CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-548

PHARMACOLOGY REVIEW

TABLE OF CONTENTS

EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

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.

1.2 Recommendation for nonclinical studies

As part of a Phase 4 Post-marketing Agreement, it is understood that the sponsor should be required to submit the currently on-going 2-year carcinogenicity study reports in mice and rats to the agency 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 90-day studies in rats to qualify the drug substance impurities

2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings

General Toxicology Study Findings: As a phosphate ester prodrug of APV, the toxicology profile of GW433908G in animals is similar to those seen in animals treated with APV. An in vitro study demonstrated both APV and GW433908X had no effect on the hERG current, but studies indicated an equivocal action potential duration (APD) shortening effect of GW433908G in isolated dog Purkinje fibres at 200 ng/mL (Report WD 2001/00683/01). Myocardial fiber degeneration and necrosis were observed in mice and rats following acute intravenous administration (mice and rats: i.v. ≥347 mg/kg), and in rats following 2-weeks repeat-dose oral administration (50mg/kg/day, 750mg/kg/day). A moderate but variable increase in QT interval, transient decreases in heart rate and blood pressure were observed in rats, which was considered unlikely to be of clinical significance because it occurred following administration of a very large dose (intraduodenal: 2000mg/kg). ECG changes (ventricular premature complexes, increases in QT interval, increases in U wave amplitude) were observed in the two-week and one-month repeat-dose oral dog studies, which were cosidered to be secondary to hypokalemia caused by the test article-induced gastrointestinal disturbances. In addition, coronary arteritis was observed in one dog (350mg/kg/day), which was considered to be a spontaneous occurrence in this species and not treatment-related. GW433908G did not show any clinically relevant cardiovascular effects in repeat dose toxicology studies in dogs with either APV or GW433908G.

The nonclinical toxicological findings with GW433908G include: (1) gastrointestinal intolerance (salivation, vomiting and faecal alterations that included soft and liquid faeces) in dogs; (2) liver toxicity in rats and dogs; (3) decreases (1% to 8%) in haematocrit and haemoglobin, 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 study.

Gastrointestinal intolerance in the dog, consisting of salivation, vomiting and soft to liquid feces, occurred consistently throughout all of the repeat dose studies with GW433908G, and led to dehydration, electrolyte loss and deterioration to moribund condition in a number of animals. Liver is the primary target organ for GW433908G toxicity in animals. Increases in serum AST, ALT, GGT or alkaline phosphatase activity, and increases in liver weights associated with hepatocyte necrosis were seen in animals treated with GW433908. In carcinogenicity studies with APV, hepatocellular adenomas were seen in male mice and rats at the high dose, consistent with the liver changes seen during the repeat dose toxicity studies with GW433908G. Consistent hematological changes between the 4-week and 6-month rat studies

included decreases (≤8%) in hematocrit and hemoglobin, and an increase (≤25%) in platelet count. All of these changes appeared to improve during the recovery period, but did not recover fully. In the 6-month study in rats, haematological changes occurred in both male and female rats at 478 mg/kg/day (320 mg/kg/day APV equivalents). Exposure (AUC) to APV on Day 179 of this study in males at this dose (46.2 µg•h/mL, lowest exposure) was approximately 0.6 times human exposure at the MRHD. The GW433908X exposure at this dose in males (0.23 µg•h/mL) was approximately 3.7 times human exposure.

Reproductive Toxicity Study Findings
In the rabbit embryofetal study, systemic exposure (AUC) to APV at the high dose on Day 20 of gestation was approximately 0.3 times the exposure in humans treated at the proposed recommended human daily dose. Note that higher doses were not used since the existing high dose caused relatively severe maternal toxicity in the form of reduced food consumption and reductions in body weight gain or losses in absolute body weights. The increased incidence of abortions in the rabbit embryofetal study at the high dose is considered related to this severe maternal toxicity. The abortions occurred late in gestation (Days 21 to 29) and after the dose administration phase of the study was finished. 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 at all doses in both male and female pup body weights at weaning. The reduction in body weights 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 at the high dose when the F1 generation was mated. GW433908G did not cause irritation when applied to the rabbit eye, but was classified as a mild irritant to rabbit skin. GW433908G showed no sensitizing potential in guinea pigs.

Immunotoxicology Study Findings
The effect of GW433908G on the immune system in the repeat dose toxicity studies was a reduction in thymus weights and the microscopic correlate of thymic atrophy in the 9-month dog study. However, both of these findings are probably attributable to stress from daily emetic episodes, and hence the thymus is not considered a primary toxicity target organ. To assess the effect of GW433908G on the immune system, an immunotoxicity study in rats will be conducted after approval of this NDA as a phase 4 commitment. The sponsor will submit the final report to the division for review after completion of the study.

Genotoxicity and Carcinogencity Study Findings
GW433908A or GW433908G have been examined in a battery of in vitro and in vivo genetic toxicity assays. The in vitro tests were carried out in the absence and presence of a rat liver-derived S9, and all studies included appropriate vehicle and positive controls. All results were negative. Additional studies on impurities of synthesis were also negative. Carcinogenicity studies in rats and mice with GW433908G are currently being initiated 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.

Issues Regarding Drug Substance Impurities
The impurity profile of GW433908G is different to APV and none of the impurities identified for APV have been observed in batches of GW433908G. To ensure adequate qualification of impurities in the drug substance, all of the impurities, except [redacted] were tested at a concentration equivalent to or greater than the proposed drug substance specification for GW433908G in nonclinical toxicity studies in rats and dogs. The sponsor also compared the toxicological profile of GW433908G with results from drug substance batches purposely spiked with potential impurities. These batches were also examined for mutagenicity in 3 bacterial reverse mutation studies. 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. Note that the sposnor calculated the drug substance qualification levels based on the high dose rather than the NOAEL in the non-clinical toxicology studies. In general, such a calculation is not acceptable because at such doses toxicity was seen in animals. The sponsor will carry out studies to qualify the above impurities as a phase 4 commitment.

Pharmacokinetics
As a phosphate ester prodrug of APV, GW433908G is rapidly and extensively hydrolyzed to APV by alkaline phosphatases in the gastrointestinal system in the human. Pharmacokinetic studies have been carried out to describe the disposition and metabolism of both APV and GW433908X (the free ester) following oral administration of GW433908G to pre-clinical species. In general, the disposition of GW433908X and APV was similar in mice, rats, dogs and humans. APV is rapidly absorbed after oral administration of GW433908G. Hepatic clearance is the principal route of elimination of APV, with metabolism via the cytochome P450 isoform CYP3A4 and excretion in the feces for rats, dogs and humans. The main products of metabolism in rats were a di-oxidation on the tetrahydrofuran moiety of the molecule and an additional site of oxidation on the aniline ring portion of the molecule. Quantitatively, the exposure ratios of GW433908X to APV were ≤2%. Systemic exposure (AUC) to APV after repeat oral administration of GW433908G to mice, rats or dogs increased with increases in dose, but not dose-proportionally. In pregnant rabbits, systemic exposure increased in a greater than dose-proportional manner. In rabbits, GW433908X to APV exposure ratios were variable, ranging from 2.9 to 39.8%, indicating that the conversion of GW433908X to APV may be less efficient in the rabbit. In mice, rats and dogs, 3-13% of the GW433908G dose was excreted in the urine, with APV being a minor component in the urine. In dogs, the balance of the dose was excreted in feces, with APV being the major component in the feces. Plasma protein binding studies indicated displacement of 4-6% by ritonavir at high doses.

The relative bioavailability of APV was re-evaluated in dogs treated with GW433908G after pre-dosing with dilute HCl. The results showed that pre-dosing with dilute HCl in dogs treated with GW433908G increased the systemic exposure (AUC) and relative bioavailability of APV. APV bioavailability when delivered by GW433908G in humans may be affected if gastric pH is raised, either artificially such as following use of H2-receptor antagonists or in achlorhydric patients.

2.2. Pharmacologic activity
Both in vitro and in vivo pharmacological studies included in this NDA demonstrated that the hydrolysis of GW433908G to APV was mediated by intestinal alkaline phosphatase. There was minimal systemic exposure to GW433908G. Additionally, a range of in vitro and in vivo studies has been carried out to investigate the general pharmacological activity and the safety pharmacology. GW433908G had no clinical relevant effect on CNS, respiratory or cardiac function in vivo. Studies with APV indicated a shortening of the repolarization phase of the cardiac action potential, but these results were not seen in toxicity studies in dogs. GW433908G had no effect on the hERG current in vitro. GW433908G did not prolong QTc interval.

2.3.1 Nonclinical safety issues relevant to clinical use

Gastrointestinal toxicity: Salivation, vomiting and soft and liquid faeces were seen in dogs with GW433908G which led to dehydration and electrolyte loss of the animals. Gastrointestinal effects have been reported during clinical trials with both GW433908G and APV, which are reversible on discontinuation of treatment.

Liver toxicity: Liver is the primary target organ for GW433908G toxicity in animals.

Reproductive Toxicity: In the rabbit embryofetal study, systemic exposure (AUC) to APV at the high dose on Day 20 of gestation was approximately 0.3 times the exposure in humans treated at the proposed recommended human daily dose. The high dose caused relatively severe maternal toxicity (reduced food consumption and reductions in body weight gain or losses in absolute body weights) and an increased incidence of abortions in gestation (Days 21 to 29). 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 at all doses in both male and female pup body weights at weaning. The reduction in body weights was accompanied by a delay in the appearance of several developmental markers. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus. Moreover, the presence of APV in maternal milk may account for the reduction in mean body weights seen in these animals. GW433908G will be contraindicated during human lactation due to the possibility of transferring the HIV virus from mother to child.

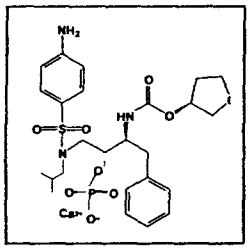
Other Issues Relevant to Clinical Use: Pre-dosing with dilute HCl in dogs treated with GW433908G increased the systemic exposure (AUC) and relative bioavailability of APV. Thus, APV bioavailability when delivered by GW433908G in humans may be affected if gastric pH is raised, either artificially such as following use of H2-receptor antagonists or in achlorhydric patients.

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY

Amprenavir (APV, 141W94) is a peptidomimetic inhibitor of HIV protease for HIV treatment, which was marketed in the US in 1999 and in the EU in 2000 as AGENERASE capsules and oral solution. The AGENERASE formulation contains a high ratio to excipients to drug substance because of the extremely low solubility of APV. Therefore, the sponsor initiated clinical development of an APV prodrug (fosmaprenavir, GW433908G) with increased water solubility for treatment of HIV infection. Fosamprenavir (GW433908G, the monocalcium salt of the phosphate ester of APV) was developed as the form proposed for the clinical use.

NDA number: 021,548, Original
Sequence number/date/type of submission: 1/15/03
Information to sponsor: Yes (X)
Sponsor and/or agent: GlaxoSmithKline, Research Triangle Park, NC 27709
Manufacturer for drug substance: The Wellcome Foundation Ltd (trading as Glaxo Wellcome Operations), Temple Hill, Dartford, Kent DA1 5AH, UK
Reviewer name: Hao Zhang, M.D.
Division name: Division of Antiviral Drug Products
HFD #: 530
Review completion date: August 31, 2003
Drug:
Trade name: Lexiva
Generic name: Fosamprenavir calcium, Fosamprenavir
Code name: GW433908G
Chemical name: Carbamic acid, [(1*S*, 2*R*)-3{{(4-aminophenyl) sulfonyl] (2-methylpropyl) amino]-1-(phenylmethyl)-2-(phosphonooxy)propyl]-C-[(3S)-tetrahydro-3-furanyl] ester, calcium salt (1:1)
CAS registry number: 226700-81-8
Molecular formula/molecular weight: $C_{25}H_{34}CaN_3O_9PS$; MW: 623.
Structure:



Relevant INDs/NDAs/DMFs: IND 58,627
Drug class: A phosphate ester prodrug of APV, an Anti-HIV protease inhibitor
Indication: Treatment of HIV infection
Clinical formulation: 700 mg Tablets
Route of administration: Oral

Proposed use: The proposed clinical dosing regimen is either GW433908G alone (1400 mg BID, equivalent to 1200 mg APV BID) or with ritonavir (either 700 mg GW433908G, equivalent to 600 mg APV + 100 mg ritonavir BID; or 1400 mg GW433908G + 200 mg ritonavir, QD). The total pill count for these regimens is 4 tablets/day (either 4 GW433908G 700 mg tablets or 2 GW433908G 700 mg tablets and 2 ritonavir 100 mg capsules).

Disclaimer: *Tabular and graphical information is from sponsor's submission unless stated otherwise.*

Studies reviewed within this submission:

Pharmacology Studies

1. In vitro permeability of GW433908A across caco-2 cells monolayers (Report No. RD2002/00489/00)
2. Mechanism of Hydrolysis of GW433908A to Amprenavir in vitro with intestinal alkaline phospatase and intestinal brush border membrane vesicles (Report No. RD2002/00142/00; Study 02AVT0010)
3. Secondary pharmacological evaluation of the HIV protease inhibitor Amprenavir and its prodrug GW433908G in radioligand binding assays (Report No. RH2002/00022/00)

Safety Pharmacology Studies

4. Study R40357 - GW433908A: overt central and peripheral pharmacodynamic effects following acute oral administration in the conscious han wistar rat (Report No. RD1998/00541/00)
5. Study S22240 - GW433908 A: cardiovascular and respirator effects following acute intra-duodenal administration in the anaesthetised rat (Report No. WD1999/00154/00)
6. Study V23092 - 141W94: effect of 141W94 on action potential parameters in dog isolated cardiac purkinje fibres (Report No. WD2001/00838/01)
7. Study V23093 - GW433908G: effect of GW433908G on action potential parameters in dog isolated cardiac purkinje fibres (Report No. WD2001/00683/01)
8. Study G01177 - Effect of 141W94 and GW433908G on the human cardiac Ikr (HERG) channel (Study Number CD2001/00015/00)
9. Study S22241 - GW433908A: cardiovascular effects following oral administration in conscious, telemetered beagle dogs (Report No. WD1998/00543/00)
10. Study S22365 - GW433908A: cardiovascular effects following intravenous administration in conscious telemetered beagle dogs (Report No. WD1998/00588/01)
11. Study S22321 - GW433908A: overt central and peripheral pharmacodynamic effects following acute oral administration in conscious beagle dogs (Report No. WD1999/00155/00)

Pharmacokinetics Studies

12. Study 98AVV0018 – Disposition and metabolic profiling in wistar hannover rats after oral administration of the calcium salt of [^{14}C] GW433908 (Report No. RD2002/00725/00)
13. Study 98AVV0017 – Disposition and metabolic profiling in beagle dogs after oral administration of the calcium salt of [^{14}C] GW433908 (Report No.RD2002/00724/00)
14. Study 98APK0034 - Pharmacokinetics and relative bioavailability of the free acid and various salts of GW433908 in male beagle dogs (Report No.RD1998/03011/01)
15. Study 99APK0030 - Pharmacokinetics and relative bioavailability of GW433908G liquid formulations after single oral doses to beagle dogs (Report No. RD1999/00927/00)
16. Study 01AVT0028 - Determination of human plasma protein binding interaction between GW433908G and Amprenavir (141W94) (Report No. RD2001/01671/00)
17. Study 01AVT0013 – Determination of human plasma protein binding interaction between Amprenavir (141W94) and the Amprenavir metabolites GW549445X and GW549444A (Report No. RD2001/00984/00)
18. Study 02APK0018 – The pharmacokinetics of GW549445X and GW549444X in rats, dogs and humans following oral administration of Amprenavir or GW433908G (Report No.RD2002/00576/00)
19. Pharmacokinetic study after oral administration of GW433908G to portal vein-cannulated han wistar rats and a beagle dog (Report No. RD1998/02935/01; Study 98APK0135)
20. Study M40725 - The enzyme induction of GW433908G in the CD-1 mouse following oral administration of GW433908G during a 13-week pilot carcinogenicity study (Report No. RD2002/00646/00)
21. rHuCYP3A4-Like immunoreactivity in rat liver microsomes from 3-month Amprenavir (TOX771) and 1-month GW433908G (R40427) Toxicology studies (Report No.RD1999/02460/02)

Toxicology Studies

22. Study M40367 - GW433908A: Acute oral toxicity study in mice (Report No. RD1998/00776/00)
23. Study M40426 - GW433908G: A single-dose oral toxicity study in CD-1 mince (Report No. RD1999/00017/00)
24. Study M40370 - GW433908A: Acute intravenous toxicity study in CD-1mice (Report No. RD1998/00657/00)

25. Study M40431 - GW433908A: Single-dose intravenous toxicity study in CD-1 mice (Report No. RD1998/02552/00)
26. Study R40368 - GW433908A: Acute oral toxicity study in rats (Report No. RD1998/00777/00)
27. Study R40425 - GW433908G: A single-dose oral toxicity study in han wistar rats (Report No. RD1999/00018/00)
28. Study R40371 - GW433908A: Acute intravenous toxicity study in han wistar rats (Report No. RD1998/00656/00)
29. Study R40432 - GW433908A: Single-dose intravenous toxicity study in han wistar rats (Report No. RD1998/02551/00)
30. Study R40364 - GW433908A: A 2-Week oral toxicity study in male han wistar rats (Report No. RD1998/00711/00)
31. Study R40427 - GW433908G: A 4-week oral gavage toxicity study in han wistar rats (RD1998/02573/00)
32. Study R40417 - GW433908G: Six month oral gavage toxicity study in han wistar rats (Report No. RD1998/02858/01)
33. Study R40877 - GW433908G: Two week oral gavage pilot toxicity study in neonatal and juvenile wistar hannover rats (Report No. RD2000/02506/00)
34. Study R40576 - GW433908G: Oral gavage pilot toxicity study in neonatal rats (Report No. RD1999/02344/00)
35. Study R40860 - GW433908G:Thirteen week oral gavage toxicity study in neonatal and juvenilewistar hannover rats (Report No.RD2002/00045/00)
36. Study D40350 - GW433908A: 14-day oral gavage toxicity studyin beagle dogs (Report No. RD1998/00487/01)
37. Study D40436 - GW433908G: A one month oral gavage toxicity study in beagle dogs (Report No. RD1998/02605/00)
38. Study D40418 - GW433908G: Nine-month oral toxicity study in beagle dogs (Report No. RD1998/02861/01)

Genetic Toxicity Studies

39. Study V40351 - GW433908A: Salmonella-escherichia coli/mammalian-microsome reverse mutation plate incorporation and pre-incubation assays (Report No. RD1998/00935/00)
40. Study V40707 - GW433908G (batch number DNPIA/38/25/3): Salmonella-escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report No. RD1999/02761/00)
41. Study V40708 - GW433908G (Batch number DNPIA/38/25/1): Salmonella escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report No. RD1999/02762/00)
42. Study V40706 - GW433908G (Batch number DNPIA/38/25/2): Salmonella and escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay (Report RD1999/02763/01)
43. Study V40376 - GW433908A: L5178Y/TK+/- mouse lymphoma in vitro mammalian cell mutagenesis assay (Report No. RD1998/01213/00)
44. Study R40476 - GW433908G: Micronucleus frequencies in bone marrow polychromatic erythrocytes from male han wistar rats following oral administration (Report No. RD1999/00412/00)

Carcinogenicity Studies

45. Study M40725 – GW433908G: 13-week oral gavage pilot carcinogenicity study in mice (Report No. RD2000/02408/00)

Reproductive and developmental toxicology

46. Study R40458 - GW433908G: oral male and female fertility study in CD (Sprague Dawley) rats (Report No. RD1999/01281/00)
47. Study R40470 - GW433908G: Oral embryo-fetal development study in CD rats (Report No. RD1999/02690/00)
48. Study L40459 - GW433908G: Oral dose range-finding study in nonpregnant New Zealand white rabbits (Report No.RD1999/00465/00)

49. Study L40460 - GW433908G: Oral dose range-finding study in pregnant New Zealand white Rabbits (Report No. RD1999/00716/00)
50. Study L40461 - GW433908G: Oral embryo-fetal development study in New Zealand white rabbits (Report No. RD1999/01035/00)
51. Study R40486 - GW433908G: Oral pre- and postnatal development study in CD (Sprague-Dawley) rats (Report No. RD1999/01282/00)

Local tolerance

52. Study L40478 - GW433908G: Acute dermal irritation study in the New Zealand white rabbit (Report No. RD1999/00553/00)
53. Study L40479 - GW433908G: Acute eye irritation study in the New Zealand white rabbit (Report No. RD1999/00551/00)
54. Study G40477 - GW433908G: skin sensitization (buehler method) study in the guinea- pig (Report No. RD1999/00552/00)

Special Toxicity Studies

55. Study R40857 - GW433908G: 14-day oral toxicity study in wistar hannover rats to assess the effects of synthetic material containing the impurities GW634519,GW569684, GW635116, GW635117, GW587304, GW453999, GW63849 and GW638468 (Report No.RD2000/01884/00)
56. Study R40917 - GW433908G: 14-day oral toxicity study in wistar hannover rats to assess the effects of synthetic material containing the impurity GW638468 (Report No. RD2001/00212/01)

Studies <u>not</u> reviewed within this submission:

Pharmacology Studies:

57. Aqueous pH-solubility profile of GW433908X and GW433908G in radioligand binding assays (Report No. RD2002/00935/00)
58. In vitro interaction of GW591198X with P-glycoprotein (Pgp) (Study Number 02AVT0088; RD2002/01464/00)
59. Investigation of the inhibition of human intestinal alkaline phosphatase by fosamprenavir (GW433908), Amprenavir (GI268188), Lopinavir (GW591198X), and Ritonavir (GW278007X) in vitro (Study Number 03DMR008; RD2003/00302/00)
60. CYP3A4 induction potential of GW591198X in human PXR assay (Study Number 03AVT0014; RH2003/00024/00)
61. Human in vitro bone marrow progenitor toxicity of anti-HIV protease inhibitor GW433908 (Report No. RR1999/00077/01)
62. The effects of GW433908G on growth-inhibition of human leukemic (B and T) and lymphomic cell lines (Report No. RH2002/00030/00)

Pharmacokinetics Studies

63. Study 01AVV0004 – Disposition and metabolic profiling in CD-1 mice after oral administration of the calcium salt of [^{14}C] GW433908 (Report No.RD2001/00560/00)
64. Study RD20010161801 – The identification of the metabolites of GW433908 in rat feces and urine (Report No. RD2001/01618/01)
65. Study RD20000237001 – The identification of the metabolites of GW433908 in dog feces and urine (Report No. RD2000/02370/01)
66. Study 02AVV0014 – The profiling and identification of metabolites of 141W94 in the mouse (Report RD2002/00504/00)
67. Study 02AVV0015 – The profiling and identification of metabolites of GW433908G in the mouse (Report No.RD2002/00505/00)
68. Study 01AVV0003 – Disposition and metabolic profiling in CD-1 mice after oral administration of [^{14}C] Amprenavir (Report RD2001/00558/00)
69. Study 99AVV0027 – Characterization of major human metabolites of Amprenavir (Report No. RD1998/00831/01)
70. Pharmacokinetics and relative bioavailability of the free acid and various salts of GW433908 in male beagle dogs Report No. RD1998/03011/01; Study 98APK0034)

71. Study RD20010052701 - Determination of human plasma protein binding interaction between Amprenavir (141W94) and ritonavir (GW278007X), delavirdine (GW305334X) and efavirenz (GW410886X) (Report No. RD2001/00527/01)

Special Toxicity Studies

72. Study S40528 -- In vitro interference study for clinical chemistry assays ALT, AST, and ALP for GW433908G and 141W94 (RD1999/01432/01)

PHARMACOLOGY

3.2.1. Brief summary

Fosamprenavir is the phosphate ester prodrug of APV. Fosamprenavir is primarily converted to APV by alkaline phosphatase at or in the apical endothelium of the intestinal membrane. APV inhibits the HIV-1 aspartyl protease in HIV-1 infected cells, resulting in an inability to process *gag* and *gag-pol* polyproteins. APV has synergistic activity with nucleoside analogues including AZT, ddI and abacavir, and the protease inhibitor, saquinavir.

3.2.2. Primary pharmacodynamics

Mechanism of action: In vitro, APV inhibited the HIV-1 aspartyl protease. Fosamprenavir is the phosphate ester prodrug of APV, which is hydrolysed to APV and inorganic phosphate as it is absorbed through the gastrointestinal epithelium.

Drug activity related to proposed indication: Fosamprenavir has a similar efficacy profile compared to APV.

Drug activity related to toxicity: In human bone marrow progenitor cells the IC_{50} values of GW433908G were 50 µM for the two colony types, CFU-GM (colony forming unit granulocyte macrophages) and BFU-E (burst forming unit erythroid).

3.2.3. Secondary pharmacodynamics

In vitro, APV at 1 and 10 µM had an inhibitory effect on isoproterenol-induced chronotropy of the significant binding interactions towards 60 target receptor sites at concentrations up to 10 µM, equivalent to approximately 5 µg/mL APV or 6 µg/mL GW433908X.

3.2.4. Safety pharmacology

Five safety pharmacology studies were conducted in rats and dogs with GW433908 to assess the effects of the test article on the central and peripheral nervous systems and cardiovascular and respiratory systems. Principles of GLP regulations were followed during these studies, which are considered valid in the assessment of the safety of GW433908G.

Neurological effects: The overt pharmacodynamic effects of GW433908A on the central nervous system (CNS) and major peripheral systems of the conscious rat (Report RD1998/00541/00) and dog (Report WD1999/00155/00) have been studied following acute oral administration. In rats, no treatment-related behavioural or overt pharmacological effects were seen at doses up to 2000 mg/kg GW433908A (1493 mg/kg APV equivalents).

Cardiovascular effects: GW433908A caused a moderate increase in QT and QTc interval in anaesthetised male rats following single intraduodenal doses of up to 2000 mg/kg (1493 mg/kg APV equivalents), a dose approximately 32 times greater than the maximum human dose of GW433908G (1400 mg BID, or 47 mg/kg/day assuming a 60 kg human). Note that the increase was variable and observed in only the high dose group (Report WD 1999/00154/00). In conscious, telemetered, male dogs, single doses of GW433908A up to 2000 mg/kg (1493 mg/kg APV equivalents) orally and 30 mg/kg (22 mg/kg APV equivalents) intravenously had no significant cardiovascular effects other than those attributed to severe emesis (Report WD 1998/00543/00).

In *in vitro* studies, GW433908G had an equivocal action potential duration (APD) shortening effect in isolated dog Purkinje fibres at the highest concentration tested, 200 ng/mL (Report WD 2001/00683/01).

APV showed a dose-related decrease in upstroke amplitude (UA) and maximum rate of depolarisation (MRD) and action potential duration (APD) at ≥ 5 µg/mL (Report WD 2001/00838/01). Additional increases in the plateau phase of the cardiac action potential at 15 and 50 µg/mL indicated an effect on potassium channels. However, a further in vitro study demonstrated both APV and GW433908X had no effect on the hERG current. GW433908G did not show any clinically relevant cardiovascular effects in repeat dose toxicology studies in dogs with APV or GW433908G (Report CD 2001/00015/00).

Pulmonary effects: GW433908A caused no respiratory effects in anaesthetised male rats following single intraduodenal doses of up to 2000 mg/kg (1493 mg/kg APV equivalents) (Report WD 1999/00154/00).

Renal effects: not determined

Gastrointestinal effects: Single intravenous doses of 100 mg/kg GW433908A (75 mg/kg APV equivalents) resulted in gastrointestinal disturbances, behavioral changes and marked increases in blood pressure and heart rate that were considered secondary to the emesis. In addition, gastrointestinal disturbances (emesis and loose or watery feces) and behavioural effects were similar to those observed during oral repeat dose toxicity studies with GW433908G in the dog and in previous studies in the dog with APV.

Abuse liability: Not determined

Other: N/A

Safety pharmacology summary and conclusions: In vitro studies demonstrated both APV and GW433908X had no effect on the hERG current, but studies indicated an equivocal APD shortening effect of GW433908G in isolated dog Purkinje fibres at 200 ng/mL (Report WD 2001/00683/01). GW433908G did not show any clinically relevant cardiovascular effects in repeat dose toxicology studies in dogs with either APV or GW433908G.

Pharmacodynamic drug interactions: APV has synergistic activity with nucleoside analogues including AZT, ddI and abacavir, and the protease inhibitor, saquinavir.

Pharmacology Studies:

1. **Study 02ARS0078 - In vitro permeability of GW433908A across caco-2 cells monolayers (Report No. RD2002/00489/00)**

Method

The *in vitro* cell permeability of GW433908A was investigated with the cultured human Caco-2 cell monolayers treated with either 10 µM or 100 µM GW433908A in Modified Eagle's Medium (MEM) containing 10% (v/v) fetal bovine serum and 1% non-essential amino acids. Transflux of GW433908A and the appearance of APV, and flux in both the absorptive direction (apical to basolateral) and the secretory direction (basalateral to apical) were determined by an [redacted] method.

Results

GA433908A was found to have low transepithelial flux, with an apparent permeability coefficient (P_{app}) of <2 nm/sec at both concentrations and in both directions. This low P_{app} value indicates that very little GW433908A crosses the monolayers as intact compond. APV was directed in the reciever compartments at the higher levels relative to GW433908A, especially after dosing with 100 µM GW433908A in which 30- to 50- fold higher amounts of APV were found.

2. **Study 02AVT0010 – Mechanism of hydrolysis of GW433908A to Amprenavir in vitro with intestinal alkaline phospatase and intestinal brush border membrane vesicles (Report No. RD2002/00142/00)**

Method and Results

An *in vitro* study was performed to investigate the enzymatic kinetics of GW433908A hydrolysis using isolated intestinal alkaline phosphatase and intestinal brush border membrane vesicles (BBMV).

Isolated intestinal alkaline phosphatase assay: GW433908A was converted to amprenavir by incubation at pH 10.4 with intestinal alkaline phosphatase isolated from rat and dog. Reactions were concentration-dependent and saturable in the range of 1.33 to 18 mM and 0.5 to 10 mM GW433908A for rat and dog intestinal alkaline phosphatase, respectively. Estimates of Vmax and Km were 19.7 nmol/min/U and 8.5 mM with isolated at intestinal alkaline phosphatase, and 11.6 nmol/min/U (38.3 nmol/min/mg) and 4.5 mM with isolated dog intestinal alkaline phosphatase, respectively.

BBMV assay: The estimates of Km and Vmax determined in BBMV studies are shown in Table 1. Results show that BBMV catalyzed the conversion of GW433908A to amprenavir at pH 10.2, and generally correlated with known expression of alkaline phosphatase (duodenum>jejunum>ileum) in the intestinal tract. Reactions were generally concentration-dependent and saturable in the range of 0.5 to 10 mM GW433908A. Estimates of Vmax were similar in rat and human BBMV, and nearly 10-fold higher in dog BBMV. Estimates of Km ranged from 0.2 to 13.3 mM, with duodenal Km estimates ranging from 1.2 to 3.8 mM. After conversion of the dog BBMV Vmax estimates to nmol/mg/min, the values were similar to the Vmax estimate from isolated dog intestinal alkaline phosphatase (38.8 to 87.2 vs 38.3 nmol/min/mg, respectively).

Table 1. Enzymatic kinetics of GW433908A hydrolysis in BBMV in rats, dogs, and humans

Species	Intestinal Segment	Km (mM)	Vmax (nmol/mg/20 min)
Rat	Duodenum	3.8 ± 3.0	113 ± 37
	Jejunum	3.4 ± 0.9	102 ± 10
Dog	Ileum	13 ± 35	42 ± 73
	Duodenum	2.4 ± 0.4	1743 ± 89
	Jejunum	0.6 ± 0.4	776 ± 104
Human	Ileum	0.9 ± 0.4	886 ± 101
	Duodenum	1.2 ± 0.9	68 ± 13
	Jejunum	0.2 ± 0.1	154 ± 7.0
	Ileum	0.5 ± 0.1	104 ± 5.

Comments

The data confirm that intestinal alkaline phosphatase can convert GW433908A to amprenavir in vitro.

3. Secondary pharmacological evaluation of the HIV protease inhibitor Amprenavir and its prodrug GW433908G in radioligand binding assays (Report No. RH2002/00022/00)

Method

The secondary pharmacological profiles (the binding interactions with various physiological receptors and ion channels) of amprenavir and its prodrug GW433908G were investigated at a concentration of 10 µM in a battery of 60 radioligand-binding assays. These included adenosine, adrenergic, bradykinin, cannabinoid, chemokine, cholecystokinin, cholinergic, dopamine, endothelin, glutamate, glycine, histamine, leukotriene, opioid, purinergic, serotonin, steroid, sigma, tachykinin and vasoactive intestine peptide (VIP) receptors; monoamine transporter sites; and ion channels (L-type and N-type calcium, chloride-GABA (γ-ainobutyric acid) A, potassium- Kv, and sodium).

Results

APV or GW433908G at 10 µM did not alter the binding of any of the tested radioligands to the respective binding site.

Safety Pharmacology Studies:

4. Study R40357 - GW433908A: overt central and peripheral pharmacodynamic effects following acute oral administration in the conscious han wistar rat (Report No. RD1998/00541/00)

GW study No.: R40357; Conducting facility: Glaxo Wellcome Inc., Research Triangle Park, NC 27709; Date Initiation: 10 November 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R4283/31/1

Method
Groups of three male Han Wistar rats received GW433908A at oral doses of 0 (vehicle, 150, 550, or 2000mg/kg). Animals were observed for the first 30 minutes after dosing and at 1,2,4,24, 48 hours post dosing. The effects of treatment on respiratory rate, the gastrointestinal tract, autonomic nervous system (pupil size, lacrimation, salivation, urination) and CNS (behavioral effects, locomotor co-ordination, skeletal muscle tone, reflexes and other neurological changes) were recorded. Plasma samples were obtained from all animals for analysis of both GW433908 and amprenavir concentrations.

Results
GW433908 caused no treatment related overt effects on the central nervous system and major peripheral systems following a single oral dose of up to 2000 mg/kg in conscious rats. Mild changes in vocalization, spontaneous activity, reactivity, body sag and grip strength were noted one hour post-dose at 150mg/kg, which is not treatment related.

Comments
GW433908 has no overt effects on the central nervous system and major peripheral systems following a single oral dose of up to 2000 mg/kg in conscious rats.

5. Study S22240 – GW433908A: cardiovascular and respiratory effects following acute intra-duodenal administration in the anaesthetised rat (Report No. WD1999/00154/00)

IND No.: 58627; Serial No.: 000; Vol. No.: 2 of 16; Pages 64-115; GW report No.: RD1999/00154/00; GW study No.: S22240; Conducting facility: Glaxo Wellcome Research and Development, Ware, Hertforshire; Date Initiation: 19 March 1998; GLP Compliance: Yes () No (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A in sterile water for irrigation (batch no. 12710)

Method
Groups of three male Han Wistar rats were anesthetized with an intraperitoneal injection of pentobarbitone sodium (30mg/kg) and intramuscular injection of ketamine (40mg/kg) and xylazine (8mg/kg). Animals received GW433908A at intraduodenal doses of 0 (vehicle), 150, 550, or 2000mg/kg in a dose volume of 10 ml/kg. Animals were observed for the last 30 minutes before and after dosing. The effects of treatment on respiratory rate, the gastrointestinal tract, autonomic nervous system (pupil size, lacrimation, salivation, urination) and CNS (locomotor co-ordination, skeletal muscle tone, reflexes and other neurological changes) were recorded. Plasma samples were obtained from all animals for analysis of both GW433908 and amprenavir concentrations.

Results
GW433908 caused no treatment related overt effects on the central nervous system and major peripheral systems following a single intraduodenal dose of up to 550mg/kg in anesthetized rats. One animal died of progressive decrease in blood pressure and heart rate at 2000mg/kg. Two animals at this dose had cardiovascular effects with slight decreases in blood pressure and heart rate and a moderate increase in QT interval.

6. Study V23092 - 141W94: effect of 141W94 on action potential parameters in dog isolated cardiac purkinje fibres (Report No. WD2001/00838/01)

Method
Action potential parameters were recorded from Purkinje fibres, isolated from four male beagle dogs, electrically paced at 1 or 0.5 Hz in this study. Fibres were incubated with 5, 15 or 50 µg/mL formulated in 0.1% v/v DMSO in physiological saline. The effects of *dl*-sotalol hydrochloride (50 µM) were evaluated at each pacing frequency (a repolarising K^+ channel antagonist as a positive control). The maximal rate of depolarisation at a pacing frequency of 3 Hz was also measured following incubation with either the highest test compound concentration or vehicle control, to assess any effects on sodium channels.

Results
At all doses and both pacing frequencies, a concentration-dependent statistically significant decrease in upstroke amplitude (UA) and maximum rate of depolarisation (MRD, with the exception of 5 µg/mL,

0.5Hz) was seen. A statistically significant shortening of action potential duration (APD) was seen at all concentrations that was more pronounced at APD60 than APD90. Note that the value for the MRD was not determined at 3 Hz stimulation frequency. Percentage changes between the steady state maximum rate of depolarisation values recorded at 1 and 3 Hz stimulation frequencies for both vehicle and 50 µg/mL treated fibres therefore were not compared.

Comments

These results indicate APV interacts with cardiac calcium channels and the transient outward potassium channel responsible for the initial repolarisation (phase 1) of the cardiac action potential. The effect observed on MRD also indicates an interaction of APV with cardiac sodium channels.

7. Study V23093 - GW433908G: effect of GW433908G on action potential parameters in dog isolated cardiac purkinje fibres (Report No. WD2001/00683/01)

Method

Isolated dog cardiac purkinje fibres were incubated with 20, 60 or 200 ng/mL GW433908X (equivalent to 25, 74 or 246 ng/mL GW433908G, or 17, 52 or 173 ng/mL APV). GW433908G was formulated in 0.1, 0.3 or 1% v/v DMSO in physiological saline, respectively. The effect of 0.1, 0.3 or 1% v/v DMSO at each pacing frequency was studied as the negative controls.

Results

No effect on resting membrane potential or action potential duration at 90% of repolarisation (APD90) was seen at any dose level at either 1 or 0.5 Hz pacing frequency. The maximum rate of depolarisation was also unaffected by GW433908G at any dose level at 3, 1 or 0.5 Hz. At 200 ng/mL GW433908X, two out of four fibres showed a decrease in upstroke amplitude (UA) and shortening of APD60 at 0.5 Hz. The UA decrease was statistically significant. No effects on these parameters were seen at 20 or 60 ng/mL GW433908X at 0.5 Hz, or at any dose level at 1 Hz. The positive control *dl*-sotalol hydrochloride caused a prolongation of the action potential duration that was inverse-frequency dependant, an effect consistent with its known activity as a repolarising K+ channel antagonist.

8. Study G01177 - Effect of 141W94 and GW433908G on the human cardiac Ikr (HERG) channel (Report No. CD2001/00015/00)

Method

The study was performed in HEK293 cells, a human kidney epithelial cell line stably transfected with hERG cDNA to determine the effects of APV and GW433908G on the human cardiac IKr (hERG) channel. The concentration response of hERG currents was measured using a pulse pattern with fixed amplitudes (depolarization: +20 mV for 2 s; repolarisation: -50 mV for 2 s) repeated at 10 s intervals. The concentration response was assessed following incubation of cells with 40, 400 or 4000 nM APV or GW433908G (in DMSO), respectively. Terfenadine was used as a positive control at 20, 50 and 500 nM.

Results

Neither APV nor GW433908G produced concentration-dependant inhibition of hERG currents. Terfenadine produced a strong concentration-dependant inhibition of hERG currents with an estimated IC50 of 16.8 nM. The maximum APV and GW433908G concentrations tested were 3.7 and 2396 times the maximal free plasma concentrations at the proposed therapeutic dose assuming 93 and 96% protein binding of APV and GW433908X, respectively.

9. Study S22241 - GW433908A: cardiovascular effects following oral administration in conscious, telemetered beagle dogs (Report No. WD1998/00543/00)

IND No.: 58627; Serial No.: 000; Vol. No.: 2 of 16; Pages 116-189; GW report No.: RD1999/00543/00; GW study No.: S22241; Conducting facility: Glaxo Wellcome Research and Development, Ware, Herts, UK; Date Initiation: 13 May 1998; GLP Compliance: No (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A in sterile water for irrigation (batch no. 12710)

Method

One group of two male beagle dogs (body weight: 12.3-12.9 kg, age: 53-64 week old) received a single dose of vehicle on Day 1. Two days later (Day 3) the animals received a single dose of 550mg/kg GW433908A. On day 7, the animals received a dose of 150mg/kg GW433908A. On Day 10, the animals received a dose of 2000mg/kg GW433908A. For each dose, data were collected at least 30 minutes before dosing and ended at 6 hours after dosing. The effects of treatment on arterial blood pressure, heart rate and lead II ECG were recorded.

Results

Oral administration of GW433908A, at doses of 150, 550 and 2000mg/kg, did not result in any notable alterations in arterial pressure, heart rate and lead II ECG variables when compared with the time-matched vehicle controls. Vomiting in both dogs at 150mg/kg and liquid feces at doses of 550 and 2000 mg/kg in one of the two dogs, however, was noted.

10. Study S22365: cardiovascular effects following intravenous administration in conscious telemetered beagle dogs (Report No. WD1998/00588/01)

IND No.: 58627; Serial No.: 000; Vol. No.: 2 of 16; Pages 116-189; GW report No.: RD1998/00588/00; GW study No.: S22365; Conducting facility: Glaxo Wellcome Research and Development, Research Triangle Park, NC, USA; Date Initiation: 13 May 1998; GLP Compliance: Yes (x) No (); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A in sterile water for irrigation (batch no. 12710)

Methods

One group of three male beagle dogs (body weight: 12.3-12.9 kg, age: 53-64 week old) received a single dose of 30mg/kg of GW433908 on Day 1. Two days later (Day 3) the animals received a single dose of 100mg/kg GW433908A. Adverse reaction following 100 mg/kg GW433908A resulted in the termination of the study. GW433908A was administered intravenously over a 10-minute infusion. For each dose, data were collected at least 30 minutes before dosing and at 1, 6 and 24 hours after dosing. The effects of treatment on arterial blood pressure, heart rate and lead II ECG were recorded. Venous blood samples were collected for GW433908 plasma level analysis, prior to dosing and at 0, 15, 30, 60, 120 and 240 minutes after the end of intravenous infusion.

Results

At 100mg/kg, GW433908 resulted in overt behavioral changes. Retching and unsteady gait followed by ataxia were noted in all animals. Marked increases in both arterial blood pressure and heart rate were also noted. Post-mortem examination of the euthanized animals revealed reddening of the duodenum consistent with prolonged vomiting. Toxicokinetic parameters, C_{max} and AUC_{∞} were calculated for the full dosing period as shown in Table 11.

Comments

A dose of 100mg/kg resulted in overt behavioral changes including vomiting, unsteady gait, ataxia and prostration in dogs, which were associated with marked increases in arterial blood pressure and heart rate.

Table 11. Toxicokinetics parameters of GW433908A and amprenavir (141W94) in male beagle dogs after a single i.v. administration of GW433908A

Dose *(mg/kg)*	AUC_{∞} *(µg*h/ml)*		C_{max} *(µg/ml)*		T_{max} *(hour)*	
	I	II	I	II	I	II
30	33.2	44.8	17.2	147	0.34	0.17
100	190	289	55	545	0.84	0.17

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

11. Study S22321 - GW433908A: Overt central and peripheral pharmacodynamic effects following acute oral administration in conscious beagle dogs (Report No. WD1999/00155/00)

IND No.: 58627; Serial No.: 000; Vol. No.: 2 of 16; Pages 37-63; GW report No.: RD1998/00155/00; GW study No.: S22321; Conducting facility: Glaxo Wellcome Research and Development, Bury Green, Herts, UK; Date Initiation: 10 November 1998; GLP Compliance: Yes (), No (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A in sterile water for irrigation (batch no. 12710)

Method
Groups of two male beagle dogs (body weight: 9.08-11.18 kg) received GW433908A at oral doses of 0 (vehicle), 150, 550, or 2000mg/kg. Animals were observed for the first hour after dosing, at 2, 3 and 5, 24 hours and 48 hours after dosing. The effects of treatment on respiratory rate, heart rate and body temperature were recorded. Plasma samples were obtained from all animals for analysis of both GW433908 and amprenavir concentrations.

Results
GW433908A caused gastrointestinal toxicity including emesis at ≥150mg/kg (plasma APV and GW433908 concentrations: >11.6 µg/ml and >0.16µg/ml, respectively), loose feces and diarrhea at ≥550mg/kg (plasma APV and GW433908 concentrations: >10 µg/ml and >0.2µg/ml, respectively) in dogs following oral administration.

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary
The sponsor conducted the following PK/TK studies in male animals only because no significant sex differences were apparent in pharmacokinetic studies with APV:

- Single dose PK studies to determine PK parameters, metabolism and excretion profiles following with oral GW433908G dosing
- TK and enzyme induction studies during repeat dose toxicity studies with GW433908G
- An in vitro study has determined protein-binding interaction between GW433908X and APV

3.3.3 Absorption
APV is rapidly absorbed in mice, rats and dogs following oral administration of GW433908. Bioavailability of APV was lower after administration of GW433908G compared to administration of equivalent doses of APV. Following single oral administration of ^{14}C-GW433908G to mice, rats or dogs, APV was rapidly absorbed with Tmax values of < 1 hour. Pharmacokinetic estimates for GW433908X in mice, rats and dogs were similar, with Tmax values of < 1 hour. GW433908X:APV exposure ratios were <1.0%. In humans, estimates of APV pharmacokinetics after administration of GW433908G showed APV plasma half-life values of approximately 5 to 7 hours.

3.3.4 Distributionl
Because very little GW433908X is available systemically, in vivo whole body distribution studies were not performed with GW433908G. Following single oral administration of ^{14}C-GW433908G to mice, rats or dogs, GW433908X:APV exposure ratios were <1.0%. Exposure (AUC_{24}) to total radiocarbon was 2 times that of APV in mice and rats, and 4 times that of APV in dogs. Additionally, APV plasma half-life estimates of approximately 2 to 4 hours were calculated in this study; plasma half-life estimates for GW433908X could not be estimated as GW433908X was measurable at 0.5 and 1.0 hours only. In humans, GW433908X could only be quantified in plasma samples from approximately 50% of the subjects (Re: APV10001), and the exposure ratio of GW433908X to APV was <0.17% for any subject.

3.3.5 Metabolism
The routes of metabolism are qualitatively similar in mice, rats, dogs and humans. Quantitatively, the main products of metabolism in rats are similar to humans, being a di-oxidation on the tetrahydrofuran moiety of the molecule and an additional site of oxidation on the aniline ring portion of the molecule. Up to 32 metabolites have been characterised after oral administration of [^{14}C]-GW433908G to mice, rats and dogs. GW549445 and GW549444 were two major radiolabelled metablolites (20-24% of the dose, respectively) in rats and humans (Figure 1). In dogs, APV was the major component in feces. The mono-oxidation product BD/8064/106/1 was the next most abundant fecal component. However, this metabolite was not seen in dogs following administration of APV. The reason for the slight difference in metabolic profile following GW433908G or APV administration in dogs is unclear. In a metabolite pharmacokinetic study, the ratio of GW549445 AUC to APV AUC in humans was 3.2% after single dosing with GW433908G, and 4.0% at steady state following administration of APV. In rats, the ratio at steady state was similar to that seen in humans (2.2 to 2.9%), whereas in dogs the ratio at steady state was approximately 10-fold lower than that seen in rats (0.2%). Exposure to GW549444 in humans was 4- to

15-fold lower than GW549445. In rats, GW549444 was only detected at low levels at single time points in some animals. GW549444 was below the limit of quantification in dog plasma. These results are consistent with the excreta metabolite identification data. Although there were some quantitative species differences in the metabolites present in excreta, the metabolite profiles in animals and human were qualitatively similar. All of the metabolites seen in humans were also seen in either rats or dogs. Additionally, repeat administration of APV induced cytochrome P450 3A in rats and mice. Hepatic enzyme induction studies with GW433908G in mice following 13 weeks administration and in rats following 4 weeks administration showed GW433809G induced cytochrome P450 3A (increases in CYP450 content and 3A enzyme activities, increases in hepatic weight, thyroid changes).

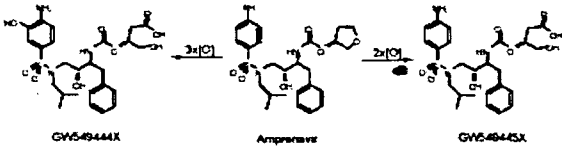


Figure 1. Major Excreted Metabolites of APV and GW433908 in Rats and Humans

3.3.6 Excretion

Hepatic clearance is the principal route of excretion for APV after administration of GW433908G. Approximately 14% of the dose in humans and 3 to 13% of the dose in rats and dogs is excreted in urine, with unchanged drug being a minor component in the urine in all cases. The majority of the dose (80% or greater) is excreted in feces, with amprenavir being a major component in dog feces, and a minor component in both rat and human feces. GW433908G metabolites were predominantly eliminated in feces in mice. Although many metabolites were not identified, the metabolite profiles in the excreta of mice were very similar after oral administration of either APV or GW433908G. Approximately 80% or greater of the administered dose was recovered in feces in mice. Excretion of APV accounted for approximately 11% of the dose in feces collected 0 to 8 hours post-dosing in mice. Excretion of unchanged GW433908X accounted for approximately 17% of the dose in feces. In rats and dogs, following oral administration of GW433908G, 17% and 28% of the dose was excreted as APV, respectively.

3.3.7 Pharmacokinetic drug interactions

The *in vitro* human plasma protein binding of GW433908G was 96% at clinically and toxicologically relevant concentrations, and decreased as concentrations of GW433908G increased and was approximately 90% at 5 µg/mL. Displacement of protein-bound GW433908G by APV was approximately 1% at APV concentrations of 1 and 10 µg/mL, respectively. The plasma protein binding of APV was reduced by up to 4% in the presence of the metabolites GW549445 and GW549444. Human plasma protein binding displacement interactions between APV and the selected antiviral drugs (ritonavir, delavirdine and efavirenz) were determined in vitro over a 10-fold concentration range, which included therapeutic concentrations (Report RD2001/00527/01). Displacement was greatest in the presence of 10 µg/mL ritonavir (approximately 6%), but APV displacement was seen with the other antivirals at between 2% and 5%. Decreases of 4% to 6% in APV protein binding at high concentrations of APV might be expected to have detectable effects on clinical pharmacokinetic parameter estimates. APV did not displace ritonavir from plasma proteins.

APV was highly bound to plasma proteins, especially α1-AAG, and differences in α1-AAG concentrations or changes in diseases that affect concentrations of α1-AAG could have an effect on the pharmacokinetics of APV. Metabolic interactions with APV are mostly likely to involve CYP3A. Drugs that affect or are affected by this enzyme have a potential to interact with APV. The extent and clinical importance of these interactions are likely to be similar to those seen with the marketed HIV PIs indinavir and nelfinavir.

Tables and figures to include comparative TK summary

Interspecies comparisons of systemic exposure for APV and GW433908X following oral dosing with GW433908G are presented in Table 1.1 and Table 1.2, respectively. The repeat-dose toxicokinetics of GW433908X and APV over a range of doses of GW433908A or GW433908G were investigated in toxicity

studies in mice, pregnant and non-pregnant rats, pregnant and non-pregnant rabbits, and dogs. All repeat dose studies used a BID dosing regimen, with the second portion of the daily dose given approximately 6 hours after the first to increase exposure to APV. The 6-hour interval was selected tofacilitate technical resource scheduling. Cmax and AUC to APV after repeat oral administration of GW433908A or GW433908G to mice, rats or dogs were generally dose-related, but not dose-proportional. In general, the exposure ratios of GW433908X to APV were 2% or less. Systemic exposure to APV and GW433908X is similar after oral dosing of pregnant and non-pregnant rats. The extent of exposure to GW433908X and APV in these nonclinical test species relative to clinically relevant exposures in humans is discussed in context with the safety data in the toxicology section of this summary.

Table 1.1. Amprenavir: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	APV Cmax (µg/mL)				APV AUC (h*µg/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Rat 4 week (RD1998/02573/00)	149	3.00	2.42	1.83	1.76	32.0	31.2	19.9	23.0
	478	5.61	7.12	3.48	4.30	62.4	83.1	33.8	49.9
	1493	9.60	11.1	5.38	4.89	224	133	47.2	66.1
	2240	11.3	10.4	4.54	5.38	184	234	53.1	80.8
Rat 6 month (RD1998/02858/01)	149	2.55	3.32	2.90	2.31	12.7	33.3	19.8	22.7
	478	7.54	8.52	5.09	5.23	84.4	74.1	46.2	54.3
	1493	9.70	13.1	5.33	7.28	154	237	57.0	82.3
	2240	8.57	9.85	5.28	7.68	243	253	54.9	107
Dog 4 week (RD1998/02606/00)	75	5.40	5.21	5.60	5.55	27.5	32.6	29.8	35.0
	194	10.4	10.7	10.4	12.2	86.4	87.7	68.2	56.8
	523	15.4	15.1	18.4	24.5	145	129	95.6	239
	747	15.5	16.5	31.5	23.4	133	123	209	156
Humans (Protocol APV20001)	58*	-		5.16		-		35.4	
Humans (APV10009 or APV10013)	1395 mg + RTV 200 mg	-		7.57		-		83.2	

Key:
‡ = AUC∞ on Day 1, AUC24 at steady state; ND = Not determined.

Table 1.1 (continued). Amprenavir: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	APV Cmax (µg/mL)				APV AUC (h*µg/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Dog 9 month† (RD1998/02861/01)	75	4.55	5.86	3.98	4.55	24.1	30.0	22.9	30.6
	194	11.7	8.22	14.4	20.5	64.2	62.6	113	143
	750/525/337	12.0	12.5	17.9	25.1	86.3	129	159	257
Rat organogenesis (RD1999/02690/00)	300	-	4.71	-	2.07	-	68.5	-	26.9
	820	-	7.84	-	3.55	-	126	-	43.2
	2240	-	8.52	-	5.94	-	229	-	57.1
Rabbit organogenesis (RD1999/01035/00)	74.8	-	0.01	-	0.22	-	0.03	-	1.81
	224.3	-	0.59	-	0.57	-	2.11	-	3.88
	672.8	-	4.16	-	3.33	-	22.2	-	25.8
Humans (APV20001)	1395 mg	-		5.16		-		35.4	
Humans (APV10009 or APV10010)	1395 mg + RTV 200 mg	-		7.57		-		83.2	

Key:
† = On Days 1 to 23 dogs were dosed with either 525 or 750 mg/kg/day. Due to severe intolerance, dosing was suspended on Day 24 and resumed on Day 29 with the two high doses combined and the dose reduced to 337 mg/kg/day.
‡ = AUC∞ on Day 1, AUC24 at steady state. ND = Not determined.

Table 1.2. GW433908X: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	GW433908X Cmax (µg/mL)				GW433908X AUC‡ (h*ug/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Rat 4 week (RD1998/02573/00)	149	2.15	0.438	0.144	0.010	15.3	3.18	0.546	0.067
	478	0.341	0.132	0.570	0.062	2.83	0.9931.77	1.65	0.636
	1493	0.146	0.488	0.276	0.281	1.57	1.77	0.786	1.25
	2240	0.122	0.174	0.147	0.420	1.42		1.20	1.58
Rat 6 month (RD1998/02858/01)	149	0.021	0.010	0.015	0.102	0.052	0.034	0.030	0.271
	478	0.036	0.132	0.032	0.381	0.156	0.299	0.229	3.27
	1493	0.050	0.069	0.102	0.184	1.58	4.72	0.930	1.00
	2240	0.047	0.124	0.117	0.143	0.616	1.26	1.08	1.82
Dog 4 week (RD1998/02605/00)	75	0.010	0.020	0.009	0.009	0.023	0.020	0.011	0.009
	194	0.016	0.147	0.034	0.053	0.041	0.365	0.049	0.059
	523	0.142	0.056	0.257	0.700	0.582	0.176	0.622	2.64
	747	0.187	0.542	0.783	0.890	0.386	1.37	1.64	2.21
Humans (APV20001)	1395 mg	-		0.024		-		0.062	
Humans (APV10010 and APV10009)	1395 mg + RTV 200 mg	-		0.015		-		0.024	

Key:
‡ = AUC∞ on Day 1, AUC24 at steady state; ND = Not determined.

Table 1.2 (continued). GW433908X: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	GW433908X Cmax (µg/mL)				GW433908X AUC‡ (h*µg/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Dog 9 month† (RD1998/02861/01)	75	0.012	0.013	0.058	0.041	0.038	0.039	0.028	0.048
	194	0.029	0.019	0.114	0.292	0.062	0.060	0.127	0.381
	750/525/337	0.301	0.134	0.375	0.850	0.627	0.335	0.556	1.63
Rat organogenesis (RD1999/02690/00)	300	-	0.013	-	0.038	-	0.055	-	0.330
	820	-	0.047	-	0.131	-	0.650	-	0.668
	2240	-	0.145	-	0.149	-	1.32	-	1.91
Rabbit organogenesis (RD1999/01035/00)	74.8	-	0.006	-	0.013	-	0.015	-	0.043
	224.3	-	0.017	-	0.034	-	0.069	-	0.158
	672.8	-	0.084	-	0.190	-	0.648	-	0.881
Humans (APV20001)	1395 mg	-		0.024		-		0.062	
Humans (APV10010 and APV10009)	1395 mg + RTV 200 mg	-		0.015		-		0.024	

Key:
† = On Days 1 to 23 dogs were dosed with either 525 or 750 mg/kg/day. Due to severe intolerance, dosing was suspended on Day 24 and resumed on Day 29 with the two high doses combined and the dose reduced to 337 mg/kg/day.
‡ = AUC∞ on Day 1, AUC24 at steady state. ND = Not determined.

Systemic exposure to APV, after oral administration of GW433908G to animals generally increased with increases in dose, but not dose-proportionally. In mice given doses of 400 to 3200 mg/kg/day GW433908G for 13 weeks (281 to 2250 mg/kg/day APV equivalents), the estimates for APV AUC_{24h} on Day 90 decreased compared to AUC estimates on Day 1 by 5 to 60%. In rats given GW433908G ranging from 50 to 2240 mg/kg/day (37.3 to 1500 mg/kg/day APV equivalents) in 4-week or 6-month toxicity studies), AUC values decreased by as much as 80% compared to Day 1 values following repeated administration. These decreases in bioavailability of APV, accompanied by increased liver weights, hepatocellular hypertrophy, increased thyroid weights and follicular cell hypertrophy in the treated animals, suggested enzyme autoinduction was a possible mechanism.

Systemic exposure to APV after oral administration of GW433908G to dogs did not decrease, but rather increased after the first dose and then generally stabilized during 4-week and 9-month studies. This increase in AUC over time was possibly due to saturation of metabolism in dogs after repeat dosing. APV exposure appeared to plateau between doses of 523 and 747 mg/kg/day GW433908G (350 and 500 mg/kg/day APV equivalents) in the 4-week toxicity study in dogs, and similar levels of exposure were achieved with 337 mg/kg/day GW433908G (225 mg/kg/day APV equivalents) in the 9-month toxicity study.

Exposure to APV after GW433908G dosing in rats was approximately 50% lower than in repeat-dose studies with APV at equivalent APV doses. Systemic exposure to GW433908X after oral administration of GW433908G generally increased, but not proportionally, with increasing dose in mice, rats and dogs. Plasma concentrations of GW433908X were highly variable; no consistent pattern of decrease or increase in systemic exposure to GW433908X was observed over time (4-weeks to 9 months). In general, the exposure ratios of GW433908X to APV were 2% or less. Systemic exposure to GW433908X and APV was similar after oral dosing of pregnant and non-pregnant rats or rabbits. In rabbits, GW433908X to APV exposure ratios were generally >3% indicating conversion of GW433908X to APV may be less efficient in the rabbit than other animals. Plasma concentrations of GW433908X and APV were higher after oral administration in neonatal/juvenile rats compared to sexually mature rats.

Pharmacokinetics Studies

12. Study 98AVV0018 – Disposition and metabolic profiling in wistar hannover rats after oral administration of the calcium salt of [^{14}C] GW433908 (Report No. RD2002/00725/00)

[redacted] Study No.: AFAR-113: [redacted] Final Report: AFAR-113-98-383; Conducting facility [redacted], Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709; Date Initiation: October 6, 1998; GLP Compliance: No (X); Drug Lot No.: R2826/128/2, purity [redacted] Radioactive Drug Lot No.: R3877/37/1, purity [redacted], sp. act. 18.2 µCi/mg, uniformly labelled in the aniline ring

Method

Eleven fasted male Wistar Hannover rats (age: 7 weeks; approximate weight: 0.214-0.247 kg; 4 rats/group) received a single oral dose (110 mg/kg) of [^{14}C] GW433908G in 0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Tween-80 by gavage. Blood, urine and feces samples (0.5 mL) were collected for analysis. Amprenavir and GW433908X concentrations in plasma were determined by [redacted] Fecal and urine samples (0 to 24 hours and 24 to 48 hours) were extracted and profiled by using [redacted]. A representative urine pool and a fecal homogenate pool extract were analyzed by [redacted] to identify the major metabolites of GW433908 in the rat. [redacted] analysis was also used to further identify drug-related material in the representative samples.

Results

The estimate of C_{max} for GW433908X after oral administration of 110 mg/kg [^{14}C]GW433908G to male Wistar Hannover rats was 12.5 ng/mL, and the estimate of T_{max} was 0.5 hours. The estimated AUC_{24h} for GW433908X was 28.6 hr•ng/mL. The exposure ratio of GW433908X to amprenavir was 0.07%. The recovery of radiocarbon in the excreta of male Wistar Hannover rats after oral administration of 110 mg/kg [^{14}C]GW433908G showed that the majority of the dose recovered in the feces and urine was excreted within 24 hours post-dose (87.4% and 2.5%, respectively). Unchanged drug (GW433908X) was not detected in feces extract samples. The percentage of the dose that was eliminated as amprenavir in the

feces was 17.1%. All metabolites were present in feces in quantities less than or equal to 8% of the dose, except for GW549445 and GW549444, which accounted for approximately 18% and 23% of the dose, respectively. After oral administration of [^{14}C]amprenavir to Wistar Hannover rats, the percentages of the dose eliminated as GW549445 and GW549444 were 48% and 6%, respectively. All other identified metabolites present in feces after oral administration of [^{14}C]GW433908G were also seen after oral administration of [^{14}C]-amprenavir. All urine metabolites were present in quantities less than 1.0% of the dose. All identified metabolites present in urine after oral [^{14}C]-GW433908G dosing were also seen after oral administration of [^{14}C]-amprenavir.

Comments

Note that similar metabolite profiles were observed in feces extracts and urine after oral administration of [14C]-GW433908G compared to metabolite profiles after oral administration of [14C]-amprenavir (Re: RD1998/00070/00).

13. Disposition and metabolic profiling in beagle dogs after oral administration of the calcium salt of [^{14}C] GW433908 (Report No.RD2002/00724/00)

GW Study No.: 98AVV0017; [redacted] Study No.: AFAR-113; [redacted] Final Report: AFAR-112-98-386; Conducting facility: [redacted], Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709; Date Initiation: October 6, 1998; GLP Compliance: No (X); Drug reference No.: GW433908G; Drug Lot No.: Batch R2826/128/2, purity [redacted] Radioactive Drug reference No.: [^{14}C]GW433908G, GW433908J; Radioactive Drug Lot No.: R3877/37/1, purity > [redacted] sp. act. 18.2 µCi/mg, uniformly labelled in the aniline ring

Method

Three male beagle dogs (9.4 to 12.6 kg) fasted overnight received a single oral dose (24 mg/kg) of [^{14}C]GW433908G in 0.1 N HCl by gavage. Blood samples (2.5 mL/sample) were collected prior to dose administration, and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose. Plasma samples were stored for analysis. Urine and feces were collected pre-dose and post-dose at 24-hour intervals up to 168 hours post-dose. Amprenavir and GW433908X concentrations in plasma were determined by [redacted]. Metabolite profiling and identification in dog fecal and urine samples was performed. All samples were profiled by using [redacted] monitoring carbon-14 related material. After [redacted], urine and fecal extract sampels were analyzed using an [redacted] method for metabolite identification.

Results

The mean estimate of C_{max} for amprenavir was 3.66 µg/mL, and estimates of Tmax ranged between 0.5 and 1.0 hour. The mean estimated AUC_{24h} for amprenavir was 8.02 hr•µg/mL. The mean estimate of C_{max} for total radiocarbon was 6.06 µg/mL, and estimates of T_{max} ranged between 0.5 and 1.0 hour. The proportion of amprenavir to radiocarbon at C_{max} was approximately 60%. The mean estimated AUC_{24}h for total radiocarbon was 34.2 hr•µg/mL, and the proportion of exposure of amprenavir to total radiocarbon was approximately 24%. The elimination of amprenavir and total radiocarbon was similar through 3 hours post-dose. A similar exposure ratio of 27% between amprenavir and total radiocarbon after oral administration of approximately 25 mg/kg [^{14}C]-amprenavir has been shown previously (RD1996/00289/00). The mean estimate of C_{max} for GW433908X after oral administration of approximately 24 mg/kg [^{14}C]GW433908G to male beagle dogs was 88.0 ng/mL, and estimates of T_{max} were 0.5 hour. The mean estimated AUC_{24h} for GW433908X was 55.4 hr•ng/mL. The exposure ratio of GW433908X to amprenavir was 0.13%.

After oral administration of approximately 24 mg/kg [^{14}C]GW433908G, 95.0% of the dose was recovered, with 80.0% of the recovered dose in the feces and 13.0% in the urine. The majority of the dose recovered in the feces and urine was excreted within 48 hours post-dose (77.5% and 12.5%, respectively).

The results indicate that metabolism and excretion of amprenavir is similar after oral administration of amprenavir or GW433908G in beagle dogs. Unchanged drug (GW433908X) was not detected in feces. All metabolites were present in feces in quantities less than or equal to 11% of the dose, except one metabolite, BD/8064/106/1, that accounted for approximately 50% of the dose for dog #1002. This

metabolite is a result of mono-oxidation of amprenavir, most likely at the aliphatic region of the molecule. The predominance of this metabolite in this sample is suspected to be the result of microbial contamination during sample handling. The percentage of the dose that was eliminated as amprenavir in the feces was 27.3%. Note that after oral administration of [^{14}C]-amprenavir to beagle dogs, the percentage of the dose eliminated as amprenavir was 52% (RD1996/00289/00). All other identified metabolites present in feces, after oral dosing of [^{14}C]-amprenavir, were also seen after oral administration of [^{14}C]-amprenavir to beagle dogs.

Quantities of amprenavir in urine were less than 0.5% of the dose. All urine metabolites were present in quantities less than 2.0% of the dose. All identified metabolites present in urine were seen in dogs after oral administration of [^{14}C]-amprenavir. Note that GW433908X was seen in the urine sample for dog #1001, which was most likely due to contamination of the urine with vomitus.

14. Study 98APK0034 - Pharmacokinetics and relative bioavailability of the free acid and various salts of GW433908 in male beagle dogs Report No. RD1998/03011/01)

IND No.: 58627; Serial No.: 122; Vol. No.: 6 of 6; Page 134; GW report No.: RD1998/03011/01; GW study No.: 98APK0034; Conducting facility: , Glaxo Wellcome Inc.; Date Initiation: 9 April 1998; GLP Compliance: N/A; Drug: GW433908A, GW433908G; GW433908X Formulation: GW433908A soft gel capsules; GW433908G tablets, and GW433908X soft gel capsules

Methods

Three male Beagle dogs (body weight: 10-14 kg; , were used in this study. Each dog received each of the GW433908 formulations with at least 7 days washout period between doses. Dogs were dosed with 360 mg/dog GW433908X and 250 mg/dose433908A orally in hand-filled soft gel capsules. GW433908G was administered in tablets at a dose of 418 mg/dog, either alone or with a 100 mL gavage of 0.05 N HCl 15 to 30 minutes prior to tablet administration. Finally, GW433908G was administered in tablets with citric acid at a dose of 434 mg/dog. Following each administration, blood samples were collected predose and at 0.25 to 24 hours postdose for pharmacokinetic analysis. Plasma concentrations of GW433908X and amprenavir were measured by ,nethods.

Results

Relative bioavailability compared to APV was calculated with reference to an APV AUC value determined following oral dosing in a previous study (Report RD1998/00328/00 in NDA 21-007) and was summarized in Table 2

Table 2. Pharmacokinetic parameters following single oral administration of GW433908G to dogs

Formulation	Dose (APV equivalents) *mg/dog*	Pharmacokinetic parameters				
		AUC_{24h} (µg•h/mL)	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	Relative Bioavailability (%)
APV	**300**	**26.2**	**7.23**	**1-2**	**3.4**	**100**
GW433908X	**360 (311)**	**16.8**	**3.28**	**1-6**	**3.0**	**64**
GW433980A	**250 (201)**	**20.3**	**7.68**	**2-3**	**1.9**	**82**
GW433908G	**418(294)**	**6.94**	**1.43**	**1-4**	**3.6**	**24**
GW433908+HCL	**418(294)**	**15.8**	**4.48**	**2-4**	**1.4**	**59**
GW433908G+Citric acid	**434(305)**	**7.94**	**2.37**	**0.5-2**	**2.8**	**29**

Pharmacokinetics and relative bioavailability of GW433908G liquid formulations after single oral doses to beagle dogs (Report No. RD1999/00927/00)

Study No.: Study No. 99APK0030; Conducting facility: DMPK, Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park (RTP), North Carolina 27709; Date Initiation: May 11, 1999; GLP Compliance: No (X); Drug reference and Lot No.: Unknown; Formulation: GW433908G suspensions (Batch Nos. 15300-046 and 15300-050), and amprenavir CTM 150 mg soft-gel capsules (Lot No. 034125), and oral solution (Lot No. 10505784). Suspension Batch No. 15300-046 contained and Batch No. 15300-050 contained . Both suspension batches contained equivalent amounts of GW433908G (GW433908X - 46.4 mg/mL).

Amprenavir CTM (soft-gel capsules) was supplied as 150-mg amprenavir formulated in Vitamin E-

TPGS, PEG 400, and propylene glycol. Amprenavir CTM oral solution, 15 mg/mL, was formulated in propylene glycol, PEG 400, and Vitamin E-TPGS, and

Method

The pharmacokinetics of amprenavir was determined after oral administration of two GW433908G suspension formulations (with and without and two amprenavir clinical trial material (CTM) formulations to beagle dogs. Three male beagle dogs (weighing 9 to 12 kg) received GW433908G in each of the two suspension formulations that contained approximately an 800-mg amprenavir-equivalent dose, administered by gavage. A 50-mL portion of 0.1 N HCl solution was administered to each dog by gavage prior to dosing the GW433908G suspension formulations. Each dog also received two 150 mg soft-gel amprenavir capsules and 20 mL of the amprenavir oral solution (300 mg amprenavir) as control arms for the study. There was a 7-day washout period between doses. Blood samples (2.5 mL) were taken at 0 (predose), 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12, and 24 hours after the dose. Whole blood and plasma samples were prepaired and transferred to for analysis by

Results

The pharmacokinetic parameters are shown (Table). Dose-adjusted estimates of C_{max} after oral administration of GW433908G suspensions with and without were 30 to 40% lower than estimates of C_{max} after oral administration of amprenavir soft-gel capsules and solution. Estimates of Tmax were similar across all formulations, and estimates of $t_{1/2}$ were similar between the GW433908G suspensions and increased after administration of amprenavir soft-gel capsules. Dose-adjusted estimates of AUC after oral administration of GW433908G suspensions with and without were similar to AUC estimates after administration of amprenavir soft-gel capsule and solution formulations.

Pharmacokinetic Parameters of Amprenavir

Formulation	Amprenavir Dose (mg)		AUC_{∞} (µg*h/mL)	C_{max} (µg/mL)	T_{max} (h)	$t_{½}$ (h)
GW433908G with [redacted]	800	Mean	23.9	4.86	2.67	3.24
		SD	19.1	3.02	1.53	0.39
GW433908G without [redacted]	800	Mean	21.1	5.07	2.67	2.42
		SD	7.58	2.24	1.53	0.36
Amprenavir CTM soft-gel capsules	300	Mean	21.8	6.94	2.00	5.66
		SD	5.87	2.02	0	5.14
Amprenavir CTM oral solution	300	Mean	21.2	7.72	1.00	9.61
		SD	9.92	3.46	0	1.95

n = 3 males
1. values adjusted to a 300 mg-equivalent dose of amprenavir

Comments

These results indicated that or) in the GW433908G suspensions had no effect on the pharmacokinetic parameter estimates of amprenavir, and that systemic exposure to amprenavir was similar after administration of the GW433908G suspensions and the amprenavir formulations.

16. Determination of human plasma protein binding interaction between GW433908G and Amprenavir (141W94) (Report No. RD2001/01671/00)

Study No.: Study No. 01AVT0028; Conducting facility: DMPK, Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park (RTP), North Carolina 27709: Date Initiation: December 18, 2001; GLP Compliance: N/A; Drug reference and Lot No.: Amprenavir (141W94, AWS 2159), purity: [redacted] [^{14}C]GW433908G (GW433908J, R6834/181/5, radioactive purity: > [redacted] uniformly labelled in the aniline ring)

Method

The extent of human plasma protein binding of GW433908G (calcium salt), and the displacement of GW433908G by amprenavir were investigated in this study. Plasma samples spiked with [^{14}C]GW433908G (final concentrations: 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 µg/mL) were incubated at 37°C for

15 minutes in the absence or presence of amprenavir, free compound was separated from protein-bound compound by ultrafiltration ([redacted] devices, [redacted] and concentrations of free and total radiolabelled compound were determined by using liquid scintillation counting. For studies of APV protein binding displacement of GW433908G, spiked plasma samples (0.1 and 1.0 µg/mL of GW433908G) were incubated at 37°C for 15 minutes prior to addition of APV (1.0 or 10 µg/mL), followed by an additional incubation at 37°C for 30 minutes prior to ultrafiltration. The percentages of unbound (free) and bound drug were calculated as follows: % unbound (free) = [(dpm/mL in filtrate)/(dpm/mL in plasma)] x 100%; bound % = (100 % - % unbound).

Results

Plasma protein-binding of [^{14}C]GW433908G was approximately 96% at 0.2 µg/mL, approximately 92% between 0.5 and 2 µg/mL GW433908G, and approximately 90% at 5 µg/mL GW433908G. Plasma protein-binding of [^{14}C]GW433908G at 0.1 µg/mL could not be determined due to analytical limits of quantitation. Displacement of protein-bound [^{14}C]GW433908G at the high concentration (1µg/mL) was not observed with 1µg/mL amprenavir, although at 10 µg/mL the presence of amprenavir caused a decrease in the plasma protein binding of [^{14}C]GW433908G of approximately 1%.

Comments

The effect of GW433908 plasma protein binding is negligible, because plasma concentrations of GW433908X (free acid) in clinical studies are generally no more than 1% of amprenavir plasma concentrations, and generally less than 0.1 µg/mL,

17. Determination of human plasma protein binding interaction between Amprenavir (141W94) and the Amprenavir metabolites GW549445X and GW549444A (Report No. RD2001/00984/00)

Study No.: 01AVT0013; Conducting facility: DMPK, Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park (RTP), North Carolina 27709; Date Initiation: December 18, 2001; GLP Compliance: N/A; Drug reference and Lot No.: Amprenavir (141W94, AWS 2159, purity: [redacted] [^{14}C]GW549445X (R5547/143/7, purity: 90..5%); GW549444A (R6755/20/12, purity: 97.6%) and [^{14}C]GI268188 (141W94, 17704/14/1, radioactive purity: [redacted]; Radiolabelled amprenavir ([uC]GI268188, 1 µCi/µL, 2.5 µL) was combined with normal pooled human plasma (HMPLCIT-M, Lot #BRH01941, [redacted]

Method

The extent of human plasma protein binding displacement of radiolabelled amprenavir by amprenavir metabolites GW549445X and GW549444A was determined in this study. Radiolabelled samples were incubated at 37°C for 30 minutes in the absence or presence of displacer, followed by an additional incubation at 37°C for 30 minutes prior to ultrafiltration ([redacted] devices; [redacted] Free compound was separated from protein-bound compound by ultrafiltration, and concentrations of free and total radiolabelled compound were determined by liquid scintillation counting. The percentages of unbound (free) and bound drug were calculated as follows: % unbound (free) = [(dpm/mL in filtrate)/(dpm/mL in plasma)] x 100; % bound = 100% - % unbound.

Results

Results of protein binding studies to examine displacement of radiolabelled amprenavir (1.0 and 10 µg/mL) by GW549445X and GW549444A indicated that the binding of radiolabelled amprenavir in the absence of other compounds was at least 91%, and similar to estimates from previous protein binding studies with radiolabelled amprenavir (RD1996/00128/00, RD1997/01807/00). Plasma with 1.0 µg/mL [^{14}C]amprenavir was 92.4% protein-bound, and had binding values of 90.0% to 90.7% in the presence of GW549445X and GW549444A (decreased approximately 2%). Plasma with 10 µg/mL [^{14}C]amprenavir was 90.9% protein-bound, and had binding values of 86.8% to 87.6% in the presence of GW549445X and GW549444A.

Comments

Decreases of 2% in amprenavir protein binding at 10 µg/mL of amprenavir might have detectable effects on clinical pharmacokinetic parameter estimates.

18. Study 02APK0018 – The pharmacokinetics of GW549445X and GW549444X in rats, dogs and humans following oral administration of Amprenavir or GW433908G (Report No.RD2002/00576/00)

Conducting facility: [redacted]t, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park (RTP), North Carolina 27709; GLP Compliance: N/A; Initiation Date: 5/3/02

Method

The concentration-time profile and pharmacokinetic parameter estimates for amprenavir metabolites GW549445X and GW549444X after administration of amprenavir or GW433908G in male and female Wistar Hannover rats and beagle dogs, and male and female volunteers and HIV positive patients were investigated in this study (RD2002/00576/00). Samples were selected from studies R40427, D40418, ESS40006, and APV10008. These were completed studies with plasma/serum samples collected after oral administration of amprenavir soft-gel capsules (co-administered with 100 mg ritonavir) or GW433908G (the calcium salt of the phosphate prodrug of amprenavir) tablets or suspension. Rat plasma samples were obtained on Day 1 and 23 from male and female Wistar Hannover rats administered 1493 or 2240 mg/kg/day GW433908G (1000 or 1500 mg/kg/day amprenavir) by oral gavage for one month (Study R40427). Dog plasma samples were obtained on Day 95 from male and female beagle dogs administered 337 mg/kg/day GW433908G (225 mg/kg/day amprenavir) by oral gavage for nine months (Study D40418). Human serum samples were obtained during Weeks 24, 32, or 40 from HIV-positive patients administered 600 and 900 mg amprenavir/100 mg ritonavir BID by oral administration (Study ESS40006). Human plasma samples were obtained from male and female volunteers administered oral doses of 1400 mg GW433908G (1200 mg amprenavir) as tablets or suspension (Study APV10008). Concentrations of GW549445X and GW549444X were determined in plasma/serum by a validated [redacted] method with [redacted] The validated calibration range for this method was [redacted] in human plasma and [redacted] in rat and dog plasma with a sample volume of 250 µL.

Results

Plasma concentrations of GW549445X were detectable in rats, dogs and humans. No gender-related differences were seen in plasma concentrations of GW549445X. AUC estimates for GW549445X in male and female Wistar Hannover rats decreased slightly between Day 1 and Day 23. Due to the amprenavir AUC estimates decreased over this period, the AUC ratio (GW549445X AUC/amprenavir AUC) increased approximately twofold. Steady-state GW549445X exposure ratios in dogs on Day 95 were approximately tenfold lower compared to steady-state exposure in rats on Day 23. Estimates of GW549445X exposure ratios in humans administered amprenavir or GW433908G were similar regardless of single dose or multiple dose (steady-state). Plasma concentrations of GW549444X in rats were measurable occasionally, but estimates of AUC could not be determined. Plasma concentrations of GW549444X in dogs were below the lower limit of quantitation established for the method. Plasma concentrations were measurable in humans, and exposure to GW549444X in humans was 75% to 95% less than GW549445X exposure. These data provide evidence of exposure to and pharmacokinetic estimates of GW549445X in rats, dogs and humans and exposure to and pharmacokinetic estimates of GW549444X in humans in this study. Pharmacokinetics parameters of amprenavir, GW549444 and GW54445X in rats, dogs and humans were summarized in the following tables.

Pharmacokinetic Parameters of Amprenavir in Rats, Dogs, and Humans

Species	Sex	GW433908G (mg/kg/day)	Amprenavir[1] (mg/kg/day)	Day (Week)	C_{max} (ng/mL)	T_{max} (h)	AUC_{tau} (ng*h/mL)
Rat	M	1493	1000	1	9600	2	224000
	F	1493	1000	1	11100	1	133000
Rat	M	1493	1000	23	5380	1	47200
	F	1493	1000	23	4890	4	68100
Rat	M	2240	1500	1	11300	2	184000
	F	2240	1500	1	10400	6	234000
Rat	M	2240	1500	23	4540	6	53100
	F	2240	1500	23	5380	1	80900
Dog	M	337	225	95	21840 ± 5791	1.6 ± 0.55	240000 ± 112446
	F	337	225	95	21960 ± 4869	1.4 ± 0.55	222000 ± 45380
M/F	M/F	ND[2]	600 or 900 mg BID[3]	(24, 32, 40)	14623 ± 3309	1.35 ± 0.84	64143 ± 26434
M/F	M/F	1400 mg/day QD	1200 mg QD	1	7281 ± 2878	1.25 ± 0.50	23304 ± 10574

1. Amprenavir or amprenavir equivalents based on GW433908G dose
2. Not determined
3. Co-administered with 100 mg ritonavir BID

Pharmacokinetic Parameters of GW549444X in Rats, Dogs, and Humans

Species	Sex	GW433908G (mg/kg/day)	Amprenavir[1] (mg/kg/day)	Day (Week)	C_{max} (ng/mL)	T_{max} (h)	AUC_{12hr} (ng*h/mL)	AUC Ratio[2] (%)
Rat	M	1493	1000	1	12.5	18	ND	ND
	F	1493	1000	1	BQL[3]	ND[4]	ND	ND
	M	1493	1000	23	BQL	ND	ND	ND
	F	1493	1000	23	10.8	1	ND	ND
	M	2240	1500	1	12.4	18	ND	ND
	F	2240	1500	1	16.2	18	ND	ND
	M	2240	1500	23	BQL	ND	ND	ND
	F	2240	1500	23	BQL	ND	ND	ND
Dog	M	337	225	95	BQL	ND	ND	ND
	F	337	225	95	BQL	ND	ND	ND
Human	M/F	ND	600 or 900 mg BID[5]	(24, 32, or 40)	22.4 ± 19.0	0.75 ± 0.25	173 ± 210	0.18 ± 0.18
	M/F	1200 mg/day QD	1200 mg/day QD	1	42.6 ± 16.3	1.40 ± 0.42	189 ± 90.7	1.03 ± 0.82

1. Amprenavir or amprenavir equivalents based on GW433908G dose
2. AUC ratio = (GW549444X AUC/amprenavir AUC) * 100
3. Below the lower limit of quantitation [redacted] for rat and dog plasma)
4. Not determined
5. Co-administered with 100 mg ritonavir BID

Pharmacokinetic Parameters of GW549445X in Rats, Dogs, and Humans

Species	Sex	GW433908G (mg/kg/day)	Amprenavir[1] (mg/kg/day)	Day (Week)	C_{max} (ng/mL)	T_{max} (h)	AUC_{12hr} (ng*h/mL)	AUC Ratio[2] (%)
Rat	M	1493	1000	1	198	2	3161	1.41
	F	1493	1000	1	79.2	4	1359	1.02
	M	1493	1000	23	118	1	1362	2.89
	F	1493	1000	23	107	4	1498	2.20
	M	2240	1500	1	176	2	2950	1.82
	F	2240	1500	1	165	18	2211	0.94
	M	2240	1500	23	104	2	1453	2.74
	F	2240	1500	23	109	0 (18)	2012	2.49
Dog	M	337	225	95	53.8 ± 37.9	2	605 ± 416	0.24 ± 0.06
	F	337	225	95	42.6 ± 10.4	2.20 ± 1.10	449 ± 149	0.21 ± 0.07
Human	M/F	ND[3]	600 or 900 mg BID[3]	(24, 32, or 40)	582 ± 677	1.25 ± 0.50	2673 ± 4237	3.22 ± 3.71
	M/F	1400 mg/day QD	1200 mg/day QD	1	282 ± 86.2	1.50 ± 0.50	750 ± 181	4.03 ± 2.27

1. Amprenavir or amprenavir equivalents based on GW433908G dose
2. AUC ratio = (GW549445X AUC/amprenavir AUC) * 100
3. Co-administered with 100 mg ritonavir BID

19. Study 98APK0135 - Pharmacokinetic study after oral administration of GW433908G to portal vein-cannulated han wistar rats and a beagle dog (Report No. RD1998/02935/01)

IND No.: 58627; Serial No.: 122; Vol. No.: 6 of 6; Page 121; GW report No.: RD1998/02935/01; GW study No.: 98APK0135; Conducting facility: [redacted], Glaxo Wellcome Inc.; Date Initiation: 14 January 1999; GLP Compliance: N/A; Drug reference No.: GW433908G; Drug Lot: 4286/88/8, 98.5% purity, 81.5% GW433908X); Formulation: GW433908G suspension (28 mg/mL) in 0.5% HPMC (hydroxypropylmethyl cellulose) with 0.1% Tween®80

Methods

Overnight fasted male portal vein cannulated Han Wistar rats (n = 7; body weight: 220-260 g; [redacted] were orally administered by gavage a single dose of GW433908G (112 mg/kg, 4mL/kg). One male portal vein cannulated Beagle dog (body weight: 12.4 kg; [redacted] was orally administered by gavage a single dose of GW433908G (35 mg/kg, 1.25 mL/kg), preceded by a 100 mL of 0.05N HCL. Blood samples from rats (0.5 mL) were collected from portal cannulated vein predose and at 0.25, 0.5, 1,2, 3, 4, 6, 8, and 12 hours postdose. . Blood samples from the dog (1.8 mL) were collected from portal cannulated vein predose and at 0.25, 0.5, 1,2, 3, 4, 6, 8, 12 and 24 hours postdose. Plasma concentrations of GW433908X and amprenavir were measured by an [redacted] method.

Results

Plasma concentrations of APV and GW433908X in the portal vein samples indicated that GW433908x was absorbed quickly and converted to APV (T_{max} for rats and dogs were 0.5 to 2 hours and 0.25 to 0.5 hours, respectively). Concentration ratios of GW433908X to APV were 2.2% and 2.5% in rats and dogs, respectively. Exposure ratios of GW433908X to APV were approximately 0.3% and 0.85 in rats and dogs, respectively (Table 1).

Table 1. Pharmacokinetic parameters following single oral administration of GW433908G to portal vein cannulated rats

GW433908G (mg/kg)	Analyte	Pharmacokinetic parameters				
		AUC_{24h} (µg•h/mL)	C_{max} (µg/mL)	T_{max}	$T_{1/2}$ (h)	AUC ratio* (%)
Rat						
112	APV	44.6	7.0	2.0	1.6	
	GW433908X	0.156	0.064	0.5	--	0.30
Dog						
35	APV	8.6	6.0	0.5	0.5	
	GW433908X	0.085	0.120	0.25	0.4	0.85

*GW433908X:APV

Comments

The hepatic first pass metabolism contributed to the conversion of GW433908X to amprenavir and reduced the potential systemic exposure to GW433908X in rats and dogs.

20. Study M40725 - The enzyme induction of GW433908G in the CD-1 mouse following oral administration of GW433908G during a 13-week pilot carcinogenicity study (Report No. RD2002/00646/00)

Conducting facility: [redacted] GLP Compliance: N/A

Method

Mice received repeated daily oral doses of GW433908G for 13 weeks. Microsomes were obtained from two groups (vehicle treated controls and those treated with 3200 mg GW433908G/kg/day) of male and female mice. Livers were removed at terminal necropsy and frozen for subsequent analysis (RD2002/00646/00). Microsomes were prepared from pools of 3 male or 3 female control livers and from 7 individual livers from the treated group, and assayed for protein and cytochrome P450 (CYP) content, and the activities of CYP1A, CYP2B and CYP3A.

Results

CYP3A activity was increased 2.9-fold in treated male mice and 4.6-fold in treated female mice Tables 1 and 2). An effect on cytochrome P450 content or CYP 1A or CYP 2B mediated activities could not be demonstrated in this study. Thus, GW433908G is considered as an inducer of CYP3A in CD-1 mice.

Table 1

P450 Content and Enzyme Activities Following 13-Week Repeat Oral Administration of GW433908G to Mice

Group Code	Group		P450 Concentration[a]	Ethyresorufin Dealkylase Activity[b]	Pentoxyresorufin Dealkylase Activity[c]	6β-testosterone Hydroxylase Activity[d]
Pooled Male Controls	Male	Control	1.01	17.2	7.33	1.97
5M		3200 mg/kg/day	1.02 ± 0.21	15.5 ± 9.34	4.34 ± 0.93	5.68 ± 1.62
Pooled Female Controls	Female	Control	0.73	24.8	3.68	1.49
5F		3200 mg/kg/day	1.00 ± 0.21	17.3 ± 7.17	5.53 ± 3.99	6.86 ± 1.74

[a] nmol/mg protein; P450 was determined by the carbon monoxide difference spectrum.
[b] pmol/mg protein/min, a marker for 1A activity
[c] pmol/mg protein/min, a marker for 2B activity
[d] nmol/mg protein/min, a marker for 3A activity

Table 2

Individual Mouse P450 Content and Enzyme Activities Following 13-Week Repeat Oral Administration of GW433908G

Group Code	Treatment	Mouse Number	P450 Concentration[a]	Ethylresorufin Deallkylase Activity[b]	Pentoxyresorufin Deallkylase Activity[c]	6β-testosterone Hydroxylase Activity[d]
Pooled Males	Control	97				
5M	3200 mg/kg/day	43				
		48				
		49				
		58				
		62				
		65				
		67				
Pooled Females	Control	92				
5F	3200 mg/kg/day	38				
		41				
		44				
		46				
		51				
		56				
		61				

[a] nmol/mg protein, P450 was determined by the carbon monoxide difference spectrum.
[b] pmol/mg protein min, a marker for 1A activity.
[c] pmol/mg protein min, a marker for 2B activity
[d] nmol/mg protein min, a marker for 3A activity

21. Study 99 AVV0024 - rHuCYP3A4-Like immunoreactivity in rat liver microsomes from 3-month Amprenavir (TOX771) and 1-month GW433908G (R40427) toxicology studies (Report No. RD1999/02460/02)

Conducting facility: DMPK, Development, GlaxoSmithKline, US Research and Development, 3030 Cornwallis Road, Research Triangle Park (RTP), North Carolina 27709; GLP Compliance: N/A

Method

The amount of recombinant human CYP3A4-like immunoreactivity (rHuCYP3A4-LI) in liver microsomes from rats in repeat dose toxicity studies was determined in this study. Rat liver microsome preparations were obtained from studies in Han Wistar rats given amprenavir (TOX771) at 50, 160, or 500 mg/kg/day for 3 months or GW433908 (R40427) at 149, 478, 1493, and 2240 mg/kg/day (100, 320, 1000, 1500 mg amprenavir-equivalents /kg/day, respectively) for 1 month. A fluorescence immunoassay was used to determine the amount of rHuCYP3A4-LI in TOX771 or R40427 rat liver microsome samples.

Results

In this study, the data show that CYP3A4-LI levels were increased in samples from amprenavir-treated rats and that levels of CYP3A4 increased with increasing amprenavir dose (Table 1).

Table 1 Averaged Concentrations of rHuCYP3A4-LI in Rat Liver Microsomes from TOX771 [TBZZ/96/0004/00]

	µg rHuCYP3A4-LI/mg microsomal protein ± Std	
Amprenavir Dose (mg/kg/day)	Male	Female
vehicle	BQL*	BQL
water	BQL	BQL
50	0.47	0.35
160	3.67 ± 0.77	4.78 ± 1.46
500	7.97 ± 1.39	9.55 ± 2.52

* = Below the lower limit of quantitation for the assay

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

The sponsor conducted oral single dose (mouse and rat) and intravenous (mouse and rat) studies, and oral repeat dose studies of up to 6 months duration (rat) and 9 months (dog) with GW433908G or GW433908A. GW433908G has been evaluated for potential genotoxicity both *in vitro* and *in vivo*. GW433908G has been evaluated for reproductive and developmental toxicities in the rat and rabbit. Carcinogenicity studies in rats and mice with GW433908G are currently being carried out and final reports will be available in 2006. Note that carcinogenicity studies for APV have been complete and the data have been reviewed recently by the division (Reference Reports RD1998/02066/01 and RD1998/01521/01). A range of special toxicity studies has also investigated for eye and dermal irritancy in the rabbit, and potential sensitisation in the guinea pig. Potential drug substance impurities of GW443908G have been evaluated for potential mutagenicity and for toxicity in the rat and dog. The intravenous single dose studies were all conducted with GW433908A because of its high solubility in saline. Intravenous studies were not performed with GW433908G. All definitive studies were performed in compliance with Good Laboratory Practice (GLP) regulations. Note that the toxicity profile of GW433908G is essentially identical to APV and APV in the clinic with ritonavir, and no significant liver toxicity has been noted with or without ritonavir co administration. Thus, the toxicity of GW433908G in combination with ritonavir (or other compounds) was not evaluated in pre-clinical animal studies.

The oral route was used for the majority of toxicity studies because this is the proposed clinical route of administration. The majority of animal studies were conducted with twice daily administration (BID) to increase systemic exposure to APV. Total daily doses, expressed as mg/kg/day of GW433908G, were divided in half and administered approximately 6 hours apart. The definitive studies with GW433908G in adult and neonatal/juvenile animals used a standard vehicle for preparing suspensions that contained 0.5% w/w hydroxypropylmethylcellulose (HPMC) and 0.1% w/w Tween® 80. In the 4-week and 9-month studies in dogs, a dilute HCl flush of the gavage tube was incorporated into the dosing procedure to ensure maximal bioavailability of APV. General toxicology studies with GW433908A or GW433908G in rodents used Wistar Hannover rats or CD-1® mice, and studies in non-rodents were performed in beagle dogs. Reproductive toxicity was assessed using CD® rats and New Zealand white rabbits. The general toxicology rat studies were conducted in Wistar Hannover rats. The strain of CD rats was used in the reproductive toxicity studies as Wistar Hannover rats have a relatively small average litter size and reproductive outcome and fetal development of CD® rats are well-documented in US laboratories.

The overall toxicity profile of GW433908G was similar to the well-established toxicity profile of APV. GW433908G has a low order of oral, single dose toxicity. The Maximum Non-Lethal Dose (MNLD) was >2000 mg/kg (highest dose tested) for the disodium salt (GW433908A) and >2986 mg/kg (highest dose tested) for GW433908G in both mice and rats. Gastrointestinal intolerance in dogs, consisting of salivation, vomiting and fecal alterations (soft to liquid feces), occurred consistently throughout all of the repeat dose studies with GW433908G. This led to dehydration, electrolyte loss and deterioration to moribund condition in a number of animals. However, intravenous administration of GW433908A caused animal deaths and clinical signs at much lower doses, including decreased activity, ataxia and changes in breathing patterns (mice: i.v. ≥217mg/kg; rats: female rats: i.v. ≥ 217mg/kg; male rats: i.v.≥ 347mg/kg).

The nonclinical toxicological findings with GW433908G include: (1) gastrointestinal intolerance (salivation, vomiting and faecal alterations that included soft and liquid faeces) in dogs; (2) liver toxicity in rats and dogs; (3) decreases (1% to 8%) in haematocrit and haemoglobin, 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 study.

Gastrointestinal intolerance in the dog, consisting of salivation, vomiting and soft to liquid feces, occurred consistently throughout all of the repeat dose studies with GW433908G, and led to dehydration, electrolyte loss and deterioration to moribund condition in a number of animals.

Liver is the primary target organ for GW433908G toxicity in rats and dogs. The hepatic toxicity consisted of increases in serum AST, ALT, GD, GGT or alkaline phosphatase activities, increased liver weights and microscopic findings, including hepatocyte necrosis. An increase in hepatocellular mitotic figures, hepatocellular hypertrophy, and periportal hepatocellular vacuolation were observed in animals following the acute oral and intravenous administration of GW433908A or GW433908G (mice: p.o. ≥2000 mg/kg, i.v. ≥347 mg/kg; rats: p.o.≥ 2986 mg/kg, i.v. ≥347 mg/kg). Hepatocellular hypertrophy, focal centrilobular vacuolation, and increases in liver weights were observed in animals following 2 or 4-weeks duration of oral GW433908 administration, which were reversible on discontinuation of treatment (rats: ≥149mg/kg, dogs: ≥75mg/kg). Liver related changes included decreases in serum triglyceride concentrations, increases in serum cholesterol and total bile acid levels, and autoinduction of drug metabolizing enzymes (rats: ≥149mg/kg, dogs: ≥75mg/kg). In carcinogenicity studies with APV, hepatocellular adenomas were seen in male mice and rats at the high dose, consistent with a continuum of liver changes seen during the repeat dose toxicity studies with APV and GW433908G. Some of the liver findings and the weak thyroid response in rats may be the result of induction of drug metabolising enzymes.

A slight increase in the incidence of thyroid follicular cell hypertrophy was observed in one-month repeat-dose studies (rats: ≥478 mg/kg/day; dogs: 194mg/kg/day), correlated with induction of microsomal enzymes.

Myocardial fiber degeneration and necrosis were observed in mice and rats following acute intravenous administration (mice and rats: i.v. ≥347 mg/kg), and in rats following 2-weeks repeat-dose oral administration (50mg/kg/day, 750mg/kg/day). A moderate but variable increase in QT interval, transient decreases in heart rate and blood pressure were observed in rats, which was considered unlikely to be of clinical significance because it occurred following administration of a very large dose (intraduodenal: 2000mg/kg). ECG changes (ventricular premature complexes, increases in QT interval, increases in U wave amplitude) were observed in the two-week and one-month repeat-dose oral dog studies, which were cosidered to be secondary to hypokalemia caused by the test article-induced gastrointestinal disturbances. In addition, coronary arteritis was observed in one dog (350mg/kg/day), which was considered to be a spontaneous occurrence in this species and not treatment-related.

In the longer-term rat studies with GW433908G, minor haematological changes were seen. Consistent haematological changes between the 4-week and 6-month rat studies were seen, including decreases in haematocrit and haemoglobin (↓1 to 8%), and an increase (↑7 to 25%) in platelet count. All of these changes appeared to improve during the recovery period, but did not recover fully.

In the rabbit embryofetal study, systemic exposure (AUC) to APV at the high dose on Day 20 of gestation was approximately 0.3 times the exposure in humans treated at the proposed Maximum Recommended Human Dose (MRHD). Higher doses were not used because the existing high dose caused relatively severe maternal toxicity in the form of reduced food consumption and reductions in body weight gain or losses in absolute body weights. The increased incidence of abortions in the rabbit embryofetal study at the high dose is considered related to this severe maternal toxicity. The abortions occurred late in gestation (Days 21 to 29) and after the dose administration phase of the study was finished. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus. 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 at all doses in both male and female pup body weights at weaning. 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 at the high dose 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. GW433908G should not be used during human lactation due to the possibility of transferring the HIV virus from mother to child.

The proposed therapeutic dosing regimen with GW433908G is either with or without ritonavir. The highest arithmetic mean exposure (AUC_{24ss}) to APV in humans occurs following the once daily regimen with ritonavir (1400 mg GW433908G and 200 mg ritonavir QD), and is 83.2 hr•µg/mL (Protocol APV10009) The highest arithmetic mean exposure (AUC_{24ss}) to GW433908X in humans occurs following the twice daily regimen with GW43908G alone (1400 mg GW433908G BID), and is 0.062 hr•µg/mL (Protocol

APV20001). These figures are used for exposure comparison to arithmetic mean exposure in animals. Exposure data (AUC) for APV and GW433908X from the main repeat dose toxicity studies are summarised and compared to exposure levels in humans in Table 1.1 and Table 1.2, respectively.

3.4.2 Single-dose toxicity

The oral, single dose studies were conducted with GW433908A in male rats and mice (Reports RD1998/00776/00 and RD1998/00777/00). The intravenous, single dose studies were conducted with GW433908A because of the poor aqueous solubility of GW433908G in both mice (Reports RD1998/00657/00 and RD1998/02552/00) and rats (Reports RD1998/00656/00 and RD1998/02551/00). Following selection of GW433908G for development, the oral single dose studies were repeated in both male and female animals (Reports RD1999/00017/00, and RD1999/00018/00). Both GW433908A and GW433908G have a low order of acute oral toxicity in mice and rats. Following oral administration, the Maximum Non-Lethal Dose (MNLD) was determined to be >2000 mg/kg (1493 mg/kg APV equivalents) for GW433908A and >2986 mg/kg (2000 mg/kg APV dose equivalents) for GW433908G in both mice and rats. GW433908A has a low order of acute intravenous toxicity in mice and rats. Intravenous administration of GW433908A caused deaths at ≥347 mg/kg (≥256 mg/kg APV equivalents) in both mice and rats. Microscopic liver changes were seen after both oral and intravenous administration, and included hepatocellular mitotic increase (oral only), hepatocellular hypertrophy (intravenous only) and reduced/depleted glycogen (both routes). These changes are consistent with the established toxicology profile of APV. Myocardial fibre degeneration and necrosis were seen in both species following intravenous administration, and myocardial mineral deposits and inflammation were seen in mice only. All findings were observed on Day 3, but not on Day 15, indicating reversibility.

3.4.3 Repeat-dose toxicity

Studies of 14 days, 4 weeks and 6 months duration were conducted in Wistar Hannover rats (Reports RD1998/00711/00, RD1998/02573/00 and RD1998/02858/01). Studies of 14 days, 4 weeks and 9 months duration (Reports RD1998/00487/01, RD1998/02605/00 and RD1998/02861/01) were conducted in beagle dogs. The 14-day rat and dog studies were conducted with GW433908A. The 4-week, 6-month and 9-rnonth studies were completed with GW433908G. The 14-day studies with GW433908A were not repeated with GW433908G because studies of longer duration superseded these results.

Liver toxicity in rats and dogs

Liver is a target organ for toxicity in both rats and dogs. GW433908G treatment-related serum clinical chemistry changes in rats included increased cholesterol, decreased triglycerides. In the 6 month rat study (Report RD1998/02858/01), increased serum activity of AST, ALT, GD and GGT were seen. The activity of AST, ALT and GD were generally higher at the end of the recovery period than they were during the dose administration phase of the study. Increases in absolute (4 to 61%) and relative (3 to 69%) liver weights were consistently attributed to GW433908G in all of the repeat dose rat studies. Microscopic findings were generally associated with the increased liver weights. The predominant microscopic finding was hepatocyte hypertrophy in the shorter-term studies and the additional findings of multinucleated hepatocytes, individual hepatocyte necrosis, increased hepatocyte pigment, increased Kupffer cell pigment, and hepatocellular vacuolation, following administration of GW433908G in the longer-term studies. Following the recovery periods in rats, the increased liver weights and most of the microscopic liver findings were improving but were not completely recovered to control levels.

In the dog, increases (23 to 272%) in serum AP concentrations were seen in all of the longer-term dog studies. ALT was also increased in the 9-month dog study only. After repeated administration to the dog, increases in liver weights were associated with GW433908G administration in the 14-day and 4-week studies, but not in the 9-month study. Hepatocellular pigmentation was evident in the 9-month study. Following the recovery periods in dogs, the increased liver weights were improving but were not completely recovered to control levels. The microscopic liver findings (pigmentation) did not recover, although hepatocellular pigment would not be expected to entirely recover within a period of 4 weeks.

APV exposure comparisons to humans are based on the 6-month rat study. There was no margin of safety seen. For example, liver-related changes in the 6-month rat study (Report RD1998/02858/01) occurred in both males and females at ≥149 mg/kg/day (≥100 mg/kg/day APV equivalents). Exposure (AUC) to APV on Day 179 of this study in male rats at this dose (19.8 hr•µg/mL, lowest exposure) was

approximately 0.2 times the exposure at the proposed MRHD (Table 1.1). APV exposure comparisons to humans are based on the 9-month dog study. There was no margin of safety seen. The GW433908X exposure in male dogs at 149 mg/kg/day (0.030 hr•µg/mL) was approximately 0.5 times the human daily exposure (Table 1.2).

Gastrointestinal toxicities in dogs

Gastrointestinal intolerance in dogs, consisting of salivation, vomiting and fecal alterations (soft to liquid feces), occurred consistently throughout all of the repeat dose studies with GW433908G. This led to dehydration, electrolyte loss and deterioration to moribund condition in a number of animals. The salivation and vomiting were not seen during the off-dose recovery period. In the 9-month study, due to gastrointestinal intolerance, some animals at the high were euthanised prior to the end of the treatment period, and the two high dose level groups were combined and the dose level was reduced (Report RD1998/02861/01). Several of the dogs that were killed in moribund condition had clinical pathology findings that were indicative of dehydration (increased haematocrits) and electrolyte imbalances (decreased serum potassium concentrations). The dehydration and electrolyte loss are mostly likely the result of the decreased food consumption in combination with the recurrent daily episodes of vomiting and diarrhea. All of these clinical signs were reversible during the recovery period. The salivation was also seen in rats. The vomiting was seen in an intravenous safety pharmacology study in dogs (Re: Report WD1998/00588/01). The exact mechanism for the vomiting observed in dogs is unknown. The gastrointestinal toxicities were seen at all dose levels in the 9-month study in dogs. Exposure (AUC) to APV in male dogs at the 75 mg/kg/day (50 mg/kg APV equivalents) in this study (22.9 hr•µg/mL) was 0.3 times that seen at the MRHD (Table 1.1). The GW433908X exposure in male dogs at 75 mg/kg/day (0.028 hr•µg/mL) was 0.5 times human exposure (Table 1.2). Note that gastrointestinal effects have been reported during clinical trials with both GW433908G and APV.

Cardiovascular effects in dogs

Electrocardiographic changes, including ventricular bigeminy, frequent ventricular premature complexes, relative increases in QT intervals (although normal limits were not exceeded), increased U wave amplitude and T wave notching, were seen in the 14-day and 4-week dog studies. These changes were produced by an electrolyte imbalance (hypokalemia) in the dogs that was caused by decreased food consumption, vomiting and fecal alterations (soft to liquid feces). Serum potassium concentrations were decreased in both the 14-day and 4-week dog studies, but not the 9-month study. No EKG changes were noted in the 9-month dog study where the clinical management of the gastrointestinal intolerance was optimised with supplements.

Hematological changes in rats and dogs

In the longer-term rat studies with GW433908G, minor haematological changes were seen. Consistent hematological changes between the 4-week and 6-month rat studies were seen, including decreases in haematocrit and haemoglobin (↓1 to 8%), and an increase (↑7 to 25%) in platelet count. All of these changes appeared to improve during the recovery period, but did not recover fully. In the 6 month study in rats (Report RD1998/02858/01), haematological changes occurred in both male and female rats at ≥478 mg/kg/day (320 mg/kg/day APV equivalents). Exposure (AUC) to APV on Day 179 of this study in males at this dose (46.2 h*µg/mL, lowest exposure) was approximately 0.6 times human exposure at the MRHD (Table 1.1). The GW433908X exposure at this dose in males (0.229 hr•µg/mL) was approximately 3.7 times human daily exposure (Table 1.2). In the dog, no haematological changes were directly attributed to the administration of GW433908G. Some hematologic parameters were indirectly affected by the reduced hydration state of the dogs following repeated vomiting and fecal alterations.

Possible enzyme induction and secondary liver and thyroid effects in rats

Concomitant findings of increased liver weights and hepatocyte and thyroid follicular hypertrophy were seen in the 4-week rat study and were most likely the result of induction of liver drug metabolising enzymes by APV. The interrelationship of these liver and thyroid findings in the rat is the result of increased metabolism and excretion of T3 and T4 in the liver and the rebound increase in TSH, which drives the thyroid follicular cells into a hypertrophic state. This is considered a rat specific effect, and no changes in thyroid hormone levels were seen in humans during clinical trials with APV (Reference Protocol PROA2002). The microscopic changes in the thyroid gland (diffuse and multifocal follicular cell hypertrophy) were found in the 4-week rat study but not in the 14-day or 6-month studies. The reason this

change was not found in the longer-term study is not clear. A combination of events such as variations between test facilities and an adaption of the hypothalamic-pituitary-thyroid (HPT) axis to long-term exposure to APV are the most likely explanation.

3.4.4 Genetic Toxicity Studies

GW433908A or GW433908G have been examined in a battery of both in vitro and in vivo genetic toxicity tests. All results were uniformly negative. GW433908 was devoid of mutagenic potential in the plate incorporation or pre-incubation Ames assay (>5800 µg/plate), the L5178Y/tk$^{+/-}$ mouse lymphoma assay (>5000 µg/ml), and micronucleus assay. The in vitro tests were carried out in the absence and presence of a rat liver-derived metabolising system (S9), and all studies included appropriate vehicle and positive controls (Reports RD1998/00935/00, RD1998/01213/00 and RD1999/00412/00). Additional studies on [redacted] impurities of GW433908G were also uniformly negative (Reports RD1999/02761/00, RD1999/02762/00 and RD1999/02763/01).

3.4.5. Carcinogenicity Studies

Carcinogenicity studies in rats and mice with GW433908G are currently being initiated and final reports will be available in 2005. Dose levels were selected based on results from a pilot 13-week study in mice (Report RD2000/02408/00) and from the 6-month study in rats. Both carcinogenicity studies are based on a toxicity end point approach (MTD). Carcinogenicity studies with APV in mice and rats have been completed and submitted for review (Reference Reports RD1998/02066/01 and RD1998/01521/01).

3.4.6. Reproductive and Developmental Toxicology

Orally administered GW433908G was evaluated for effects on fertility, organogenesis and peri-natal and post-natal behaviour and development. GW433908G doses were administered twice daily approximately 6 hours apart.

Fertility and early embryonic development to implantation in rats

The potential effects of GW433908G on fertility and early embryonic development to implantation were evaluated in a combined male and female rat study (Report RD1999/01281/00). All male reproductive indices concerning mating success were similar to control values. At necropsy of the male rats, paired testes (4 to 7% increase in absolute weights) and paired epididymides (0 to 2% increase in absolute weights) weights were increased, however, microscopic examination of the testes and epididymides from males in the high dose (2240 mg/kg/day) group did not show any changes compared to controls. These changes did not interfere with the ability of any male rat in this study to successfully mate and sire pregnancy. All female reproductive indices concerning estrous cyclicity, mating success and viability of the offspring were similar to control values. At caesarean sectioning of the female rats, the weight of the gravid uterus was decreased (0 to 16%), and the number of corpora lutea (mean/dam) and the number of implantations (mean/dam) were also decreased. All of these findings are most likely interrelated in that the decrease in the gravid uterine weights is probably due to the reduced number of corpora lutea and the subsequent smaller number of uterine implantation sites. The reduced gravid uterine weights are not due to early embryonic death since the number of resorptions and both pre- and post-implantation embryo loss were not increased in the GW433908G-treated female rats. Mean systemic exposure (AUC) in male rats to APV on Day 27 prior to mating was 89.6 hr•µg/mL at the high dose of 2240 mg/kg/day, or approximately 1.1 times the exposure at the proposed MRHD. The GW433908X exposure ratio between male rats and humans was approximately 84.7 times on the same day. Exposure ratios between high dose female rats (Day 13) and humans were 1.3 and 54.5 times for APV and GW433908X, respectively.

Embryofetal development in rats

There were no effects on embryofetal development or uterine parameters in rats following oral administration of GW433908G at doses up to 2240 mg/kg/day (1498 mg/kg/day APV equivalents). No fetal malformations were observed in this study, and fetal variations were unrelated to GW433908G administration (Report RD1999/02690/00). Systemic exposure (AUC) to APV on Day 17 of gestation was approximately 0.7 times the exposure at the proposed MRHD (Table 1.1). Exposure to APV on Day 6 of

gestation was approximately 2.8 times the human exposure, but, as in other rat studies, exposure decreased with repeated administration. The GW433908X exposure ratio between rats and humans was approximately 30.8 times on Day 17 (Table 1.2).

Embryofetal development in rabbits

GW433908G dose levels for the definitive study were chosen following preliminary studies in the non-pregnant (Report RD1999/00465/00) and pregnant (Report RD1999/00716/00) rabbit. In the definitive study (Report RD1999/01035/00), GW433908G was administered on Days 7 to 20 of pregnancy at 74.8 to 672.8 mg/kg/day (50 to 450 mg/kg/day APV equivalents). Caesarean sections were conducted on Day 29 of pregnancy. At the high dose of 672.8 mg/kg/day, one rabbit died and five others aborted during the study. The doses of 224.3 and 672.8 mg/kg/day caused maternal toxicity in the form of reduced body weight gain and decreased food consumption. There were no effects on embryofetal development or uterine parameters at any dose. No GW433908G-related changes were noted in fetal external, soft tissue or skeletal evaluations. The increased incidence of abortions at the high dose is considered related to the maternal toxicity at this dose. The abortions occurred late in gestation (Days 21 to 29) and after the dose administration phase of the study was finished. Systemic exposure (AUC) to APV on Day 20 of gestation was approximately 0.3 times the exposure at the proposed MRHD (Table 1.1). The GW433908X exposure ratio was approximately 14.2 times on Day 20 (Table 1.2). Limited systemic exposure in pregnant rabbits has also been noted with the protease inhibitors nelfinavir mesylate (Viracept®) and indinavir sulphate (Crixivan®).

Pre-natal and post-natal development including maternal function in rats

GW433908G was assessed in rats for effects on pre-natal and post-natal development of the F1 and F2 pups, and the F0 maternal function (Report RD1999/01282/00). In the F0 females, GW433908G did not cause any abnormalities in F0 reproductive performance, including the fertility index, percent pregnant, gestation length, litter size and the number of stillbirths. In the F1 generation, GW433908G decreased pup survival at the high dose of 2240 mg/kg/day (1498 mg/kg/day APV equivalents) and pup body weights at weaning at all doses. Note that APV and APV metabolites have been shown to be excreted into rat milk, and to be slightly concentrated in the milk versus plasma (see Reference Report RD1997/03812/00). Therefore, the presence of APV in maternal milk may account for the reduction in mean body weights seen in these animals. The reduced body weight effect was seen in the F1 male and female pups persisted in both sexes. Prolonged precoital interval, prolonged gestation period and a slight reduction in the mean number of implantation sites were also seen at the high dose of 2240 mg/kg/day when the F1 generation was mated. Administration of GW433908G did not adversely affect the body weights or survival of the F2 generation.

Toxicity studies in neonatal and juvenile rats

[redacted] for GW433908G, the sponsor conducted toxicity studies in neonatal and juvenile rats (starting at 4 days of age). GW433908G was evaluated for toxicity in three neonatal and juvenile rat studies. The first two studies were dose range finding studies that were used to select doses for the third, and pivotal rat study. Doses were administered using the same BID regimen as in the general toxicology studies in adults. with two equal daily doses administered 6 hours apart. Note that the [redacted] GW433908G [redacted] suspension formulation contains [redacted] in generally accepted concentrations, and the [redacted] suspension formulation was not used in neonatal and juvenile rat studies. Note that the suspension formulation (0.5% w/w hydroxypropyl methylcellulose and 0.1% w/w Tween® 80) was used. In the initial pilot study (Report RD1999/02344/00), GW433908G doses of 5 to 160 mg/kg/day (3 to 107 mg/kg/day APV equivalents) were administered to male and female rat pups starting on lactation Day 5 (Day 0 = day of birth) and continuing for 31 days. Mortality in this study was slightly higher in the female pups treated with 160 mg/kg/day than at other doses. The relationship of GW433908G administration to this mortality was not clear since only 2 of 20 female pups died and no male pups died in the same dose group. Because of the equivocal relationship of mortality to GW433908G administration in the first study, a second pilot study (Report RD2000/02506/00) was conducted in neonatal and juvenile rats. GW433908G doses of 61 to 1105 mg/kg/day (43 to 777 mg/kg/day APV equivalents) were administered to male and female rats pups starting on lactation Day 4 (Day 0 = day of birth) and continuing for 15 days. Mortality in this study was increased at ≥553 mg/kg/day and the NOAEL for this study was set at 184 mg/kg/day (130 mg/kg/day APV equivalents; GW433908 human equevalent dose: 30 mg/kg/day). In the pivotal neonatal and juvenile

rat study (Report RD2002/00045/00), GW433908G was administered at doses of 100 to 300 mg/kg/day (71 to 213 mg/kg/day APV equivalents) to rat pups starting on lactation Day 4 (Day 0 = day of birth) and continuing for 91 days. The clinical observation of suspected empty stomachs (decreased amount of milk or no milk) was seen in male and female pups in the high dose 300 mg/kg/day (213 mg/kg/day APV equivalents) group during the first 2 to 3 days of treatment. Both male and female pups in the high dose group showed slightly lower mean body weights during the first 4 weeks of treatment. In the high dose group in this study, increases in liver weights, AST and ALT levels were seen during the treatment period, which generally increased further by the end of the recovery period and increases in liver weights were reversible. No microscopic changes were noted in the liver that correlated with these findings. The finding of higher AST and ALT levels at the end of the recovery period was also seen in the 6-month rat study in adult animals (see Section 3.4.3). The mechanism is unclear at this time. The NOAEL for this study was 175 mg/kg/day (GW433908 human equevalent dose: 30 mg/kg/day). A new GW433908G-related microscopic finding of hyaline droplet accumulation in the cortical tubule epithelial cells was noted in male rats in all dose groups in this study and was reversible. This finding has not been described in any of the previous repeat dose rat studies with either APV or GW433908G. The finding was considered likely due to male rat specific metabolism of α2u-globulin and is of limited toxicological relevance to humans (Durham and Swenberg, 2002).

3.4.7. Local Tolerance

GW433908G did not cause irritation when applied to the rabbit eye (Report RD1999/00551/00), but was classified as a mild irritant to rabbit skin (Report RD1999/00553/00). GW433908G showed no sensitising potential in guinea pigs (Report RD1999/00552/00).
I. PharmTox Summary

3.4.8. Special Toxicology

Immunotoxicity in dogs: Thymus was identified as a target organ following repeated administration of GW433908G in dogs. In dogs, decreases in thymus weights were associated with GW433908G administration in the 14-day and 4-week studies. Microscopic examination of the thymus revealed thymic atrophy, which is probably the result of stress from the daily emetic episodes described earlier. However, decreases in thymus weights and thymic atrophy were not seen in the 9-month dog study, where clinical management of emetic events and the general condition was more effective. The thymic changes were seen at all dose levels in the 4-week study (Report RD1998/02605/00). The sponsor stated that both of these findings are probably attributable to stress from daily emetic episodes, and hence the thymus may not be considered a primary toxicity target organ. However, according to the sponsor, an immunotoxicology study will be conducted in rats because GW433908G will be administered to HIV patients. The final report will be submitted after completion of the study.
Toxicology studies of drug substance impurities : Based on current ICH Guidelines (ICH Q3A) for drugs with a total daily dose of >2 g, drug substance impurities in excess of 0.05% w/w were identified and examined for toxicological effects. To ensure adequate qualification of these impurities, two additional oral studies in rats were conducted to compare the well-established toxicological profile of GW433908G and APV with results from drug substance batches purposely spiked with potential impurities (Table 1.3). These batches were also examined for mutagenicity in 3 bacterial reverse mutation studies (Reports RD1999/02761/00, RD1999/02762/00 and RD1999/02763/01).

The impurity profile of GW433908G is different to APV and none of the impurities identified for APV have been observed in batches of GW433908G. To ensure adequate qualification of impurities in the drug substance, all of the impurities, except [redacted] were tested at a concentration equivalent to or greater than the proposed drug substance specification for GW433908G in nonclinical toxicity studies in rats and dogs. The sponsor also compared the toxicological profile of GW433908G with results from drug substance batches purposely spiked with potential impurities. These batches were also examined for mutagenicity in 3 bacterial reverse mutation studies.

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 the nonclinical 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. Note that the sposnor calculated the drug substance qualification levels based on the high dose rather than the NOAEL in the non-clinical toxicology studies. In general, such a calculation is not acceptable because at such doses toxicity was seen in animals. As part of a Phase 4 Post-marketing Agreement, it is recommend that the sponsor conduct a 90-day study in rats studies to qualify the drug substance impurities

Table 1.1. Amprenavir: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	APV Cmax (µg/mL)				APV AUC (h*ug/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Rat 4 week (RD1998/02573/00)	149	3.00	2.42	1.63	1.76	32.0	31.2	19.9	23.0
	478	5.61	7.12	3.48	4.30	62.4	83.1	33.6	49.9
	1493	9.80	11.1	5.38	4.89	224	133	47.2	68.1
	2240	11.3	10.4	4.54	5.38	184	234	53.1	80.6
Rat 6 month (RD1998/02856/01)	149	2.55	3.32	2.90	2.31	12.7	33.3	19.8	22.7
	478	7.54	8.52	5.09	5.23	84.4	74.1	46.2	54.3
	1493	9.70	13.1	5.33	7.28	154	237	57.0	62.3
	2240	8.57	9.85	5.28	7.68	243	253	54.9	107
Dog 4 week (RD1998/02605/00)	75	5.40	5.21	5.60	5.55	27.5	32.6	29.6	35.3
	194	10.4	10.7	10.4	12.2	86.4	87.7	68.2	56.8
	523	15.4	15.1	18.4	24.5	145	129	95.9	239
	747	15.5	18.5	31.5	23.4	135	123	209	156
Humans (Protocol APV20001)	56*	-		5.16		-		35.4	
Humans (APV10009 or APV10010)	1395 mg + RTV 200 mg	-		7.57		-		83.2	

Key:
‡ = AUC - on Day 1. AUC24 at steady state ND = Not determined.

Table 1.1 (continued). Amprenavir: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	APV Cmax (µg/mL)				APV AUC (h*ug/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Dog 9 month† (RD1998/02861/01)	75	4.55	5.86	3.98	4.55	24.1	30.0	22.9	30.6
	194	11.7	8.22	14.4	20.5	64.2	62.6	113	143
	750/525/337	12.0	12.5	17.9	25.1	86.3	129	159	257
Rat organogeness (RD1999/02656/00)	300	-	4.71	-	2.07	-	68.5	-	26.9
	820	-	7.64	-	3.55	-	126	-	43.2
	2240	-	8.52	-	5.94	-	229	-	57.1
Rabbit organogeness (RD1999/01035/00)	74.8	-	0.01	-	0.22	-	0.03	-	1.81
	224.3	-	0.59	-	0.57	-	2.11	-	3.88
	672.8	-	4.16	-	3.33	-	22.2	-	25.8
Humans (APV20001)	1395 mg	-		5.16		-		35.4	
Humans (APV10009 or APV10010)	1395 mg + RTV 200 mg	-		7.57		-		83.2	

Key:
† = On Days 1 to 23 dogs were dosed with either 525 or 750 mg/kg/day. Due to severe intolerance, dosing was suspended on Day 24 and resumed on Day 29 with the two high doses combined and the dose reduced to 337 mg/kg/day.
‡ = AUC - on Day 1, AUC24 at steady state ND = Not determined.

Table 1.2. GW433908X: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	GW433908X Cmax (ug/mL)				GW433908X AUC‡ (h*ug/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Rat 4 week (RD1998/02573/00)	149	2.15	0.438	0.144	0.010	15.3	3.18	0.546	0.067
	478	0.341	0.132	0.570	0.052	2.83	0.9931.77	1.65	0.536
	1493	0.146	0.466	0.276	0.281	1.57	1.77	0.786	1.25
	2240	0.122	0.174	0.147	0.420	1.42		1.20	1.58
Rat 6 month (RD1998/02856/01)	149	0.021	0.010	0.015	0.102	0.052	0.034	0.030	0.271
	478	0.036	0.132	0.032	0.381	0.156	0.299	0.229	3.27
	1493	0.052	0.069	0.102	0.184	1.56	4.72	0.930	1.00
	2240	0.047	0.124	0.117	0.143	0.615	1.26	1.08	1.82
Dog 4 week (RD1998/02605/00)	75	0.010	0.020	0.009	0.009	0.023	0.020	0.011	0.009
	194	0.016	0.147	0.034	0.053	0.041	0.365	0.049	0.089
	523	0.142	0.056	0.257	0.700	0.582	0.176	0.622	2.64
	747	0.187	0.542	0.783	0.890	0.388	1.37	1.64	2.21
Humans (APV20001)	1395 mg	-	-	0.024		-		0.062	
Humans (APV10010 and APV10009)	1395 mg + RTV 200 mg	-		0.015		-		0.024	

Key:
‡ = AUC - on Day 1. AUC24 at steady state. ND = Not determined

Table 1.2 (continued). GW433908X: Toxicokinetic Data From Selected Oral Toxicity Studies (Arithmetic Means)

Study (Report Number)	GW433908G Dose Levels (mg/kg/day)	GW433908X Cmax (µg/mL)				GW433908X AUC‡ (h*µg/mL)			
		Study Start		Study End		Study Start		Study End	
		M	F	M	F	M	F	M	F
Dog 9 month† (RD1998/02861/01)	75	0.012	0.013	0.058	0.041	0.038	0.039	0.026	0.048
	194	0.029	0.019	0.114	0.292	0.062	0.060	0.127	0.381
	750/525/337	0.301	0.134	0.375	0.850	0.627	0.335	0.556	1.63
Rat organogenesis (RD1999/02690/00)	300	-	0.013	-	0.038	-	0.055	-	0.330
	820	-	0.047	-	0.131	-	0.650	-	0.868
	2240	-	0.145	-	0.149	-	1.32	-	1.91
Rabbit organogenesis (RD1999/01035/00)	74.8	-	0.006	-	0.013	-	0.015	-	0.043
	224.3	-	0.017	-	0.034	-	0.069	-	0.156
	672.8	-	0.084	-	0.190	-	0.648	-	0.881
Humans (APV20001)	1395 mg	-		0.024		-		0.062	
Humans (APV10013 and APV10009)	1395 mg + RTV 200 mg	-		0.015		-		0.024	

Key:

† = On Days 1 to 23 dogs were dosed with either 525 or 750 mg/kg/day. Due to severe intolerance dosing was suspended on Day 24 and resumed on Day 29 with the two high doses combined and the dose reduced to 337 mg/kg/day.

‡ = AUC∞ on Day 1, AUC24 at steady state. ND = Not determined.

Table 1.3. Highest Concentrations of Drug Substance Impurities Tested in Nonclinical Toxicity Studies

Drug Substance Impurity	Highest Concentration Tested in Repeat Dose Rat Study (% w/w)‡	Current Drug Substance Specification (% w/w)	Study Type	Report No
[redacted]			4 week rat & In vitro bacterial mutation tests	RD1998/02573/00 (4 week rat) & RD1998/02761/00 RD1998/02762/00 RD1998/02763/01 (mutation tests)
			14 day rat & In vitro bacterial mutation tests	RD2000/01864/00 (14 day rat) & RD1998/02761/00 RD1998/02762/00 RD1998/02763/01 (mutation tests)
			14 day rat & In vitro bacterial mutation tests	RD2001/00212/01 (14 day rat) & RD1998/02761/00 RD1998/02762/00 RD1998/02763/01 (mutation tests)
			6 month rat & In vitro bacterial mutation tests	RD1998/02858/01 (6 month rat) & RD1998/02761/00 RD1998/02762/00 RD1998/02763/01 (mutation tests)

Key: [redacted]

Single-dose toxicity studies in mice and rats:

22. GW433908A: Acute oral toxicity study in mice (Report No. RD1998/00776/00)

GW study No.: M40367; Conducting facility: Glaxo Wellcome Inc; Date Initiation: 24 March 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A solution in reverse osmosis treated water

Key study findings: The oral NOEL of GW433908A in male mice was established to be **≥2000 mg/kg**. This is equivalent to a human dose of approximately **≥183 mg/kg/day** based on body surface area.

Methods

A group of 5 male CD-1 mice (body weight: 29.3 to 33.5 g) received a single dose of 2000mg/kg of GW433908A (dose volume: 10 ml/kg) by oral gavage. After 14 days observation, the animals were euthanized for post mortem examination macroscopically.

Results

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

23. GW433908G: A single-dose oral toxicity study in CD-1 mince (Report No. RD1999/00017/00)

GW study No.: M40426; Conducting facility: Glaxo Wellcome Inc; Date Initiation: 26 January 1999; GLP Compliance: Yes (X), No ( ); Drug reference No.: GW433908G; Drug Lot: R4283/31/1; Formulation: GW433908G in 0.5% (w/w); hydroxypropylmethylcellulose in 0.1% (w/w) Tween 80

Key study findings: The oral **NOAEL** of GW433908G in mice was established to be ≥2986mg/kg. This is equivalent to a human dose of approximately ≥209 mg/kg/day based on body surface area.

Methods

Three male and three female CD-1 mice were dosed at 2986mg/kg of GW433908G and observed for 7 days thereafter in a range-finding study. Six CD-1 mice per sex group (body weight for males: 26.1-35g; for females: 22.8-31.2; Age: 6-7 weeks) received a single dose of 2986mg/kg of GW433908G (dose volume: 10ml/kg), or 0.5% (w/w) hydroxypropyl-methylcellulose in 0.1% (w/w) Tween 80 by oral gavage. The mice were observed daily for clinical signs for 14 days thereafter. Body weights were measured weekly. At necropsy, all mice were examined macroscopically. All tissues from scheduled deaths were microscopically examined.

Results

Clinical signs: No treatment-related clinical signs were noted.
Body weights: There was no effect of test article treatment on body weight.
Gross pathology: There were no dose-related macroscopic findings.
Histopathology: An increase in mitotic figures in hepatocytes and peripherolobular depletion were observed in mice treated with the test article, which returned to normal microscopic appearance 14 days after treatment.

24. GW433908A: Acute intravenous toxicity study in CD-1mice (Report No. RD1998/00657/00)

GW study No.: M40370; Conducting facility: Glaxo Wellcome Inc; Date Initiation: 1 April 1998; GLP Compliance: Yes (X); Drug reference No.: GW433908A; Drug Lot: R2826/7/1; Formulation: GW433908A solution in 0.9% sodium chloride solution (20 mg/ml)

Key study findings: The intravenous **NOEL** of GW433908A was established to be **≥200 mg/kg**. This is equivalent to a human dose of approximately **≥18.3 mg/kg/day** based on body surface area.