenicity:

Carcinogenicity studies in rats and mice with GW433908G are currently being carried out and final reports will be available in 2005. Dose levels were selected based on results from a pilot 13-week study in mice and from the 6-month study in rats.

3.4.6. Reproductive and developmental toxicology:

46. GW433908G: oral male and female fertility study in CD (sprague dawley) rats (Report No. RD1999/01281/00)

GW Study No.: R40458; [redacted] Study No.: 65C-07498; Conducting facility: [redacted] Date Initiation: 2 August 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; 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:

- The parental NOAEL was determined to be equivalent to 201 mg/kg/day APV (300 mg/kg/day GW433908; APV AUCs: 87.4, 46.0 and 49.9 µg•h/ml on Days 1, 27 and 42, respectively). Decreased body weight gain and food consumption, and increased liver weights in both sexes, as well as increased testes weights (without histological findings) in male rats were seen at 548 and 1498 mg/kg/day APV equivalence (820 and 2240 mg/kg/day GW433908, respectively).

- The male reproductive NOAEL was determined to be 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908). The female reproductive NOAEL was determined to be 548 mg/kg/day APV dose equivalence (820 mg/kg/day GW433908). The reduced gravid uterine weights and reduced numbers of ovarian corpora lutea and uterine implantation sites were seen at the 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908), a dose that produced evidence of systemic toxicity in female rats.

- The developmental NOAEL for *in utero* developmental toxicity of F1 conceptuses was considered to be equivalent to 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908), based on the absence of effects on the number of resorptions and post-implantation loss.

Methods

Dosing:

Species/Strain: Rat/CD® (Sprague-Dawley)
#/sex/group or time point (main study): 25 rats/sex/group
Satellite groups used for toxicokinetics: 16 rats/sex/group
Age: approximately 10 weeks (male) and 8 weeks (females)
Body Weight: 271 to 353 g (males); 196 to 233 g (females)
Doses in administration units: 0, 300, 820 and 2240 mg/kg/day GW433908G, equivalent to 0 (vehicle), 201, 548 and 1498 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 dosing: Males – 4 weeks pre-paring to mating (up to 42 days); females- 2 weeks pre-pairing to Day 6 of pregnancy (up to 35 days; day of mating = Day 0 of pregnancy)

Observations and times:

Clinical signs: Once 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
Caesarean sections and evaluation of uterine contents: on Gestation Day 13
Toxicokinetics: For toxicokinetic evaluation, blood samples from male rats in the control group and the test article treatment groups were collected on Dose Days 1, 27 and 42 and from females on Dose Days 1, 13 and 28, prior to dosing (0 hour) and at time points between 0 and 24 hours after the first daily dose.

Results

Mortality: No treatment-related mortality or morbidity was observed in male and female rats.

Clinical signs: Treatment-related pale feces and piloerection were observed in parental female rats at doses of 2240mg/kg/day GW433908 (1498 mg/kg/day APV dose equivalence). The toxicological significance of these findings is unclear since they were seen only in females in this study and not in previous repeat dose studies with GW433908G.

Body weight: Treatment-related reductions in body weight gain were seen in males and females at doses of ≥820mg/kg/day GW433908 (≥548 mg/kg/day APV dose equivalence). A slight reduction in body weight gain was seen in male rats at 300mg/kg/day GW433908 (201 mg/kg/day APV dose equivalence) during the first 7 days of treatment (Table 3-1).

Food consumption: Treatment-related reductions in food consumption were seen in males and females at doses of ≥820mg/kg/day GW433908 (≥548 mg/kg/day APV dose equivalence).

Terminal and necroscopic evaluation: Treatment-related increases in relative liver weights to body weights (males: 8-18%; female: 9-30%) and in absolute liver weights (male: 4-10%, female: 8-17%) were noted in rats at all dose levels in both sexes, which may be the result of enzyme induction (Table 3-1). There were no histological findings in the liver. In males, treatment-related increases in testes weights and epididymides weights were seen. Absolute testes weights were increased (6-7%) at ≥820 mg/kg/day GW433908 (≥548 mg/kg/day APV dose equivalence). Relative testes weights increased at all doses. However, there were no histological findings in the testes or epididymides. Treatment-related reductions in gravid uterine weights were seen in female rats at 2240 mg/kg/day GW433908 (1498 mg/kg/day APV dose equivalence). This was considered most likely due to the reduced number of ovarian corpora lutea and uterine implantation sites following maternal treatment with GW433908. There are no treatment-related changes in other reproductive indices seen in males and females, including mating success, precoital interval, estrous cycle, and viability of offspring (Table 3-1).

Toxicokinetics: 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, 13 and 28. In general, exposure ratios (GW433908 to APV) were less than 0.04. Mean C_{max} and total systemic exposure in all dose groups decreased from Day 1 to Day 28, consistent with auto-induction (Table 3-2).

Reproductive and developmetal toxicology conclusion:

- The male reproductive NOAEL was determined to be 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908). The female reproductive NOAEL was determined to be 548 mg/kg/day APV dose equivalence (820 mg/kg/day GW433908).
- The developmental NOAEL for *in utero* developmental toxicity of F1 conceptuses was considered to be equivalent to 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908)
- At the developmental NOAEL (1498 mg/kg/day APV dose equivalence), pregnant rats produced exposures (AUC) to APV of 148 μg•h/ml on Day 28, which were 4 times higher than the expected therapeutic exposure (AUC) in humans following a dose of GW433908G equivalent to 2400 mg/day APV.
- The number of ovarian corpora lutea and uterine implantation sites were considered to be lower due to body weight changes in female rats at 2240 mg/kg/day GW433908 (1498 mg/kg/day APV dose equivalence).

Table 3-1 GW433908G: Oral Male and Female Fertility Study in CD-Sprague Dawley Rats

	Male					Female			
APV Base Equivalent Dose (mg/kg/day)	0	201	548	1498	APV Base Equivalent Dose (mg/kg/day)	0	201	548	1498
GW433908 (mg/kg/day)	0	300	820	2240	GW433908 (mg/kg/day)	0	300	820	2240
Body weight change (g)					Body weight change (g)				
Premating Days 1 -28	148	132	125	87*	Premating Days 0-14	32	29	24*	11.6*
Mating: Days 28-42	43	43	41*	34*	Gestation Days 0-6	29	29	26	12.3*
					Gestation Days 6-13	37	40	37	46.5*
Food consumption (g/rat/day)					Food consumption (g/rat/day)				
Premating Days 1 -28	30	29	28	25*	Premating Days 0-14	21	20	19	15.6*
Mating: Days 35-42	30	30	29	27*	Gestation Days 0-6	24	24	22	18.9*
					Gestation Days 6-13	26	27	26	25.8
Organ weight changes	Ctrl	% Change from control				Ctrl	% Change from control		
Liver (g)	23	4	10*	0	Liver (g)	16	8	14*	17*
Liver relative to BW(%)	4.7	8*	16*	18*	Liver relative to BW(%)	4.9	9*	20*	30*
Testes (g)	3.3	4	6*	7*	Gravid uterus (g)	10.9	0	-8	-16
Testes relative to BW (%)	0.7	8*	13*	26*					
Epididymides (g)	1.3	2	2	0					
Epididymides relative to BW (%)	0.3	6	8*	17*					
Reproductive					Reproductive				
# paired	25	25	25	24	# paired	25	25	25	24
# of mating	24	25	25	24	# of mating	24	25	25	24
# siring pregnancy	21	24	25	24					
Reproductive									
Estrous cycle: proportion of normal estrous cycle sequences (mean/female)						100	100	100	100
Time of mating (days)						2.9	2.5	2.6	2.9
mating index (%)						96	100	100	100
Fertility index (%)						88	96	100	100
# pregnant at cesarean section						21	24	25	24
Developmental									
Uterine parameters:									
# corpora lutea (mean/dam)						16	15.3	15.1	13.7*
# of implantation (mean/dam)						15.8	15.4	14.5	13.8*
Pre-implantation loss (%)						4.7	5.6	5.0	4.1
# live fetuses (mean/dam)						15.4	14.8	14.4	13.4
% resorption per litter (mean)						2.1	6.5	2.4	3.6
Post-implantation loss (%)						2.1	6.5	2.4	3.6

BW: body weight, * P≤0.05

Table 3-2 GW433908G: Oral Male and Female Fertility Study in CD-Sprague Dawley Rats – Toxicokinetics

		Male				Female			
APV Base Equivalent Dose (mg/kg/day)		0	201	548	1498	0	201	548	1498
GW433908 (mg/kg/day)		0	300	820	2240	0	300	820	2240
No. of Animals: TK		16	16	16	16	16	16	16	16
GW433908X									
AUC_{0-} (µg•h/mL)	Day 1	0.646*	0.714	1.068	2.052	2.533*	0.698	1.087	1.675
	Day 13	0.828*	0.544	0.738	3.378	0.191*	0.843	1.561	5.253
	Day 28	1.184*	0.559	1.076	3.427	0.528*	0.949	3.186	4.548
GW433908X									
C_{max} (µg/mL)	Day 1	0.472*	0.039	0.091	0.250	0.718*	0.267	0.221	0.192
	Day 13	0.201*	0.101	0.061	0.380	0.036*	0.084	0.153	0.536
	Day 28	0.322*	0.998	0.119	0.264	0.168*	0.144	1.395	0.363
APV									
AUC_{0-} (µg•h/mL)	Day 1	-	92	168	227	-	87	196	392
	Day 13	-	58	57	111	-	46	62	90
	Day 28	0.02*	60	64	148	-	50	89	110
APV									
C_{max} (µg/mL)	Day 1	-	8	9	15	-	8	14	15
	Day 13	-	4	5	9	-	4	5	6
	Day 28	0.01*	5	6	11	-	5	7	7

*: Due to sample contamination, plasma concentrations of GW433908G and APV in control samples were measurable.

Table 3-3 Exposure Ratio of APV in CD-Sprague Dawley 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 fertility Study RD1999/01281/00 (R40458)	300 (201)	M	5.19	49.9	1.4	0.8
		F	4.56	59.5	1.7	0.9
	820 (548)	M	7.35	88.9	2.5	1.4
		F	5.95	63.8	1.8	1.0
	2240 (1498)	M	7.43	110	3.1	1.7
		F	11.3	148	4.1	2.3
Human GW433908G study (APV20001)	(48[c])	M+F	5.30	35.8[d]	–	–
Human APV/RTV study (APV20001)	(24[e])	M+F	7.17	64.4[f]	–	–

a.: Day 17 of pregnancy; arithmetic mean values are quoted for rat data
b.: Day 17 of pregnancy; 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 3-4 Exposure Ratio of GW433908X in CD-Sprague Dawley 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 $_{0\text{-}24h}$ [b] *µg•h/mL*	Ratio of Rat to Human AUC Following GW433908G administration (APV20001)
Rat fertility Study RD1999/01281/00 (R40458)	300 (201)	M	0.14	0.95	13.6
		F	0.10	0.56	8.0
	820 (548)	M	1.40	3.19	45.5
		F	0.12	1.08	15.4
	2240 (1498)	M	0.36	4.55	65.0
		F	0.26	3.43	49.0
Human GW433908G study (APV20001)	(48[c])	M+F	0.03	0.07[d]	--

a.: Day 17 of pregnancy; arithmetic mean values are quoted for rat data
b.: Day 17 of pregnancy; 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., $AUC_{0\text{-}12h}$ (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

47. GW433908G: Oral embryo-fetal development study in CD rats (Report No. RD1999/02690/00)

GW Study No.: R40470; Study No.: 207-032; Conducting facility: [redacted]; Date Initiation: 12 July 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G suspension (14.9, 47.8. 149.3 and 224 mg/ml) in 0.5% (w/w) hydroxypropyl-methylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

- The maternal NOAEL for GW433908 could not be determined in this study as dose related increases in the incidence of alopecia, and reduced body weight gains and food consumption values were seen at all dose levels.
- The pregnancy rate (80%) in rats at ≥ 820 mg/kg/day GW433908 was below the historical range of the pregnancy rate (88-100%) and that of the control animals (92%).
- The developmental NOAEL for GW433908 was greater than 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908), based on the absence of adverse effects on embryofetal development in this study.

Methods

Dosing:

Species/Strain: Rat/CD®IGSBR VAF/Plus® (Sprague-Dawley)
#/sex/group or time point (main study): 25 rats/sex/group
Satellite groups used for toxicokinetic: 16 rats/sex/group
Age: approximately 10-11 weeks
Body Weight on Day 0 of Pregnancy: 202 to 258 g
Doses in administration units: 0 (vehicle), 300, 820 and 2240 mg/kg/day GW433908G, equivalent to 0 (vehicle), 201, 549 and 1498 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 dosing: Days 6 to 17 of pregnancy

Observations and times:
Clinical signs: Once 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
Caesarean sections and evaluation of uterine contents: Rats were killed on Day 20 of pregnancy.
Toxicokinetics: For toxicokinetic evaluation, blood samples from rats on Dose Days 6 and 17 of pregnancy, prior to dosing (0 hour) and at time points between 0 and 24 hours after the first daily dose.

Results
Mortality: No treatment-related mortality or morbidity was observed in rats.
Clinical signs: Treatment-related soft or liquid feces and alopecia were observed in rats at doses of 2240mg/kg/day GW433908 (1498 mg/kg/day APV dose equivalence). Note that alopecia was not seen in studies with GW433908 in Wistar Han rats, The toxicological significance of these findings is unclear. Treatment-related ungroomed coat and urine-stained abdominal fur each occurred in one rat at doses of 2240mg/kg/day GW433908.
Body weight: Treatment-related and dose-dependent reductions in body weight and body weight gain were seen in rats at all doses over the first 24 hours of dosing (Gestation Days 6 to 7) (Table 4-1).
Food consumption: Absolute and relative reductions in food consumption were seen in rats at all doses during the treatment period (Gestation Days 6 to 18).
In-life observations: There was a slight reduction in pregnancy rate in rats at ≥ 820 mg/kg/day GW433908 (≥ 549 mg/kg/day APV dose equivalence). The pregnancy rate (80%) in rats at ≥ 820 mg/kg/day GW433908 was below the historical range of pregnancy rate (88-100%) and that of the control animals (92%). Note that no consistent correlation with clinical signs or body weight loss early in the treatment period was seen in the non-pregnant rats. In pregnant rats, no pre-implantation loss and post-implantation loss were seen at all doses.
Terminal and necroscopic evaluation: There are no treatment-related changes in uterine parameters and embryofetal development in rats at doses of GW433908 as high as 2240 mg/kg/day (Table 4-1).

Toxicokinetics: 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 non dose-proportional manner on Days 6 and 17. In general, exposure ratios (GW433908 to APV) were less than 0.04. Mean C_{max} and total systemic exposure of APV in the 300 and 820 mg/kg/day GW433908 groups decreased from Day 6 to Day 17, consistent with auto-induction (Table 4-2).

Summary and conclusion:
- The maternal NOAEL for GW433908 could not be determined in this study as dose related increases in the incidence of alopecia, and reduced body weight gains and food consumption values were seen at all dose levels.
- The pregnancy rate (80%) in rats at ≥ 820 mg/kg/day GW433908 was below the historical range of pregnancy rate (88-100%) and that of the control animals (92%).
- The developmental NOAEL for GW433908 was greater than 1498 mg/kg/day APV dose equivalence (2240 mg/kg/day GW433908), based on the absence of adverse effects on embryofetal development in this study.
- At the developmental NOAEL for GW433908, pregnant rats produced exposures (AUC) to APV of 57.1 µg•h/ml on Day 17, which were 1.6 times higher than the expected therapeutic exposure (AUC) in humans following a dose of GW433908G equivalent to 2400 mg/day APV (AUC: 35.8 µg•h/ml; Re: APV2001) (Table 4-3).

Table 4-1 GW433908G: Oral Embryofetal Development Study in CD-Sprague Dawley Rats

	Female			
APV Base Equivalent Dose (mg/kg/day)	0	201	549	1498
GW433908 (mg/kg/day)	0	300	820	2240
# of pregnant rats	23	24	20	20
Pregnancy rate (%)	92	96	80	80
Body weight change (g)				
Days 6-7	3.5	-5.2*	-9.9*	-12.1*
Days 6-9	11.7	3.7	1.4*	-2.6
Days 6-18	82.5	76.8	75	66.2*
Days 18-21	53.4	59.1	56.6	58.6
Days 0-21	73.1	70.0	59.7*	53.6*
Food consumption (absolute) (g/rat/day)				
Days 6 -28	25.6	23.6*	22.8*	21.3*
Days 18-21	26.9	27.7	28.3	28.0
Food consumption (relative) (g/kg/day)				
Days 6 -28	85.5	80	79.8*	75.7*
Days 18-21	72.4	74.4	78.1*	78.6*
Developmental				
Uterine parameters:				
# corpora lutea (mean/dam)	16.4	16	16.8	16.6
# of implantation (mean/dam)	14	14.2	14.8	14.7
Pre-implantation loss (mean%/dam)	15.5	11.1	11.2	10.7
# live fetuses (mean/dam)	13.6	13.9	14.4	14.4
# embryo/fetal losses (mean/dam):				
Early	0.4	0.3	0.4	0.3
Late	0	0	0	0
Dead fetus	0	0	0	0
Post-implantation loss (mean%/dam)	2.7	4.3	2.6	2.3
Fetal body weight (mean/g)	5.36	5.52	5.39	5.41
Fetal sex ratio (% males)	50.0	53.5	50.7	48.0
Total # live fetuses (litters)	312 (23)	334 (24)	288 (20)	288 (20)

BW: body weight, * P≤0.05

Table 4-2 GW433908G: Oral Embryofetal Development Study in CD-Sprague Dawley Rats - Toxicokinetics

	Female			
APV Base Equivalent Dose (mg/kg/day)	0	201	549	1498
GW433908 (mg/kg/day)	0	300	820	2240
No. of Animals: TK	16	16	16	16

GW433908X AUC_{0-} (µg•h/mL) Day 6 of pregnancy Day 17 of pregnancy	 - -	 0.055 0.330	 0.650 0.668	 1.322 1.908
GW433908X C_{max} (µg/mL) Day 6 of pregnancy Day 17 of pregnancy	 - -	 0.013 0.038	 0.047 0.131	 0.145 0.149
APV AUC_{0-} (µg•h/mL) Day 6 of pregnancy Day 17 of pregnancy	 - -	 68.5 26.9	 126 43.2	 227 57.1
APV C_{max} (µg/mL) Day 6 of pregnancy Day 17 of pregnancy	 - -	 4.71 3.07	 7.64 3.55	 8.52 5.94

Day of pregnancy = Day of mating

Table 4-3 Exposure Ratio of APV in CD-Sprague Dawley 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)
Oral Embryofetal Development Study RD1999/02690/00	300 (201) 820 (549)) 2240 (1498)	F F F	2.07 3.55 5.94	26.9 43.2 57.1	0.75 1.21 1.59	0.42 0.67 0.89
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.: Day 17 of pregnancy; arithmetic mean values are quoted for rat data
b.: Day 17 of pregnancy; 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 4-4 Exposure Ratio of GW433908X in CD-Sprague Dawley 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)
Oral Embryofetal Development Study RD1999/02690/00	300 (201) 820 (549)) 2240 (1498)	F F F	0.03 0.13 0.15	0.30 0.67 1.91	4.7 9.5 27.3
Human GW433908G study (APV20001)	(48[c])	M+F	0.03	0.07[d]	--

a.: Day 17 of pregnancy; arithmetic mean values are quoted for rat data
b.: Day 17 of pregnancy; 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., $AUC_{0\text{-}12h}$ (17.89 µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours

48. GW433908G: Oral dose range-finding study in nonpregnant new zealand white rabbits (Report No.RD1999/00465/00)

GW Study No.: I 40459; Study No.: 6169-241; Conducting facility: [redacted]; Date Initiation: 25 March 1999; GLP Compliance: No (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

- A NOAEL for GW433908 could not be determined in this study due to decreased body weight gain observed in rabbits at 149.5 mg/kg/day GW433908 (100 mg/kg/day dose equivalence to APV).

Methods

Dosing:

Species/Strain: Rabbit/New Zealand White Hra:(NZW)SPF females
#/sex/group or time point (main study): 3 rabbits/group
Age: approximately 6 months
Body Weight on Day 0 of the study: 3275-4104 g
Doses in administration units: 0 (vehicle), 149.5, 373.8, 747.5, 1121.3 and 1495 mg/kg/day GW433908G, equivalent to 0 (vehicle), 100, 250, 500, 750 and 1000 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 dosing: 14 days

Observations and times:

Clinical signs: Once 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
Macroscopic examiniation: animals were killed on Day 15.
Toxicokinetics: For toxicokinetic evaluation, blood samples from animals on Days 7 and 14, prior to dosing (0 hour) and 7 hours after the first daily dose (i.e., 1 hour after the second daily dose). The low limit of quantitation is [redacted]

Results

Mortality: No treatment-related mortality or morbidity was observed in animals.
Clinical signs: Treatment-related fewer fecal pellets were seen in all rabbits at 373.8 and 747.5 mg/kg/day GW433908 (250 and 500 mg/kg/day APV dose equivalence, respectively) and 2 out of 3 rabbits at 1121.3 and 1495 mg/kg/day GW433908 (750 and 1000 mg/kg/day APV dose equivalence, respectively).
Body weight: Treatment-related and dose-dependent reductions in body weight and body weight gain were seen in animals over the study period starting at Day 7 (Table 5-1).
Food consumption: Dose-dependent reductions in food consumption were seen in animals at all doses during the treatment period.
Terminal and necroscopic evaluation: There are no treatment-related macroscopic changes in animals at all doses of GW433908.

Toxicokinetics: Toxicokinetic data demonstrated that plasma concentrations of amprenavir and GW433908G were achieved and that estimates of GW433908 and amprenavir plasma concentrations (7 hours post-dose) generally increased with increasing dose in a greater than

dose-proportional manner on Days 1 and 14. Plasma concentration ratios (GW433908 to APV) were 0.06-0.6 (Table 5-2).

SUMMARY AND CONCLUSION:

- A NOAEL for GW433908 was not achieved in this study as dose-related decreases in body weight gains and in food consumption values were seen in rabbits at 149.5 mg/kg/day GW433908 (100 mg/kg/day APV dose equivalence).
- Based on this study, dose levels of GW433908 equivalent to 50, 100, 150 and 200 mg/kg/day APV were selected for a subsequent oral dose range-finding study in pregnant New Zealand white rabbits.

Table 5-1 GW433908G: Oral Dose Range-Finding Study in Nonpregnant New Zealand White Rabbits – Toxicological Findings

Sex	Female					
APV Base Equivalent Dose (mg/kg/day)	0	100	250	500	750	1000
GW433908 (mg/kg/day)	0	149.5	373.8	747.5	1121.3	1495.0
# of rabbits	3	3	3	3	3	3
Toxicological Findings:	Control	% Change from Control				
Body weight (g)						
Day 14	4084	-7	-17	-19	-16	-25
Body weight gain (g)						
Days 1-14	293	-56	-175	-233	-208	-313
Food consumption (g/rabbit)						
Days 1-14	2379	-11	-58	-63	-63	-78

Table 5-2 GW433908G: Oral Dose Range-Finding Study in Nonpregnant New Zealand White Rabbits – Toxicokinetics

Sex	Female					
APV Base Equivalent Dose (mg/kg/day)	0	100	250	500	750	1000
GW433908 (mg/kg/day)	0	149.5	373.8	747.5	1121.3	1495.0
No. of Animals: TK	3	3	3	3	3	3
GW433908X Plasma Concentration:						
7 h post-dose (µg/mL)						
Day 1	-	0.008	0.036	0.147	0.118	0.278
Day 14	-	0.008	0.117	0.278	0.710	1.930
APV Plasma Concentration:						
7 h post-dose (µg/mL)						
Day 1	-	0.26	0.97	2.13	1.87	8.10
Day 14	-	0.14	0.39	2.09	2.50	3.07

49. GW433908G: Oral dose range-finding study in pregnant New Zealand white rabbits (GW Report No. RD1999/00716/00)

GW Study No.: LI40460; [redacted] Study No.: 6169-243; Conducting facility: [redacted], Date Initiation: 23 April 1999; GLP Compliance: No (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

- The NOAEL for F0 females and fetuses was determined to be >200 mg/kg/day dose equivalence to APV (299 mg/kg/day GW433908) in this study since no dose-related maternal or fetal toxicity was observed at any dose level.

Methods

Dosing:

Species/Strain: Rabbit/New Zealand White Hra:(NZW) SPF females
#/sex/group or time point (main study): 5 rabbits/group
Age on Day 1 of pregnancy: approximately 6 months
Body Weight on Day 1 of pregnancy: 3363 to 4082 g
Doses in administration units: 0 (vehicle), 74.8, 149.5, 224.3 and 299 mg/kg/day GW433908G, equivalent to 0 (vehicle), 50, 100, 150 and 200 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 dosing: Day 7 to 20 of pregnancy (Day of mating = Day 1 of pregnancy)

Observations and times:

Clinical signs: Once 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
Caesarean section and macroscopic examiniation: animals were killed on Day 29 of pregnancy.
Toxicokinetics: For toxicokinetic evaluation, blood samples from animals on Days 7 and 20 of pregnancy, prior to dosing (0 hour) and 7 hours after the first daily dose (i.e., 1 hour after the second daily dose). The low limit of quantitation for APV was [redacted] The low limit of quantitation for GW433908 was [redacted]

Results

Mortality: No treatment-related mortality or morbidity was observed in this study. All animals survived until their scheduled caesarean section.
Clinical signs: Treatment-related fewer or no fecal pellets for several dams in all test groups were seen during gestation Days 7 to 20. The decreased fecal excretion observed on gestation Days 26-29 or 27 to 29 in all groups, including control, is considered normal, since the rabbits were nearing delivery.
Body weight: There were no statistically significant differences in mean maternal body weight and body weight gain between the control and test groups over the study period (Table 6-1).
Food consumption: There were no statistically significant differences in food consumption between the control and test groups over the study period.
Terminal and necroscopic evaluation: There are no treatment-related macroscopic findings noted in animals at all doses of GW433908 during the maternal necropsies. The pregnancy rates were 100% for females at 0, 50 and 200 mg/kg/day APV dose equivalence and 80% for females at 100 and 150 mg/kg/day APV dose equivalence. None of the females aborted, died, or delivered early. All of the pregnant females had viable fetuses. The gravid uterine weight and the number of corpora lutea and implantation sites, preimplantation loss, percent resorptions, postimplantation loss, and number of live fetuses were generally similar among the groups and showed no evidence of treatment-related changes (Table 6-1).
A single fetus in the 100 mg/kg/day APV dose equivalent group had a rudimentary tail, which was seen in one fetus from one litter with no similar findings in any other dose group. This finding is a spontaneous background finding in this strain of rabbit and was considered not to be treatment-related. No other fetal anomalies were seen in this study.

Toxicokinetics: Toxicokinetic data demonstrated that GW433908 and amprenavir plasma concentrations (7 hours post-dose) generally increased with increasing dose in a greater than dose-proportional manner on Days 7 of pregnancy and in a less than dose-proportional manner on Day 20 of pregnancy. Plasma concentration ratios (GW433908 to APV) were 0.06-0.6 (Table 6-2).

SUMMARY AND CONCLUSION:

- The NOAEL for F0 females and fetuses was determined to be >200 mg/kg/day dose equivalence to APV (299 mg/kg/day GW433908) in this study since no dose-related maternal or fetal toxicity was observed at any dose level.
- Based on this study, dose levels of GW433908 equivalent to 50, 150 and 450 mg/kg/day APV were selected for the definitive embryofetal development study in New Zealand white rabbits.

Table 6-1 GW433908G: Oral Dose Range-Finding Study in Pregnant New Zealand White Rabbits – Toxicological Findings

Sex	Female				
APV Base Equivalent Dose (mg/kg/day)	0	50	100	150	200
GW433908 (mg/kg/day)	0	74.8	149.5	224.3	299
# of rabbits	5	5	5	5	5
Toxicological Findings:					
Body weight (g)					
Gestation Day 29	4285	4088	4116	4034	4195
Body weight gain (g)					
Gestation Day 29	522	311	381	403	431
Food consumption (g/rabbit)					
Gestation Days 7-21	175	138	157	153	147
# pregnant	5	5	4	4	5
# of deaths	0	0	0	0	0
# of abortions	0	0	0	0	0
# with live fetuses at term	5	5	4	4	5

Table 6-2 GW433908G: Oral Dose Range-Finding Study in Pregnant New Zealand White Rabbits – Toxicokinetics

Sex	Female				
APV Base Equivalent Dose (mg/kg/day)	0	50	100	150	200
GW433908 (mg/kg/day)	0	74.8	149.5	224.3	299
No. of Animals: TK	5	5	5	5	5
GW433908X Plasma Concentration:					
7 h post-dose (µg/mL)					
Day 7 of pregnancy	-	0.003	0.014	0.019	0.022
Day 20 of pregnancy	-	0.012	0.015	0.023	0.017
APV Plasma Concentration:					
7 h post-dose (µg/mL)					
Day 7 of pregnancy	-	0.02	0.06	0.22	0.40
Day 20 of pregnancy	-	0.19	0.42	0.34	0.54

50. GW433908G: Oral embryo-fetal development study in New Zealand white rabbits (GW Report No. RD1999/01035/00)

GW Study No.: L40461; [redacted] Study No.: 6169-244; Conducting facility: [redacted] Date Initiation: 2 June 1999; GLP Compliance: Yes (X); Drug reference No.: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G suspension in 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) with 0.1% (w/w) Tween 80

Key study findings:

The maternal NOAEL in the rabbit was determined to be 50 mg/kg/day dose equivalence to APV (74.8 mg/kg/day GW433908) in this study since no dose-related maternal toxicity was observed at this dose level.

The developmental NOAEL in the rabbit was determined to be >450 mg/kg/day dose equivalence to APV (672.8 mg/kg/day GW433908) in this study since no dose-related fetal toxicity was observed at any dose level.

Methods

Dosing:

Species/Strain: Rabbit/New Zealand White
#/group or time point: 25 female rabbits/group
Age on Day 1 of pregnancy: approximately 6 months
Body Weight on Day 1 of pregnancy: 2829 to 4471 g
Doses in administration units: 0 (vehicle), 74.8, 224.3 and 672.8 mg/kg/day GW433908G, equivalent to 0 (vehicle), 50, 150 and 450 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 dosing: Day 7 to 20 of pregnancy (Day of mating = Day 0 of pregnancy)

Observations and times:

Clinical signs: Once 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
Caesarean section and macroscopic examiniation: animals were killed on Day 29 of pregnancy.
Toxicokinetics: For toxicokinetic evaluation, blood samples from animals on Days 7 and 20 of pregnancy, prior to dosing (0 hour) and at timepoints between 0 and 24 hours after the first daily dose. The low limit of quantitation for APV was [redacted] The low limit of quantitation for GW433908 was [redacted]

Results

Mortality: One female at 672.8 mg/kg/day GW433908G died on Gestation Day 27, which is considered treatment-related. One control female died on Gestation Day 16. Note that the cause of death was not determined. All other animals survived until their scheduled caesarean section.
Clinical signs: Treatment-related fewer or no fecal pellets were seen in dams in the 224.3 and 672.8 mg/kg/day GW433908 groups during gestation Days 7 to 21, which were correlated with decreased food intake in these two groups.
Body weight: There were significant dose-related decreases in mean body weight and weight change in female rabbits at 224.3 and 672.8 mg/kg/day GW433908. However, body weight gain increased after treatment ended. Mean total body weight change values for Gestation Days 7 to 21 were 65, 17, and –192% of control value for the 74.8, 224.3, and 672.8 mg/kg/day GW433908G groups, respectively (Table 7-1). There were slight decreases in body weight changes in females at 74.8 mg/kg/day GW433909, but these changes were within normal variation.
Food consumption: There was a significant decrease in food consumption in females at 672.8 mg/kg/day GW433908. Mean food consumption values during Gestation Days 7 to 21 were 89, 88 and 50% of control value for the 74.8, 224.3, and 672.8 mg/kg/day GW433908G groups, respectively (Table 7-1).

In-life observation: The pregnancy rates were 96% for females at 0, 74.8, and 224.3 mg/kg/day GW433908G and 100% for females at 672.8 mg/kg/day GW433908. A total litter resorption was seen in a female rabbit that died at 672.8 mg/kg/day GW433908.
Abortion was seen in one female at 74.8 mg/kg/day GW433908 on Gestation Day 27, one female at 224.3 mg/kg/day GW433908G on Gestation Day 26, and five females at 672.8 mg/kg/day GW433908G on Gestation Day 21, 23, 26, or 29. The increased number of abortions in the 672.8 mg/kg/day group is attributed to the test article-related decreases in food consumption. None of the females aborted, died, or delivered early.
Terminal and necroscopic evaluation: There are no treatment-related macroscopic findings noted in animals at all doses of GW433908 during the maternal necropsies. The mean gravid uterine weight and corrected terminal weight were similar among the groups. All of the pregnant females at caesarean section had viable fetuses. The number of corpora lutea and implantation sites, preimplantation loss, percent resorptions, postimplantation loss, and number of live fetuses were similar among the groups and showed no evidence of treatment-related changes (Table 6-1). Mean placental weights and mean fetal body weights were generally similar among groups. There were no treatment-related findings in fetal external, soft tissue, or skeletal evaluations.
Toxicokinetics: Toxicokinetic data demonstrated that GW433908 and amprenavir plasma concentrations (7 hours post-dose) generally increased with increasing dose in a greater than dose-proportional manner on Days 7 of pregnancy and in a less than dose-proportional manner on Day 20 of pregnancy. Plasma concentration ratios (GW433908 to APV) were 0.06-0.6 (Table 7-2).

SUMMARY AND CONCLUSION:

- The maternal NOAEL in the rabbit was determined to be 50 mg/kg/day dose equivalence to APV (74.8 mg/kg/day GW433908; AUC: 1.81µg•hr/mL) in this study since no dose-related maternal toxicity was observed at this dose level.
- The developmental NOAEL in the rabbit was determined to be ≥ 450 mg/kg/day dose equivalence to APV (≥ 672.8 mg/kg/day GW433908; AUC: ≥ 25.8 µg•hr/mL) in this study since no dose-related fetal toxicity was observed at any dose level.
- At the developmental NOAEL for GW433908, pregnant rabbits produced exposures (AUC) to APV of 25.8 µg•h/ml on Day 20, which was 0.72 times the expected therapeutic exposure (AUC) in humans following a dose of GW433908G equivalent to 2400 mg/day APV (AUC: 35.8 µg•h/ml; Re: APV2001) (Table 7-3).

Table 7-1 GW433908G: Oral Embryofetal Development Study in the New Zealand White Rabbits – Toxicological Findings

Sex	Female			
APV Base Equivalent Dose (mg/kg/day)	0	50	150	450
GW433908 (mg/kg/day)	0	74.8	224.3	672.8
# of rabbits	25	25	25	25
Toxicological Findings:				
Maternal	Control	% Change from Control		

Body weight changes (g)				
Gestation Days 7-21	176.7	-35	-83*	-209*
Gestation Days 21-29	63	108	150	340*
Food consumption (g/rabbit/day)				
Gestation Days 7-21	174.9	-11	-12	-50**
Gestation Days 21-29	118.6	16	28**	36**
# of deaths	1	0	0	1
# of abortions	0	1	1	5
# with live fetuses at term	23	23	23	19
Developmental				
# of abortions	0	1	1	1
Uterine parameters				
# corpora lutea (mean/dam)	10.0	11.4	10.0	10.3
#implantations (mean/dam)	9.1	9.5	8.8	9.0
Pre-implantation loss (mean%/dam)	8.6	15.9	12.3	11.7
Total # of live fetuses/# of litters	199/23	201/23	186/23	164/19
Live fetuses (mean/dam)	8.7	8.7	8.1	8.6
# embryo/fetal losses (mean):				
Early	0.1	0.4	0.4	0.1
Late	0.3	0.3	0.3	0.3
Dead fetus	0	0.0	0.0	0.0
Post-implantation loss (mean %/dam)	4.3	8.5	7.8	3.5
Fetal body weight (g)	41.8	39.9	42.1	40.1
Fetal sex ratio (% males)	52	57	48	51

Table 7-2 GW433908G: Oral Embryofetal Development Study in the New Zealand White Rabbits – Toxicokinetics

Sex	Female			
APV Base Equivalent Dose (mg/kg/day)	0	50	150	450
GW433908 (mg/kg/day)	0	74.8	224.3	672.3
No. of Animals: TK	25	25	25	25
GW433908X:				
$AUC_{0\text{-}24h}$ (µg•hr/mL)				
Day 7 of pregnancy	-	0.015	0.069	0.646
Day 20 of pregnancy	-	0.043	0.156	0.881
C_{max} (µg/mL)				
Day 7 of pregnancy	-	0.006	0.017	0.084
Day 20 of pregnancy	-	0.012	0.034	0.190
APV:				
$AUC_{0\text{-}24h}$ (µg•hr/mL)				
Day 7 of pregnancy	-	0.03	2.11	22.2
Day 20 of pregnancy	-	1.81	3.88	25.8
C_{max} (µg/mL)				
Day 7 of pregnancy	-	0.01	0.59	4.16
Day 20 of pregnancy	-	0.22	0.57	3.33

Table 7-3. Exposure of APV in Oral Embryofetal Development Study in New Zealand White Rabbit Following Repeat Dose 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 $_{0\text{-}24h}$ [b] *µg•h/mL*	Ratio of Animal to Human AUC Following GW433908G administration (APV20001)	Ratio of Animal to Human AUC Following APV/RTV administration (APV20001)
Oral Embryofetal Development Study RD1999/01035/00	74.8 (50)	F	0.22	1.81	0.05	0.03
	224.3 (150)	F	0.57	3.88	0.11	0.06
	672.8 (450)	F	3.33	25.8	0.72	0.40
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.: Day 20 of pregnancy; arithmetic mean values are quoted for rat data; **b.:** Day 20 of pregnancy; 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., $AUC_{0\text{-}12h}$ (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 7-4 Exposure GW433908X in Oral Embryofetal Development Study in New Zealand White Rabbit Following Repeat Dose 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 $_{0\text{-}24h}$ [b] *µg•h/mL*	Ratio of Animal to Human AUC Following GW433908G administration (APV20001)
Oral Embryofetal Development Study RD1999/01035/00	74.8 (50)	F	0.01	0.33	0.6
	224.3 (150)	F	0.03	0.67	2.2
	672.8 (450)	F	0.19	1.91	12.6
Human GW433908G study (APV20001)	(48[c])	M+F	0.03	0.07[c]	--

a.: Day 20 of pregnancy; arithmetic mean values are quoted for rat data
b.: Day 20 of pregnancy; arithmetic mean values are quoted for rat data
c.: 1200 mg BID APV dose equivalence in a 50 kg person. Based on multiple dose following administration of GW433908, i.e., $AUC_{0\text{-}12h}$ (17.89 µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours

Comments

The lack of clear safety margins for APV makes extrapolation of the findings from the rat fertility study, and rat and rabbit embryofetal development studies to humans very difficult. Therefore, GW433908G should only be used in pregnancy if the potential benefits justify the possible adverse events and that women of child baring potential participating in clinical studies with GW433908G should take adequate precaution against pregnancy.

51. Study R40486 -- GW433908G: Oral pre - and postnatal development study in cd (sprague-dawley) rats (Report No. RD1999/01282/00)

Conducting Laboratory: [redacted] Sponsor: Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709; Study No.: 65C-7510; Date Initiation: 28 September 1999; GLP Compliance: Yes (X); Test Material: GW433908G; Drug Lot: R4283/34/1; Formulation: GW433908G in 0.5% (w/w) hydroxypropyl methylcellulose and 0.1% (w/w) TWEEN®80

Methods

Dams (F0)

One hundred and fifty female and one hundred male albino CD® (Sprague-Dawley) rats CD®[SD]BR; [redacted] were used to generate timed-mated females for the study. F0 sperm-positive female rats (25 rats/dose; body weights on gestational day 0 (gd

0): 245.5 to 321 g) were given vehicle (0.5% hydroxypropyl methylcellulose and 0.1% TWEEN®80; w/w) or GW433908G at doses of 300, 820 or 2240 mg/kg/day (dose volume: 5 mL/kg) twice daily by gavage (at least six hours apart), from gd 6 through postnatal day (pnd) 20, a dosing duration of 36 to 38 days which included F1 fetal development and parturation as well as postpartum lactation (a mean gestational length is 21-23 days). Clinical signs, body weight, and feed consumption were evaluated on gd 0 (pretreatment), 6, 9, 12, 15, 18, and 20 (gestation periods) and pnd 0, 4, 7, 14, and 21 (lactation period). Clinical observations were recorded four times daily. Beginning on gd 20, the F0 females were examined twice daily for littering, or signs of dystocia.

Offsprings: F1 Progeny

Pre-wean development of the F1 generation was monitored by evaluating acquisition of landmarks, beginning on pnd 1 with pinna detachment and later by eye opening (pnd 11). All pups were evaluated for development, growth, and viability through to weaning. At weaning on pnd 21, 25 F1 pups/sex/group were randomly selected from the maximum number of litters for generating the F2 animals. They were subsequently housed individually for a minimum of 49 days, until the youngest F1 offspring reached pnd 70, without dosing. The remaining unselected F1 pups were also retained without dosing and group housed by sex by litter until they reached puberty. The selected pups were examined daily for clinical signs, and mortality, and weighed once weekly. All F1 pups (selected and unselected) were evaluated for onset of puberty by determining acquisition of vaginal patency (VP; opening; on pnd 22-44) and cleavage of the balanopreputial gland (preputial separation: PPS; on pnd 35-54). Pregnant F1 females were weighed throughout gestation on day 0, 6, 9, 12, 15, 18, and 20 and during early lactation on pnd 0 and 4, while the F1 males were weighed weekly throughout mating and gestation of the F2 litters. The F1 pregnant females were monitored twice daily, beginning on gd 20, for parturition.

Pre- and postnatal development study designs and target doses were shown in Text Table A and Figure

Figure 1 Study Design

F0 Females

Text Table A. Pre- and Postnatal Development Study Design and Target Doses

Group No.	No. Animals Dosed	No. Days Exposure	Dosing Period (gd through pnd)	Total Daily Dose of GW433908G (mg/kg/day)[a]	Dose per Time (mg/kg/dose)[b]	GW433908G Dosing Concentration (mg/ml)	Dose Volume per Time (ml/kg)
1	25	36-38	6 through 20	0.0	0.0	0.0	5.0
2	25	36-38	6 through 20	300	150	30	5.0
3	25	36-38	6 through 20	820	410	82	5.0
4	25	36-38	6 through 20	2240	1120	224	5.0

[a] Conversion of GW433908 form to 141W94 can be calculated using the correction factor 1.495, i.e., 1.495 g of GW433908G is equivalent to 1.0 g 141W94.

[b] Doses were administered by gavage twice per day, once in the morning and once in the afternoon, at least six hours apart.

Key: C = Quar
M = Matir
G = Gest
gd = Gestational day
L = Lactation (21 days)
pnd = Postnatal day
N = Necropsy
PWHP = Postwean holding period (minimum 49 days, so F1 offspring are at least 70 days old at end of this period)
VCE = Vaginal cyclicity evaluation of F1 females for 21 days immediately prior to mating
Gavage dosing of F0 females, gd 6 through pnd 20

Terminial and necroscopic evaluation:

F0 Dams

On pnd 21, all F0 dams were necropsied. The thoracic and abdominal organs were examined macroscopically. Uterine implantation scars were counted. Tissues showing macroscopic abnormalities, a sample of mammary tissue, one abdominal mammary gland, ovaries and pituitary were retained in neutral buffered 10% formalin. Uteri from any F0 females who appeared non-pregnant were stained with 10% ammonium sulfide.

F1 Dams

On pnd 4 of each F2 litter, when the pups were euthanized and examined, each surviving F1 dam was

sacrificed. Non-pregnant F1 females or females whose whole litters were born dead prior to pnd 4 were sacrificed at or after the calculated pnd 4 date. The thoracic and abdominal organs were examined macroscopically. Uterine implantation scars were counted. Tissues showing macroscopic abnormalities, a sample of mammary tissue, one abdominal mammary gland, ovaries and pituitary were retained in neutral buffered 10% formalin for possible subsequent examination. Uteri from any F1 females who appeared non-pregnant were stained with 10% ammonium sulfide. The F1 males were sacrificed at or after the pnd 4 date of their F2 litter. The thoracic and abdominal organs were examined macroscopically. Pituitary, organs with gross lesions, paired testes, epididymides, seminal vesicles, and prostate were retained in neutral buffered 10% formalin for possible subsequent examination.

F1 (Progeny)
On the day of birth (pnd 0), all F1 pups were examined externally for malformations and then were counted, weighed, and sexed. Pups that were stillborn or died before pnd 4 were examined externally and viscerally, and any abnormal tissues were retained in buffered neutral 10% formalin. Pups were counted, weighted individually, sexed, and examined externally on pnd 4, 7, 14, and 21. F1 litters were not standardized during lactation. Pups that died on pnd 5-21 were necropsied; any abnormal tissues were retained in buffered neutral 10% formalin.

F2 (progeny)
On the day of parturition (pnd 0) and pnd 4, the F2 litters were examined for external malformations, pup viability, number, sex, and individual body weight. F2 pups were terminated on pnd 4.

Results

in-life observations:
F0 Dams

Mortality: One F0 female in the 820 mg/kg/day group was sacrificed on gd 12 due to a broken leg. One dam died due to a misdirected dose on pnd 9 at 2240 mg/kg/day.
Gestation-F0 females for F1 litters: No other F0 females were moribund, aborted, or delivered early during gestation. Four F0 dams at 2240mg/kg/day had dead litters on pnd 4 and 12.
Clinical observations: A dose-related increased incidence of alopecia was seen in F0 dams throughout gestation and lactation. At all doses during gestation, piloerection increased in incidence with dose. At 820 and 2240 mg/kg/day during lactation, signs were seen, including piloerection, postdose rooting behavior, and salivation.
Body weight: Reductions in body weight and weight changes were seen in the F0 females at 820 and 2240 mg/kg/day throughout gestation and lactation. Reduced gestational body weight were also seen in F0 dams at the 300 mg/kg/day in the first three days of the gestational dosing period (gd 6-20) and reduced body weight was observed on pnd 0 (Tables 1).
Food consumption: Food consumption was reduced in the F0 females at 820 and 2240 mg/kg/day during gestation, but only in the 2240 mg/kg/day group during lactation. These responses were associated with statistically significant reductions in food consumption (Table 1).
Reproductive and lactational indices: The fertility index was similar across all dose groups (87.5-92%). The gestational index was 100% for all dose groups. There were no treatment-related changes in F0 gestational and fertility indices for the F1 litters, gestational length, number of implantation sites, and percent postimplantation loss at all doses.

Toxicokinetics: not determined

Offspring:
F1 Toxicity
Mortality: Pups euthanized moribund or found dead, from pnd 0 through 21, were 5, 7, 7, and 93 at 0, 300, 820, and 2240 mg/kg/day, respectively (Table 2). No milk band, cold, inactive, emaciated, pale ears, feet and tail, and thin fur were observed in F1 males and females in a dose related manner. Four-day, as well as 7-day and 14-day survival indices were significantly reduced in the 2240 mg/kg/day group.
Clinical signs: One litter in the low dose group had pale ears, feet and tails on pnd 14 and 21 and thin fur on pnd 21.

Body weight: Average pup body weights were reduced at 820 and 2240 mg/kg/day from pnd 0 through 21 (Table 2). Additionally, average pup body weights per litter (all pups or separated by sex) were significantly reduced when compared to controls on pnd 14 and 21 for the 300 mg/kg/day group. Body weights for study days 0 and 7 (prebreed period: 0-6 days after weaning on pnd 21) for F1 males and females were decreased significantly for all dose groups. Body weights remained significantly decreased in the 820 and 2240 mg/kg/day dose group for males and females through study day 56 (mating began on study day 56) and day 77.

F1 Development: The developmental landmark of eye opening was significantly delayed (i.e., pups were older at acquisition) in the 820 and 2240 mg/kg/day. Average age at acquisition of vaginal opening and preputial separation was significantly delayed at 2240 mg/kg/day. Auditory startle was also affected, with a significant reduction in the average force of jump at all doses for males and at 820 and 2240 mg/kg/day for the females, partially due to the reduced body weights of the pups at these doses. There were no treatment-related changes in motor activity or learning and memory (Morris maze) in both sexes at all doses (Tables 3 and 4). Body weights in females were significantly lower than controls through gestation and lactation for the F2 litters.

Lactation – F1 females for F2 litters: F1 maternal gestational weight change was also significantly reduced at 2240 mg/kg/day on pnd 0 and 4. During lactation, alopecia, chromodacryorrhea, rust color fur were seen in F1 maternal animals in a nontreatment-related incidence.

Gestation - F1 females for F2 litters: Gestational length was significantly longer, and the number of uterine implantation sites per dam was significantly lower at 2240 mg/kg/day. There was no treatment-related changes in mating, fertility, pregnancy, or gestational indices in F1 animals.

F2

A slight decrease in F2 litter size was seen in the 2240 mg/kg/day group on pnd 0-4. F2 pups body weights per litter were significantly increased (all pups or separated by sex) on pnd 0 and 4 only at the 2240 mg/kg/day, associated with reduced (not statistically significant) litter size at this dose (table 5).

Terminal and Necropscopic evaluations:

Dams (F0)

No treatment –related effects on the appearance or size of any organ were seen.

F1 Parental animals:

One male dead on study 67 at 2240 mg/kg/day had congestive lungs and green mucus present in the mouth. Note that one F1 male and one F1 female were found dead shortly after weaning on study day –1 at 2240 mg/kg/day, but causes of death are unknown. No other treatment-related abnormalities were seen at necropsy.

F2 Progeny

No treatment-related findings were seen on pnd 4 for F2 pups. One dead pup on pnd 0 had patent ductus arteriosus, indicating primary pulmonary atelectasis.

Table 1. Mortality, changes in body weight, food consumption and reproductive parameters in F0 Females

F0 Females-Dosage (mg/kg/day) GW433908G 141W194 equivalent	0 (control) 0 (control)	300 201	820 548	2240 1498
No. Mated: No. of Deaths	25 0	25 0	25 1	25 1
Body weight (g): Gestation Day 20 Lactation Day 7 Lactation Day 14	 394.5 339.1 351.7	 388.3 328.2 343.3	 361.1** 309.3** 329.5**	 335.3*** 259.8*** 276.4***
Body weight gain (g) Gestation Days 6-20 Lactation Days 0-4 Lactation Days 0-21	 113.9 21.1 20.1	 109.5 29.6 40.8	 79.0** 24.3 47.8	 61.6*** 4.3*** 26.5
Food consumption (g/kg/day): Gestation Days 6-20 Lactation Days 0-21	 79.8 205.0	 75.3 214.3	 68.1** 206.2	 62.5*** 178.7***
Reproductive Gestation length (days): Number	 22.1	 22.0	 22.0	 22.1

pregnant:	23	22	21	22
Number with live litters:	23	22	21	22

**P<0.05; ***P<0.01

Table 2. F1 Litters – Study Findings

F1 Litters-Dosage (mg/kg/day) GW433908G 141W194 equivalent	0 (control) 0 (control)	300 201	820 548	2240 1498
Mean number live births/dam	14.3	15.5	15.0	15.0
Mean number still births/dam	0.1	0.1	0.0	0.1
Mean Survival Index				
Day 4	99.5	99.3	98.4	95.5*
Day 7	99.7	99.5	99.7	88.8**
Day 14	100.0	99.7	99.7	88.4**
Lactational (survival) Index				
Day 21	99.2	98.6	97.9	75.5**
Total number of pups dying				
Day 0 through 21	5	7	9	93
Mean pup body weight at weaning, male (g)	48.52	42.67**	38.65***	24.52**
Mean pup body weight at weaning, female (g)	45.75	40.88*	37.84***	24.72***
Sex ratio of live newborns (% males)	53.1	52.2	54.3	51.7
Number of litters evaluated	23	22	21	22
Developmental markers (mean litter day):				
Pinna detachment	2.72	2.70	2.85	2.91
Eye opening	13.70	13.97	14.36***	15.40***
Balano preputial separation	41.31	41.14	42.15	44.61***
Vaginal opening	31.15	31.65	31.48	35.04***

**P<0.05; ***P<0.01

Table 3. F1 Males – Study Findings

F1-Males-Dosage (mg/kg/day) GW433908G 141W194 equivalent	0 (control) 0 (control)	300 201	820 548	2240 1498
Number evaluated postweaning	25	25	25	24
No. of Deaths	0	0	0	1
Body weight				
Study Day 7 (g)	122.3	110.1**	102.5***	78.9***
Study Day 49 (g)	434.6	429.8	404.0	362.5***
Study Days 0-91 (g)	505.3	508.3	480.9	440.4***
Sensory function (auditory startle test block 1, mean force)	616.23	425.72***	407.54***	270.00***
Motor Activity	919.32	824.32	862.48	786.71
Learning and memory (Morris Water Maze, median swim time in seconds)	11	11	11	11
Number paired	25	25	25	24
Number with evidence of mating	24	24	24	24
Number siring	24	22	24	23

**P<0.05; ***P<0.01

Table 4. F1 Females – Study Findings

F1 Females-Dosage (mg/kg/day) GW433908G 141W194 equivalent	0 (control) 0 (control)	300 201	820 548	2240 1498
Number evaluated postweaning	25	25	25	24
No. of Deaths	0	0	0	1
Body weight				
Study Day 7 (g)	106.5	98.7*	91.8***	74.1***
Study Day 49 (g)	259.6	256.1	239.9*	222.5***
Body weight change (g):				
Premating	213.2	212.2	205.4	198.3
During Gestation days 0 to 20	155.0	141.5*	153.0	139.4*
Sensory function (auditory startle test block 1, mean force)	538.12	430.33	414.46*	318.51***
Motor activity	874.24	845.40	756.00	753.71
Learning and memory (Morris Water Maze, median swim time in seconds)	11	11	11	11
Number of females with abnormal estrous	0	1	0	0

cycles Precoital interval (days)	2.5	2.7	3.3	3.9
Number paired	25	25	24	24
Number pregnant	24	22	24	23
Number with live litters	24	22	24	23
Gestation length (days)	21.8	22.1	22.0	22.3**

*P<0.05; **P<0.01; ***P<0.005

Table 5. F2 Litters – Study Findings

F2 Litters-Dosage (mg/kg/day)				
GW433908G	0 (control)	300	820	2240
141W194 equivalent	0 (control)	201	548	1498
Number of litters evaluated	24	22	24	23
Mean number implantations/dam	16.58	14.59	15.92	14.26**
Mean number live births/dam	15.3	13.9	15.3	13.7
Mean number still births/dam	0.5	0.5	0.2	0.0
Mean litter size:				
Day 0	15.3	13.9	15.3	13.7
Day 4	15.1	13.6	14.8	13.6
Mean pup body weight at Day 0 (g)	6.10	6.28	6.22	6.64**
Mean pup body weight at Day 4 (g)	9.36	9.99	9.48	10.71**
Sex ratio of live newborns (% males)	52.4	48.5	50.3	45.3

**P<0.05

Reproductive and developmental toxicology summary: In this study, the NOAEL for maternal toxicity in rats was established at 300 mg/kg/day GW433908G. The minor transient decrease in body weight observed in F0 dams at this dose was not considered evidence of systemic toxicity. Reduced body weights during gestation and lactation and reduced feed consumption were seen at higher doses. GW433908G did not affect the fertility index, gestational length, and number of implants, post-implantation loss, or the number of stillbirth and live pups. A developmental NOAEL was not established in this study, but it was considered less than 300 mg/kg/day, because decreased mean F1 pup body weight at weaning and decreased jump force in auditory startle (the later in males only) were seen at 300 mg/kg/day. At 840 and 2240 mg/kg/day, decreased body weight of the F1 pups caused a jump force reduction in auditory startle, and postnatal deaths (at 2240 mg/kg/day only). Additionally, prolonged precoital interval, prolonged gestation, reduced number of uterine implantation sites per litter, and reduced maternal gestational body weights were seen in F1 females at 2240 mg/kg/day, which are likely secondary to the effect on F1 female body weights throughout the postnatal period of this dose.

Note that the sponsor did not measure the exposure of the test article in this study. However, according to the sponsor, the low dose (300 mg/kg/day) administrated to female animals in an oral embryofetal developmental study, would deliver an exposure (AUC: 26.9 µg•h/ml; RD1999/02690/00) similar to the expected clinical exposure to 141W94 following a therapeutic dose of 2400 mg/day in humans (AUC: 37µg•h/ml; APV20001). The high dose of 2240 mg/kg/day administered to rats in a 6-month toxicity study would produced an exposure (AUC: 54.7-107 µg•h/ml) 1.4 to 2.7 times the expected clinical exposure to 141W94 following a therapeutic dose of 2400 mg/day in humans (AUC=37µg•h/ml; APV20001).

Reproductive and developmental toxicity conclusion:
The NOAEL for F0 female reproductive toxicity is considered to be 2240 mg/kg/day (HED: 37 mg/kg/day). The developmental NOAEL was considered to be less than 300 mg/kg/day (HED: <5 mg/kg/day). Prolonged precoital interval, prolonged gestation, reduced number of uterine implantation sites per litter, and reduced maternal gestational body weights were seen in F1 females at 2240 mg/kg/day, which are likely secondary to the effect on F1 female body weights throughout the postnatal period of this dose.

Reproductive and developmental toxicology summary:
In the rabbit embryofetal study, systemic exposure (AUC) to APV at the high dose on Day 20 of gestation was low and only approximately 0.3 times the exposure in humans following administration of the maximum recommended human dose (MRHD). The increased incidence of abortions in the rabbit embryofetal study at the high dose is considered related to severe maternal toxicity. The abortions occurred late in gestation (Days 21 to 29) and after the dose administration phase of the study was finished. However, since amprenavir also induced abortions and the effect was seen at low exposures, the abortions will be placed into the label. In the pre- and post-natal reproduction study in rats,

GW433908G caused a reduction in F1 pup survival at the high dose of 2240 mg/kg/day and a reduction in both male and female pup body weights at weaning at all doses, which was accompanied by a delay in the appearance of several developmental markers. The reduced body weight effect noted in the F1 male and female pups persisted in both sexes and likely contributed to the effects seen on some reproductive parameters when the F1 generation was mated. The presence of APV in maternal milk may account for the reduction in mean body weights seen in these animals.

Local Tolerance

52. GW433908G: Acute dermal irritation study in the New Zealand white rabbit (Report No. RD1999/00553/00)

GW study No.: L40478; [redacted] Study No.: 6169-247; Conducting facility: [redacted] Date Initiation: 26 march 1999; GLP Compliance: Yes (X); Drug Lot: R4283/32/1

Method and results

GW433908G (0.5 g in 1.5 mL distilled water) was applied to the intact back skin (dosage: 0.5 g/6.25cm^2) in three New Zealand White albino rabbits (body weights: 2.67-2.87 kg; age: 16 weeks) and the primary dermal irritation potential of GW433908G was evaluated in animals under 4-hour semioccluded conditions. Slight erythema reaction was seen in one animal at the 0.5 to 1-hour post exposure observation period. The primary dermal irritation index (Draiz) was 0.1. Thus, the GW433908G is considiered to be a mild irritant.

53. Study L40479 – GW433908G: Acute eye irritation study in the New Zealand white rabbit (Report No. RD1999/00551/00)

GW study No.: L40479; [redacted] Study No.: 6169-248; Conducting facility: [redacted], Date Initiation: 26 march 1999; GLP Compliance: Yes (X); Drug Lot: R4283/32/1

Method and results

GW433908G (0.265 g in 1 mL distilled water) was instilled into the eyes of each New Zealand White albino rabbit (body weights: 2.73-3.05 kg; age: 17 weeks) at the 10 mg (n=1 animal) or 27 mg dose (n=3 animal). The primary eye irritation potential of GW433908G was evaluated for up to 72-hour after treatment. Slight conjunctival redness reaction was seen in one 10mg dose animal. The treated eye of these animals returned to a normal appearance within 24 hour. Slight conjunctival redness reaction was seen in all three 27-mg dose animals. All eyes treated with the 27-mg dose returned to a normal appearance by 72 hours after treatment. Thus, the GW433908G is considiered to to be a negligible risk of causing eye damage (Maximum Overal Mean Score (Draiz): 5-11; Grade 1 rating).

54. Study G40477 – GW433908G: skin sensitization (buehler method) study in the guinea- pig (Report No. RD1999/00552/00)

GW study No.: L40477; [redacted] Study No.: 6169-249; Conducting facility: [redacted]; Date Initiation: 26 march 1999; GLP Compliance: Yes (X); Drug Lot: R4283/32/1

Method and results

The delayed contact hypersensitivity potential of GW433908G was evaluated in albino guinea pig of the CrL:(HA) BR strain (body weights: 0.37-0.525 kg; age: 6-7 weeks) using modified Buehler method. Animals were devided into three groups: an irritation screening group (2/sex/group), a test group (10/sex/group), and a naïve control group (5/sex/group). GW433908G (0.4g in 0.7 mL sterile water) was administered via the dermal route to each animal in the test group during the three-application induction phase of the study. GW433908G, when applied as a 0.4-g dose at challenge two weeks following the administration of the third induction dose, did not elicit any dermal responses. Thus, the GW433908G is not considiered to be a dermal sensitizer in guinea pigs.

Special Toxicity Studies

55. GW433908G: 14-day oral toxicity study in wistar hannover rats to assess the effects of synthetic material containing the impurities [redacted] (Report No.RD2000/01884/00)

GW study No.: R40857; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 5 October 2000; GLP Compliance: Yes (X); Drug Lot: F005430 (GW433908 drug substance) or R4623/153/1 [GW433908G drug substance containing each of the impurities [redacted]; Formulation: GW433908G solution in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) and 0.1% (w/w) Tween 80 in reverse osmosis-treated water

This 14-day repeat dose toxicity study evaluated the effects of various added drug substance impurities on the toxicology and toxicokinetics of GW433908G in Han Wistar rats.

Methods

Three groups of 10 male and 10 female Han Wistar rats (Tac:Glx:WifBR; body weight: Male = 225 - 329 g; Female = 137 – 214.2 g; age: 9 – 10 weeks) received twice daily (6 hours apart) at daily doses of 0 (vehicle), or1942 mg/kg/day GW433908G alone (1368 mg/kg/day APV equivalents), or twice daily (6 hour apart) at daily doses of 1942 mg/kg/day GW433908 (1368 mg/kg/day APV equivalents) with added potential drug substance impurities, respectively, by oral gavage (10 mL/kg/day) for 14 days. The daily doses of the GW433908G and the impurities were summarized as follows:

Daily Doses of GW433908G and GW433908G with added potential drug substance impurities

Test article	Daily dose (mg/kg/day)			Impurity
	GW433908	APV equivalents	Impurities	%
GW433908 alone	1942	1368	--	--
GW433908 with impurities	1942	1368	[redacted]	

A further 4 animals/sex were included in each group for toxicokinetics. 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. Blood samples were collected at terminal necropsy for clinical chemistry and hematology analysis. Urine samples were collected and analyzed after overnight fasting on Day 12. All animals were killed at the end of the treatment period on Day 15. A complete gross examination was carried out on each animal and organ weight (adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, uterus, thyroid and parathyroid glands) 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 (Appendix Table 1). Histopathologic evaluation was performed on all prepared tissues from all three groups of animals by a pathologist.

Note that the sponsor stated that a deviation from the protocol-specified target concentration is seen due to a correction factor was not employed during test material formulation to account for the total water and solvent content (12.8%, w/w) in GW433908G. Thus, the test material formulation of GW433908G, with or without impurities, used throughout this study, was 194.2 mg/ml. The actual dose of GW433908G was 1942 mg/kg/day, rather than 2240 mg/kg/day.

Results

Clinical signs: No unscheduled deaths were seen in this study. Treatment-related moderate salivation was noted on Day 11 in one female rats given 1942 mg/kg/day GW433908G containing impurities. This finding has been observed previously (Report RD1998/02573/00 and RD1998/02858/01).
Body weights: No toxicologically significant differences in body weight or body weight change were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, body weights and body weight gain were lower for males and females treated with GW433908 (with and without the impurities) compared to the vehicle control animals throughout the treatment period. Body weight gain from day 1 to Day 15 in rats treated with GW433908G (with and without the impurities) was 57% to 66% of the corresponding control for male and 68% to 77% of the corresponding control value for females (Table 1).
Food consumption: No toxicologically significant differences in food consumption were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, food consumption was lower for both male and female rats treated with GW433908 (with and without the impurities) compared to the vehicle control animals throughout the treatment period. Food consumption from day 1 to Day 15 in rats treated with GW433908G (with and without the impurities) was 87% to 89% of the corresponding control for male and 82% to 87% of the corresponding control value for females (Table 1).
Hematology: No toxicologically significant differences in hematological parameters were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. No treatment-related statistically significant differences in hematological parameters were noted in this study.
Clinical chemistry: No toxicologically significant differences in clinical chemistry parameters were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, statistically significant decreases in serum triglyceride concentration (males and females), alkaline phosphatase (males), glucose (males and females), and albumin/globulin ratio (females), and slight, statistically significant increases in cholesterol (females) and globulin (females) were seen in rats treated with GW433908G (with and without the impurities).
Urine analysis: No toxicologically significant differences in urinalysis parameters were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, statistically significant increases in urine sodium and urobilinogen were seen in male rats treated with GW433908G (without the impurities) compared to those treated with the vehicle.
Gross pathology: No toxicologically significant differences in organ weights were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, a statistically significant increase in relative and absolute liver weights in females, relative liver weights in males and relative thyroid weights in females was seen in rats treated with GW433908G (with and without the impurities) compared to those treated with the vehicle control material (Table 2).
Histopathology: No toxicologically significant differences in microscopic changes were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities. However, minimal, diffuse hepatocellular hypertrophy and minimal to mild fore-stomach epithelial vacuolization were seen in rats treated with GW433908G (with and without the impurities). This finding has not been seen in previous studies in the rat of up to 6-month treatment duration with GW433908G.

Table 1. Food consumption and body weight changes in Han Wistar rats with oral administration of GW433908G with and without impurities (twice daily for 14-days)

Dose (mg/kg/day)	Male			Female		
	Vehicle	w/o Impurities	w/ Impurities	Vehicle	w/o Impurities	w/ Impurities
GW433908G	0	1942	1942	0	1942	1942
APV Equivalents	0	1368	1368	0	1368	1368
Body weight	Group Mean (g)					
Day 1	288	290	285	184	176	178
Day 8	313	305	292	197	182	187
Day 15	333	320	310	207	192	195
Body weight gain Day 1 through 15	45	30	26	23	16	18
Food consumption	Group Mean (g/rat/day)					
	22	19	19	15	12	13

Table 2. Organ weight changes in Han Wistar rats with oral administration of GW433908G with and without impurities (twice daily for 14-days)

Dose (mg/kg/day)	Male			Female		
	Vehicle	w/o Impurities	w/ Impurities	Vehicle	w/o Impurities	w/ Impurities
GW433908G	0	1942	1942	0	1942	1942
APV Equivalents	0	1368	1368	0	1368	1368
Organ weight	Group Mean					
Liver, absolute (g)	13.6	15.3	14.3	8.1	11.5	11.6
Liver, relative (%)*	4.0	4.7	4.6	3.9	6.0	5.9
Thyroid, absolute(g)	0.033	0.031	0.029	0.024	0.026	0.025
Thyroid, relative (%)*	0.0099	0.0095	0.0094	0.0115	0.0139	0.0128

*: Relative to body weight

Comments

In this study, Wistar Han rats receiving GW433908G containing impurities [redacted] showed no additional toxicity to rats receiving GW433908G. The toxicity profile seen in this study was consistent with the known toxicity of APV which included salivation; decreased body weights, food consumption, triglycerides, alkaline phosphatase, glucose, albumin/globulin ratio; increased cholesterol and globulin; and diffuse hepatocellular hypertrophy accompanied by increased liver weights. Note that thyroid follicular cell hypertrophy was not seen and microsomal enzyme induction was not evaluated in this study, which have been observed previously (RD1998/02573/00). Minimal to mild fore-stomach epithelial vacuolization was seen in rats treated with GW433908G (with and without the impurities). However, the relationship of this finding to GW433908G is unknown because it was not seen in previous 6-month studies in the rat with GW433908G.

Toxicokinetics

Methods

Blood samples were taken at 7 hours after the first dose (controls bled at 7 hours postdose only) on Day 1 and Day 13 for toxicokinetic evaluation. Plasma concentrations of GW433908G and amprenavir were determined, using an [redacted] method.

Results

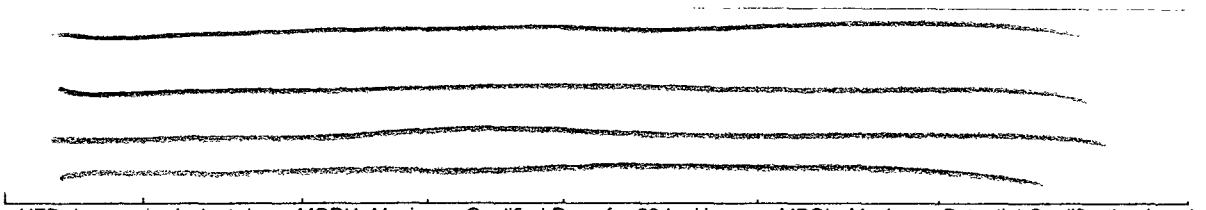
Both GW433908G, the prodrug of APV, and APV were detected in plasma in this study. No statistically significant differences in plasma GW433908G and APV levels were seen between rats treated with GW433908G and those treated with GW433908G containing the impurities (Table 3).

Table 3 Toxicokinetics parameters of GW433908G and amprenavir (141W94) in Han Wistar rats after oral administration of GW433908G with and without impurities during a 14-day toxicity study

GW433908G 1942 mg/kg/day	Plasma Concentration			
	Day 1		Day 13	
	GW433908X ng/ml	APV µg/ml	GW433908X ng/ml	APV µg/ml
GW433908G				
Male	282	12	103	4.8
Female	133	10.3	157	5.4
GW433908 + Impurities				
Male	185	13.8	173	5.4
Female	70.8	9.4	88	7.1

Maximum Theoretical Qualification Levels for Potential Drug Substance Impurities in Rats

Potential Drug Substance Impurity	Conc. in Toxicity Study (% area/area)	Daily Impurity Dose to Rats (mg/kg)	HED for 60 kg person (mg/day)	Recommended drug dose for 60 kg human (mg/day)	Maximum Potential Qualification Level (% area/area)*	Proposed Qualification Level (% area/area)*



HED: human equivalent dose; MQDH: Maximum Qualified Dose for 60 kg Human; MPQL: Maximum Potential Qualification Level; Assuming a human dose of 1400 mg/day GW433809G, BID;
*Maximum Potential Qualification Level = {(Maximum Qualification Dose for 60 kg Human/Projected Daily Dose of the Impurity in Humans) x (Concentration of Impurity in Toxicity Study)}
** ( ) = Current Drug Substance Specification (CDSS); adjusted by the level of CDSS.

56. Study R40917 -- GW433908G: 14-day oral toxicity study in wistar hannover rats to assess the effects of synthetic material containing the impurity [redacted] (Report No. RD2001/00212/01)

GW report No.: RD2001/00212/01; GW study No.: R40917; Conducting facility: Glaxo Wellcome Inc., Medicines Safety Evaluation Division, Five Moore Drive, Research Triangle Park, NC 27709; Date Initiation: 22 March 2001; GLP Compliance: Yes (X) No ( ); Drug Lot: F018622 (GW433908 drug substance) or R4623/191/2 [GW433908G drug substance containing [redacted] (the impurity) [redacted]]; Formulation: GW433908G solution in 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) and 0.1% (w/w) Tween 80 in reverse osmosis-treated water

This 14 to 15-day repeat dose toxicity study evaluated the effects of the drug substance impurity [redacted] on the toxicology and toxicokinetics of GW433908G in Han Wistar rats.

Method

Three groups of 10 male and 10 female Han Wistar rats (Tac:Glx:WifBR; body weight: Male = 233 - 356 g; Female = 153 – 228 g; age: 9 – 10 weeks) received twice daily (6 hours apart) at daily doses of 0 (vehicle), or 2240 mg/kg/day GW433908G alone (1368 mg/kg/day APV equivalents), or twice daily (6 hour apart) at daily doses of 2240 mg/kg/day GW433908 (1368 mg/kg/day APV equivalents) with added potential the drug substance impurity ([redacted]), respectively, by oral gavage (10 mL/kg/day) for 14 to 15 days. The daily doses of the GW433908G and the impurities were shown in the following Table.

Daily Doses of GW433908G and GW433908G with added potential drug substance impurities

Test article	Daily dose (mg/kg/day)			Impurity
	GW433908G	APV equivalents	Impurities	%, w/w
GW433908 alone	2240	1600	--	--
GW433908 with the impurity [redacted]	2240	1600	[redacted]	

A further 4 animals/sex were included in each group for toxicokinetics. 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. Blood samples were collected at Day 1 and Day 13 prior to terminal necropsy for clinical chemistry, hematology, and plasma drug concentration analysis. 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, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, uterus, thyroid and parathyroid glands) 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 (Appendix Table 1). Histopathologic evaluation was performed on all prepared tissues from all three groups of animals by a pathologist.

Results

Clinical signs: No unscheduled deaths were seen in this study.
Body weights: No toxicologically significant differences in body weight or body weight change were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity. However, body weights and body weight gain were lower for males and females treated with GW433908 (with and without the impurity) compared to the vehicle control animals through out the treatment period. Body weight gain from day 1 to Day 15 in rats treated with GW433908G (with and without the impurity)

was 88% to 83% of the corresponding control for male and 78% to 75% of the corresponding control value for females (Table 1).

Food consumption: No toxicologically significant differences in food consumption were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity. However, food consumption was lower for female rats treated with GW433908 (with and without the impurity) compared to the vehicle control animals through out the treatment period. Food consumption from day 1 to Day 15 in rats treated with GW433908G (with and without the impurities) was 83% to 87% of the corresponding control for females (Table 1).

Hematology: No toxicologically significant differences in hematological parameters were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity.

Clinical chemistry: No toxicologically significant differences in clinical chemistry parameters were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity. However, statistically significant decreases in serum triglyceride concentration (males and females), alkaline phosphatase (males), glucose (males and females), and albumin/globulin ratio (females), and slight, statistically significant increases in cholesterol (females) and globulin (females) were seen in rats treated with GW433908G (with and without the impurity) (Table 2).

Gross pathology: No toxicologically significant differences in organ weights were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity. However, a statistically significant increase in relative and absolute liver weights in males and females was seen in rats treated with GW433908G (with and without the impurity) compared to those treated with the vehicle control material (Table 2).

Histopathology: No toxicologically significant differences in microscopic changes were seen between rats treated with GW433908G and those treated with GW433908G containing the impurity. However, minimal, diffuse hepatocellular hypertrophy and minimal to mild fore-stomach epithelial vacuolization were seen in rats treated with GW433908G (with and without the impurity). Note that this finding has not been seen in previous studies in the rat of up to 6-month treatment duration with GW433908G.

Table 1. Food consumption, body weight changes and plasma drug levels in Han Wistar rats with oral administration of GW433908G with and without the impurity [redacted] (twice daily for 14-days)

Dose (mg/kg/day)	Male			Female		
	Vehicle	w/o Impurities	With the Impurity	Vehicle	w/o Impurities	With the Impurity
GW433908G APV Equivalents	0 0	2240 1600	2240 (8.96) 1600(8.96)	0 0	2240 1600	2240 (8.96) 1600 (8.96)
Body weight	Group Mean (g)					
Day 1 Day 8 Day 15	260 278 298	279 298 313	270 285 302	191 204 213	185 195 203	193 203 210
Body weight gain Day 1 through 15	38	34	32	22	17	16.5
Food consumption	Group Mean (g/rat/day)					
	20	20	19.7	15.7	13.1	13.6
GW433908X Conc. (ng/mL)	Group Mean					
Day 1 Day13	-- --	217 140	286 349	-- --	94 135	57 141
141W94 Conc. (µg/mL)	Group Mean					
Day 1 Day13	-- --	13 4.4	13.4 3.3	-- --	14 5.6	8 5.7

Table 2. Clinical chemistry changes, organ weight changes, and pathological findings in Han Wistar rats with oral administration of GW433908G with and without the impurity [redacted] (twice daily for 14-days)

Dose (mg/kg/day)	Male			Female		
	Vehicle	w/o Impurities	With the Impurity	Vehicle	w/o Impurities	With the Impurity

GW433908G APV Equivalents	0 0	2240 1600	2240 (8.96) 1600 (8.96)	0 0	2240 1600	2240 (8.96) 1600 (8.96)
Clinical Chemistry	**Group Mean**					
Albumin/Globulin ratio	2.2	2.3	2.2	2.7	2.4	2.4
Alkaline phosphatase (U/L)	194	139	156	100	89	70
Cholesterol (mg/dL)	62	57	63	53	67	61
Globulin (g/dL)	2	2	2	1.8	2	2
Glucose (mg/dL)	193	177	178	195	181	179
Total Protein (g/dL)	6	6	6	6.6	7	7
Triglycerides (mg/dL)	137	50	54	82	41	40
Organ weight	**Group Mean**					
Liver, absolute (g)	11.3	15.1	14.5	7.9	11.1	11.3
Liver, relative (%)*	3.8	4.8	4.8	3.7	5.5	5.3
Microscopic findings						
Liver *Diffuse hepatocyte hypertrophy, minimal*	--	10	9	--	8	10
Stomach *Forestomach, limiting ridge, epithjelium, cytoplasmic vacuolization, minimal to mild*	--	6	8	--	4	2
Thyroid *Follicular cell hypertrophy, minimal*	1	8	8	--	7	5

*: Relative to body weight

Comments

In this study, Wistar Han rats receiving GW433908G (2240 mg/kg/day) containing the impurity [redacted] showed no additional toxicity to rats receiving GW433908G. The toxicity profile seen in this study was consistent with the known toxicity of APV which included decreased body weights, food consumption, triglycerides, alkaline phosphatase, glucose, albumin/globulin ratio; increased cholesterol and globulin; and diffuse hepatocellular hypertrophy accompanied by increased liver weights. Note that thyroid follicular cell hypertrophy was seen in this study. Minimal to mild fore-stomach epithelial vacuolization were seen in rats treated with GW433908G (with and without the impurity). However, relationship of this finding to GW433908G is unknown because it was not seen in previous 6-month studies in the rat with GW433908G.

Maximum Theoretical Qualification Levels for Potential Drug Substance Impurities in Rats

Potential Drug Substance Impurity	Conc. in Toxicity Study (% area/area)	Daily Drug Impurity Dose to Rats (mg/kg)	HED of the drug impurity for 60 kg human mg/day	Recommended drug dose for 60 kg human (mg/day)	Maximum Potential Qualification Level (% area/area)*
[redacted]					

HED: human equivalent dose; MQDH: Maximum Qualified Dose for 60 kg Human; MPQL: Maximum Potential Qualification Level;
Assuming a human dose of 1400 mg/day GW433809G, BID;
Maximum Potential Qualification Level (%) = {(Human equivalent dose of the drug impurity for 60 kg person)/(Recommended daily drug dose for 60 kg person)x100}

Comments

Although the present study in rats reassured the safety of potential drug substance impurity at levels far in excess of those likely to be seen in the drug products, this same study could not reassure the safety of potential drug substance impurities in regard to the treatment duration.

To ensure adequate qualification of impurities in the drug substance, the sponsor conducted two additional oral studies in rats to compare the well-established toxicological profile of GW433908G and APV with results from drug substance batches purposely spiked with potential impurities. These batches were also examined for mutagenicity in 3 bacterial reverse mutation studies.

Summary of Special Toxicity Studies:

To ensure adequate qualification of impurities in the drug substance, the sponsor conducted two additional oral studies in rats to compare the well-established toxicological profile of GW433908G and

APV with results from drug substance batches purposely spiked with potential impurities. These batches were also examined for mutagenicity in 3 bacterial reverse mutation studies. 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.

Genetic Toxicology Qualification of Drug-related Impurities in Fosamprenavir Calcium

Impurity	Maximum Clinical Daily Dose[B]		Genetic Tox Assay	Genetic Tox Study Results	Nonclincal Report Number
	%	mg/kg			
[redacted]			Bacteria mutation test (Ames test)	- - - - - - - - -	RD1999/02761/00 RD1999/02762/00 RD1999/02763/01

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.

Qualification Master Lists (Fosamprenavir Calcium)

Impurity	Highest Clinical Conc in Clinica Studies[B]		Preclinical Dose[A]		Species	Duration	Drug substance specification (% w/w)	Safety Margin[C]	Nonclincal Report Number
	%	mg/kg	%	mg/kg (HED)					
[redacted]					Rat	6-month		0.57	RD1998/02858/01
					Rat	6-month		1.18	
					Rat	6-month		0.31	
					Rat	6-month		0.51	
					Rat	6-month		0.64	
					Rat	6-month		0.1	
					Rat	6-month		0.30	
					Rat	6-month		0.31	
					Rat	6-month		0.67	
					Rat	6-Month		0.74	

A: Preclinical Dose of Impurities (HED) in mg/kg/day = Preclincal Dose of the Test Article (mg/kg) x Highest Concentration of Impurities (% w/w); tested in repeat dose rat studies.
B: Highest clinical concentration in clinical phase II and III studies
C: Safety Margin = Preclinical dose of drug Impurities (HED) in mg/kg/day/ Maximum clinical dose of drug Impurities in mg/kg/day
D: Highest concentration tested in a repeat dose rat study is below proposed drug substance specification. When dose multiples from the rat study are considered, then the qualified impurity concentration exceeds the drug substance specification.

Comments

All of the impurities, except [redacted], were tested at a concentration equivalent to or greater than the proposed drug substance specification for GW433908G. [redacted] were qualified when toxicity study multiples were considered. In addition, these impurities at tested levels did not change the known toxicological profile of GW433908G or APV. However, by calculations based upon the Human Equivalent Doses (HED) of the impurities at the No Observed Adverse Event Level (NOAEL) of the non-clinical toxicity studies in rats and dogs, the

Maximum Qualified Dose of the impurities [redacted] are less than the proposed Dose of Impurity in Humans that the current drug substance specifications permit.

Issues regarding combination toxicity studies with ritonavir

The toxicity of GW433908G in combination with ritonavir (or other compounds) has not been evaluated in pre-clinical animal studies. The toxicity profile of GW433908G is essentially identical to APV and APV has been used extensively in the clinic with other antiretroviral drugs, including ritonavir. The sponsor considered that combination toxicity studies may produce clinically irrelevant information. For example, GW433908G and ritonavir, when co-administered, may produce additive or synergistic liver toxicity in animals. However, in clinical trials, no significant liver toxicity has been observed with or without ritonavir co- administration.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: The sponsor is requesting approval to market fosamprenavir to be administered alone or in combination with ritonavir for the treatment of HIV infection. The drug product, fosamprenavir is approvable in the perspective of non-clinical Pharmacology and Toxicology.

General Toxicology Issues: The nonclinical toxicological findings with GW433908G include: (1) gastrointestinal intolerance (salivation, vomiting and fecal alterations that included soft and liquid feces) in dogs; (2) liver toxicity in rats and dogs; (3) decreases (1% to 8%) in hematocrit and hemoglobin, and an increase (7% to 25%) in platelet count in rats in the longer-term studies; (4) an increased incidence of late gestational abortions in pregnant rabbits; and (5) decreased survival in F1 rat pups in the pre- and post-natal studies.

Recommendations: As part of a Phase 4 Post-marketing Agreement, it is understood that the sponsor should be required to submitted the currently on-going 2-year rat and mouse carcinogenicity study reports to the division for review by the CDER-CAC, when these studies are completed. As part of a Phase 4 Post-marketing Agreement, it is recommend that the sponsor conduct studies in rats to qualify the drug substance impurities [redacted]

Suggested labeling: Minor label revisions are recommended in the Carcinogenesis, Mutagenesis, and Impairment of Fertility Section (Labeling revised by the reviewer as September 30, 2003; Labeling proposed by the sponsor as January 15, 2003).

[redacted]

1 Draft Labeling Page(s) Withheld

4587 [redacted]
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4591 **New Label**

4592 **Carcinogenesis and Mutagenesis:** Carcinogenicity studies of fosamprenavir in rats and mice are in
4593 progress; however, results are available from carcinogenicity studies with amprenavir. Amprenavir was
4594 evaluated for carcinogenic potential by oral gavage administration to mice and rats for up to 104 weeks.
4595 Results showed an increase in the incidence of benign hepatocellular adenomas and an increase in the
4596 combined incidence of hepatocellular adenomas plus carcinoma in males of both species at doses that
4597 produced approximately 2 times (mice) and 4 times (rats) the human exposure (based on $AUC_{0\text{-}24hr}$
4598 measurement) at the recommended dose of 1,200 mg fosamprenair twice daily. Administration of
4599 amprenavir did not cause a statistically significant increase in the incidence of any other benign or
4600 malignant neoplasm in mice or rats. It is not known how predictive the results of rodent carcinogenicity
4601 studies may be for humans.

4602 Fosamprenavir and amprenavir were not mutagenic or genotoxic in a battery of in vitro and in
4603 vivo assays. These assays included bacterial reverse mutation (Ames), mouse lymphoma, rat
4604 micronucleus and chromosome aberrations in human lymphocytes.

4605 **Impairment of Fertility:** The effects of fosamprenavir on fertility and general reproductive performance
4606 were investigated in male (treated for 4 weeks before mating) and female rats (treated for 2 weeks before
4607 mating through postpartum day 6). Systemic exposures ($AUC_{0\text{-}24\ hr}$) to amprenavir in these studies were
4608 3 (males) to 4 (females) times higher than exposures in humans following administration of the maximum
4609 recommended human dose (MRHD) of fosamprenavir alone or similar to those seen in humans following
4610 administration of fosamprenavir in combination with ritonavir. Fosamprenavir did not impair mating or
4611 fertility of male or female rats and did not affect the development and maturation of sperm from treated
4612 rats.

4613 **Pregnancy and Reproduction**: Pregnancy Category C. Embryo/fetal development studies were
4614 conducted in rats (dosed from day 6 to day 17 of gestation) and rabbits (dosed from day 7 to day 20 of
4615 gestation). Administration of fosamprenavir to pregnant rats and rabbits produced no major effects on
4616 embryo-fetal development; however, the incidence of abortion was increased in rabbits that were
4617 administered fosamprenavir. Systemic exposures ($AUC_{0\text{-}24\ hr}$) to amprenavir at these dosages were 0.8
4618 (rabbits) to 2 (rats) times the exposures in humans following administration of the MRHD of fosamprenavir
4619 alone or 0.3 (rabbits) to 0.7 (rats) times the exposures in humans following administration of the MRHD of
4620 fosamprenavir in combination with ritonavir. In contrast, administration of amprenavir was associated with
4621 abortions and an increased incidence 3 minor skeletal variations resulting from deficient ossification of the
4622 femur, [redacted] trochlea, [redacted] in pregnant rabbits at the tested dose [redacted] approximately one
4623 twentieth of the exposure seen at the recommended human dose.

4624 The mating and fertility of the F1 generation born to female rats given fosamprenavir was not
4625 different from control animals; however, fosamprenavir did cause a reduction in both pup survival and

body weights. Surviving F1 female rats showed an increased time to successful mating, an increased length of gestation, a reduced number of uterine implantation sites per litter, and reduced gestational body weights compared to control animals. Systemic exposure ($AUC_{0\text{-}24hr}$) to amprenavir in the F0 pregnant rats was approximately 2 times higher than exposures in humans following administration of the MRHD of fosamprenavir alone or approximately the same as those seen in humans following administration of the MRHD of fosamprenavir in combination with ritonavir.

There are no adequate and well-controlled studies in pregnant women. Fosamprenavir calcium tablets should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Antiretroviral Pregnancy Registry: To monitor maternal-fetal outcomes of pregnant women exposed to [redacted], an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling 1-800-258-4263.

Nursing Mothers: The Centers for Disease Control and Prevention recommend that HIV-infected mothers not breastfeed their infants to avoid risking postnatal transmission of HIV. Although it is not known if amprenavir is excreted in human milk, amprenavir is secreted into the milk of lactating rats. Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers should be instructed not to breastfeed if they are receiving [redacted]

Reviewer Signature: Hao Zhang, M.D., Pharmacologist

Supervisor Signature: James G. Farrelly; **Concurrence**: Yes___ No ___

Appendix/attachments

References

- Borlakoglu, 1993, Expression of P450 isoenzymes during rat liver organogenesis
- Carr, 1998, A syndrome of peripheral lipodystrophy, hyperlipidemia dn insulin resistance in patients receiving HIV protease inhibitors
- Cerbai, 1990, Cardiac electrophysiologic effects of a new calcium antagonist, lacidipine Durham, 2002, Risk Assessment: The Changing Paradigm. In: Handbook of Toxicologic Pathology
- GlaxoSmithKline, 1997, Report No. RR1997/00125/00, Human in vitro Marrow Progenitor Toxicityof Anti-HIV Protease inhibitors: saquinavir (GR133358A) and indinavir (GW283913A)
- GlaxoSmithKline, 1999, Report No. RD1999/02681/00. Validation of a Method for the Determination of GW433908 and Amprenavir in Rat Plasma using [redacted] Report No. 6169-237)
- GlaxoSmithKline, 1999, Report No. RD1999/02498/00, Validation of a Method for the Determination of GW433908 and Amprenavir in Rabbit Plasma using [redacted]
- GlaxoSmithKline, 1999, Report No. RD1999/00101/00, The validation of a [redacted] method (RBMT-MET-093.01) for the Determination of GW433908 and Amprenavir in Dog Plasma (Glaxo Wellcome Study No. 99/AVS/0074)
- GlaxoSmithKline, 1999, Report No. RD1999/02148/00, The Validation of a [redacted] Method RBMT-MET-134.00 for the Determination of rHuCYP3A4-like Immunoreactivity in Rat Liver Microsomes (99AVS0048)
- GlaxoSmithKline, 2002, Report No. RD2002/00233/00, Validation of a Method for the Determination of GW433908 and Amprenavir in Mouse Plasma Using [redacted] Study No.02AVS0014)
- GlaxoSmithKline, 2002, Report No. RD2002/00232/00, Validation of a Method for the Determination of GW433908 and Amprenavir in Dog Plasma Using [redacted] Study No. 02AVS0013) Leeder, 1997, Pharmacogenetics in pediatrics
- McClain, 1989, The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. Toxicology and Applied Pharmacology, 99, 216-228
- Mitcheson, 1999, Characteristics of a transient outward current (sensitive to 4-aminopyridine) in Ca2 tolerant myocytes isolated from the rabbit atrioventricular node
- Walli, 1998, Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-1 infected patients

Reviewer: NDA No.

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