CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-226

21-251

PHARMACOLOGY REVIEW

Assigned Reviewer: Hao Zhang
Division: Division of Antiviral Drug Products, HFD-530
Date Submitted: December 28, 1999 – May 22, 2000
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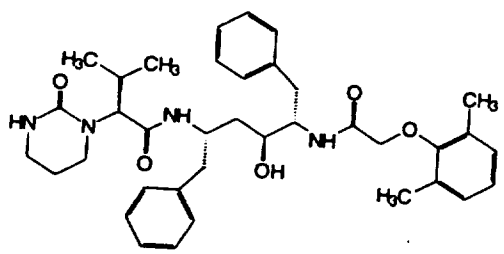
Sponsor: Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500

Drug: Lopinavir co-formulated with ritonavir as a liquid oral administration, and as a soft gelatin capsule, containing both lopinavir and ritonavir

I. Lopinavir

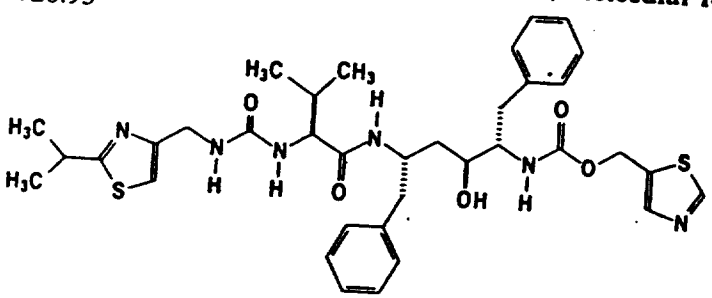
Code Name: Abbott-157378; **Generic Name:** ABT-378; lopinavir; **Chemical name:** [1S-[1R*, (R*), 3R*, 4R*]]-N-[4-[[2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5phenyl-1-(phenylmethyl)pentyl] tetrahydro-alpha-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide; **Molecular formula:** $C_{37}H_{48}N_4O_5$; **Molecular weight:** 628.80; **CAS Registry Number:** 192725-17-0



Structural Formula of Lopinavir (ABT-378)

II. Ritonavir

Code Name: Abbott-84538; **Generic Name:** ABT-538; **Trade Name:** Norvir™
Chemical name: 10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, [5S-(5R*, 8R*, 10R*, 11R*)]; **CAS Registry Number:** 155214-67-5; **Molecular formula:** $C_{37}H_{48}N_6O_5S_2$; **Molecular weight:** 720.95



Structural Formula of Ritonavir (ABT-578)

Relevant INDs/NDAs/DMFs: NDA 20-659 (Ritonavir); IND [redacted]
Drug Class: Peptidomimetic HIV protease inhibitors
Indication: HIV infection

Clinical formulation and Components: each lopinavir/Ritonavir **Soft Gelatin Capsule (Trade Name:** Kaletra) for oral administration contains lopinavir (133.3 mg) and ritonavir (33.3 mg). Inactive ingredients include oleic acid, propylene glycol, polyoxyl 35 castor oil, gelatin, sorbitol special, glycerin, titanium dioxide, and FD&C Yellow No. 6.

Lopinavir/Ritonavir (Trade Name: Kaletra) **Oral Solution** contains lopinavir (80 mg/mL) and ritonavir (20 mg/mL) in 42.4% alcohol (v/v). Inactive ingredients include alcohol, high fructose corn syrup, propylene glycol, glycerin, providone, Magnasweet-110 flavor, natural and artificial vanilla flavor, polyoxyl 40 hydrogenated castor oil, artificial cotton candy flavor, acesulfame potassium, saccharin sodium, sodium chloride, peppermint oil, sodium citrate, citric acid, and methanol.

Route of Administration Oral
Proposed clinical dose: ABT-378/ritonavir, 400 mg/100 mg, BID

INTRODUCTION

lopinavir/ritonavir (Kaletra) is a co-formulation of lopinavir and ritonavir. lopinavir (ABT-378) is a novel peptidomimetic HIV protease inhibitor. Its antiviral potency is 10-fold greater than ritonavir (ABT-538). The EC_{50} of lopinavir for wild type HIV in 50% human serum is 0.1 μM. Ritonavir inhibits the CYP3A-mediated metabolism of lopinavir. Although lopinavir showed poor bioavailability in animals when administered alone, the combination of lopinavir with ritonavir substantially improved the pharmacokinetic profile of lopinavir. Additionally, co-administration of lopinavir and ritonavir produced sustained suppression of HIV replication. Based on these results, the sponsor initiated clinical development of lopinavir/ritonavir for treatment of HIV infection [redacted] The NDA was granted fast track status by the division on August 19, 1999.

STUDIES REVIEWED WITHIN THIS SUBMISSION

Non-clinical Pharmacology Studies

1. Study of ABT-378 in various receptor binding and ion transport assays. CEREP Report: RAP-860020 S 810/830/500. [redacted] Abbott Laboratories, [redacted], 1997.
2. Effects of ABT-378 on K channels. [redacted] Abbott Laboratories, [redacted] 1997.

Safety Pharmacology Studies

3. ABT-538 and ABT-378 CNS general pharmacology profile in the mouse and the rat after P.O. co-administration. I.T.E.M.-LABO Report No D28.1697/1. [redacted] Abbott Laboratories, [redacted] 1997.
4. Burke SE, Cox BF, Polakowski JS, Preusser LC. Cardiovascular profile of ABT-378/ABT-538 in conscious rats, anesthetized dogs, and conscious dogs. [redacted] Abbott Laboratories, [redacted] 1996.
5. Burke SE, Nelson RA, Cox BF. Effect of ABT-378/ABT-538 on electrocardiographic end-points in pentobarbital-anesthetized dogs. [redacted] Abbott Laboratories, [redacted] 1997.

Non-clinical Toxicology Studies

Acute studies – Mice and Rats

6. Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Mice (Study No. TD96-220, R&D/96/458)
7. Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-218, R&D/96/456)
8. Acute Intravenous Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in mice (Study No. TA96-221, R&D/96/459)
9. Acute Intravenous Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-219, R&D/96/457)
10. Single- Dose Oral Toxicity Study of Abbott-157378 in Rats (Study No. TA96-315, R&D/96/669)

Repeat Dose Studies – Mice

11. Three-Month Oral Maximum-Tolerated Dosage Study with Abbott-157378 in Combination with Abbott-84538 in mice (Study No. TA97-029, R&D/97/501)

Repeat Dose Studies – Rats

12. Two-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Rats (Study No. TA96-079, R&D/96/300)
13. Three-Month Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Rats (with a One-Month Recovery Period), Study No. TA96-156, R&D/96/574
14. Six-Month Oral Toxicity Study with Abbott-157378 in Combination with Abbott-84538 (Ritonavir) in Rats, Study No. TA97-002, R&D/97/720

Repeat Dose Studies – Neonatal and Juvenile Rats

15. Two-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Neonatal Rats (Study No. TA98-069, R&D/98/307)
16. Four-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Juvenile Rats (Study No. TA98-022, R&D/98/375)

Repeat Dose Studies – Dogs

17. Two-Week Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs, Study No. TB96-067
18. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (With a One –Month Recovery Period), Study No. TB96-157, R&D/96/675
19. Six-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs, Study No. TB97-003, R&D/97/752
20. Nine-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs, Study No. TB98-020, R&D/99/124

Special Toxicity Studies

Repeated Dose Studies – Dogs

21. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with Impurities in Beagle Dogs, Study No. TB98-013, R&D/98/371
22. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with New Impurities in Beagle Dogs, Study No. TB98-150, R&D/99/093
23. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with related Substances in Beagle Dogs, Study No. TB99-127, R&D/00/030

Reproduction Toxicity Studies

24. Evaluation of the Effects of Orally Administered Abbott-157378 in Combination with Ritonavir (Abbott-84538) on the Reproductive Function of Male and Female Rats (Seg. I DART) (Study No. TA97-047, R&D/97/382)

25. Evaluation of the Effects of Orally Administered Abbott-157378 in Combination with Ritonavir (Abbott-84538) on the Embryonic and Fetal Development of the Rat (Seg. II DART) (Study No. TA96-162, R&D/97/335)
26. Evaluation of the Effects of Orally Administered Abbott-157378 and Abbott-84538 (ritonavir) Combination on the Embryonic and Fetal Development of the Rabbit (Seg. II DART) (Study No. TE96-152, R&D/97/365)
27. Study of the Effects of Abbott-157378 in Combination with Ritonavir on Pre- and Postnatal Development, Including Maternal Function in the Rat (Seg. III DART) (Abbott Study No. TA97-109, R&D 98/315). This study was conducted at

Genetic Toxicology Studies

28. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli)* of Abbott-157378 (Study No. TX96-114, R&D/96/439)
29. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli)* of Abbott-157378 With High Impurities (Abbott Study No. TX98-072, R&D/98/303)
30. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli)* of Abbott-157378 With High New Impurities (Abbott Study No. TX98-185, R&D/98/589)
31. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli)* of Abbott-157378 With Related Substances (Abbott Study No.TX99-137, R&D/99/447)
32. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli)* of Abbott-84538 Soft Elastic capsule (SEC) Formulation (Abbott Study No.TX96-395, R&D/96/593)
33. *In Vitro* Cytogenetics Human Lymphocyte Culture Assay of Abbott- 157378 (Study No. TX96-185, R&D/96/440)
34. *In Vitro* Cytogenetics Human Lymphocyte Culture Assay of Abbott-157378 With High Impurities (Study No. TX98-073, R&D/98/304)
35. *In Vitro* Cytogenetics Human Lymphocyte Culture Assay of Abbott-157378 With High New Impurities (Study No. TX98-186, R&D/98/645)
36. *In Vitro* Cytogenetics Human Lymphocyte Culture Assay of Abbott-157378 With Related Substances (Study No. TX99-138, R&D/99/448)
37. *In Vitro* Cytogenetics Assay in Human Lymphocytes of Abbott-84538 Soft Elastic Capsule (SEC) Formulation (Study No. TX96-401, R&D/96/746)
38. Mouse Micronucleus Assay of Abbott-157378 Alone and in Combination with Abbott-84538 (Abbott Study No. TD96-211, R&D/90/320)
39. L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay of Abbott-157378, Study No. TX96-230, R&D/96/773)

Nonclinical Pharmacokinetic Studies

Drug Metabolism Overview

40. Abbott-157378 Drug Metabolism Report No 9 – Preclinical pharmacokinetic summary of Abbott-157378 in rat, monkey and dog (Abbott laboratories Division 46 Report No. R&D/96/568, 1996)

Absorption and Pharmacokinetics

41. Abbott-157378 Drug Metabolism Report No 24 – Tabulation of plasma concentration data for three-month oral maximum tolerated dosage study of Abbott-157378 in combination with ritonavir (Abbott-84538) in mice (Protocol TD-97-029) (Abbott laboratories Division 46 Report No. R&D/97/625, 1997)
42. Abbott-157378 Drug Metabolism Report No 13 – A tabulation of plasma concentration data for three-month oral toxicity study of Abbott-157378 in combination with ritonavir (Abbott-84538) in rats (with one-month recovery period) (Protocol TD-96-156) (Abbott laboratories Division 46 Report No. R&D/97/672, 1997)
43. Abbott-157378 Drug Metabolism Report No 28 – Toxicokinetics of Abbott-157378 and ritonavir (Abbott-84538) in a six-month oral toxicity study of Abbott-157378 in combination with ritonavir in rats (Protocol TD-97-002) (Abbott laboratories Division 46 Report No. R&D/97/700, 1997)
44. Abbott-157378 Drug Metabolism Report No 20 – Tabulation of plasma concentration data for a study of oral administered Abbott-157378 in combination with ritonavir (Abbott-84538) on the embryonic and fetal development of the rat (Protocol TD-96-162) (Abbott laboratories Division 46 Report No. R&D/97/440, 1997)
45. Abbott-157378 Drug Metabolism Report No 35 – Tabulation of plasma concentration data for a four-week oral toxicity study of Abbott-157378 in combination with ritonavir (Abbott-84538) in immature (juvenile) rats (Protocol TD-98-002) (Abbott laboratories Division 46 Report No. R&D/98/363, 1998)

46. Abbott-157378 Drug Metabolism Report No 36 – Tabulation of plasma concentration data for a two-week oral toxicity study of Abbott-157378 in combination with ritonavir (Abbott-84538) in neonatal rats (Protocol TD-98-002) (Abbott laboratories Division 46 Report No. R&D/98/364, 1998)
47. Abbott-157378 Drug Metabolism Report No 14 – A tabulation of plasma concentration data for three-month oral toxicity study of Abbott-157378 in combination with ritonavir (Abbott-84538) in beagle dogs (with one-month recovery period) (Protocol TD-96-157) (Abbott laboratories Division 46 Report No R&D/96/740, 1997)
48. Abbott-157378 Drug Metabolism Report No. 27 -Toxicokinetics of Abbott-157378 and ritonavir (Abbott-84538) in a six-month oral study of Abbott-157378 in combination with ritonavir in dogs (Protocol TB97-003) (Abbott Laboratories Division 46 Report No. R&D/97/699, 1997)
49. Abbott- 157378 Drug Metabolism Report No. 38 - Tabulation of plasma concentration data for a three-month oral toxicity study of Abbott-157378 and ritonavir (Abbott-84538) combination with impurities in beagle dogs (Protocol TB98-013) (Abbott Laboratories Division 46 Report No. R&D/98/425, 1998)
50. Abbott-157378 Drug Metabolism Report No. 47 - Tabulation of plasma concentration data for a three-month oral toxicity study of Abbott-157378 and Abbott-84538 combination with new impurities in beagle dogs (Protocol TB98-150) (Abbott Laboratories Division 46 Report No. R&D/99/119, May 1999)
51. Abbott-157378 Drug Metabolism Report No. 49 -Toxicokinetics of Abbott-157378 and ritonavir (Abbott-84538) in a nine-month oral toxicity study of Abbott-157378 in combination with ritonavir in beagle dogs (Protocol TB98-020) (Abbott Laboratories Division 46 Report No. R&D/99/160, July 1999)

Protein Binding and Blood Partitioning

52. Abbott-157378 Drug Metabolism Report No. 2- Protein binding of [^{14}C] Abbott-157378 in mouse, rat, dog, monkey and human plasma (Protocol V96-009) (R&D/96/305, 1996)
53. Abbott-157378 Drug Metabolism Report No 17 - Protein binding of [^{14}C]Abbott-157378 and [^{14}C]Abbott-84538 in dog plasma (Protocol V97-020) (R&D/97/392, August 1997)
54. Abbott- 157378 Drug Metabolism Report No 55 - Effect of Abbott-157378 in the presence of ritonavir on the *in vitro* protein binding of [^{14}C] warfarin, [^{3}H] digoxin and [^{3}H] imipramine in human plasma (Protocol V99-052) (Abbott Laboratories Division 46 Report No. R&D/99/648, December 1999)
55. Abbott-157378 Drug Metabolism Report No 56 - Effect of saquinavir, amprenavir, nelfinavir and ibuprofen on the *in vitro* protein binding of [^{14}C] Abbott -157378 in human plasma in the presence of ritonavir (Protocol V99-053) (Abbott Laboratories Division 46 Report No. R&D/99/648. December 1999)
56. Abbott-157378 Drug Metabolism Report No 12 -Binding of [^{14}C]Abbott-157378 to human α_1 -acid glycoprotein and albumin (Protocol V96-0351) (Abbott Laboratories Division 46 Report No R&D/96/611, October 1996)
57. Abbott-157378 Drug Metabolism Report No. 22 -*Ex vivo* protein binding of [^{14}C]Abbott-157378 and [^{14}C] Abbott-84538 (ritonavir) in human plasma (Protocol V97-024) (Abbott Laboratories Division 46 Report No. R&D/97/608, November 1997)
58. Abbott-157378 Drug Metabolism Report No. 41 - *Ex vivo* protein binding of [^{14}C]Abbott-157378 in plasma of HIV-infected subjects (Protocol V98-046) (Abbott Laboratories Division 46 Report No. R&D/98/590, January 1999)
59. Abbott-157378 Drug Metabolism Report No. 15 -Comparison of protein binding of [^{14}C]Abbott-157378 in human plasma determined by equilibrium dialysis and ultrafiltration (Protocol V96-060) (Abbott Laboratories Division 46 Report No. R&D/97/195, June 1997)

Tissue Distribution/Accumulation

60. Abbott-157378 Drug Metabolism Report No 21– Tissue Distribution and mass balance of radioactivity after an oral dose of [^{14}C]Abbott-157378 and Abbott-84538 in male rats (Battlelle Study No. N002554A) (Abbott laboratories Division 46 Report No. R&D/97/474, 1997)
61. Abbott-157378 Drug Metabolism Report No. 45 - Lacteal excretion and fetal tissue distribution of radioactivity following a single oral dose of [^{14}C]Abbott-157378 given in combination with ritonavir in the rat (Abbott Laboratories Division 46 Report No. R&D/99/034, 1999)

Enzyme Induction/Inhibition

62. Abbott-157378 Drug Metabolism Report No. 7 - Characterization of the human liver microsomal cytochrome P450 isoforms involved in the oxidative metabolism of [^{14}C]Abbott-157378 (Abbott Laboratories Division 46 Report No. R&D/96/505, September 1996)
63. Abbott-157378 Drug Metabolism Report No. 30 - Effect of Abbott-157378 and ritonavir (Abbott-84538)

on cytochrome P450 and UDP-glucuronosyltransferase activities in cultured human hepatocytes (Abbott Laboratories Division 46 Report No. R&D/97/735, March 1998)

Metabolic Pathways and Metabolites

64. Abbott-157378 Drug Metabolism Report No. 5 - Effect of Abbott-84538 on the biliary excretion of [^{14}C]Abbott-157378 after intravenous or intraduodenal administration to chronically bile duct cannulated rats (Protocols V96-013 and V96-024) (Abbott Laboratories Division 46 Report No. R&D/96/487,1996)
65. Abbott- 157378 Drug Metabolism Report No. 26 -Metabolism and disposition of [^{14}C]Abbott-157378 given in combination with Abbott 84538 (ritonavir) in dogs/Protocols V97-002 and V97-003); Abbott Laboratories Division 46 Report No. R&D/97/668, 1998.

In Vitro Metabolism

66. Abbott-157378 Drug Metabolism Report No 4 - In vitro metabolism of [^{14}C] Abbott-157378 by mouse, rat, dog, monkey and human liver microsomes and by human liver slices and hepatocytes (Abbott laboratories Division 46 Report No. R&D/96/448, 1996)
67. Abbott-84538 Drug Metabolism Report No. 75 - The in vitro permeability and P-glycoprotein-mediated transport of Abbott-157378 and Abbott-84538 across human Caco-2 Cells (Abbott Laboratories Division 46 Report No. R&D/00/262)

Excretion

68. Abbott-157378 Drug Metabolism Report No 6 – Effect of Abbott-84538 on the metabolism and disposition of [^{14}C] abbott-157378 in rats (Protocol V 96-012 and V96-023) (Abbott laboratories Division 46 Report No. R&D/96/486, 1996)

Analytical

69. Abbott-157378 Drug Metabolism Report No. 10 -An HPLC method for the simultaneous determination of Abbott-157378 and ritonavir in human plasma using UV detection (Abbott Laboratories Division 46 Report No. R&D/96/589, September 1996)
70. Abbott-157378 Drug Metabolism Report No. 1 - The preparation of [^{14}C]Abbott-157378 (Abbott Laboratories Division 4 Report No. R&D/96/279, May 1996); non-GLP; Vol. 39, Page 234-245

PHARMACOLOGY

Mechanism of Action

Lopinavir (ABT-378) is a novel peptidomimetic HIV protease inhibitor with 10-fold greater potency than ritonavir (ABT-538). It prevents cleavage of the gag-pol polyprotein, thereby blocking HIV-1 and HIV-2 maturation. The oral bioavailability of ABT-378 is very poor when administered alone. Combination of ABT-378 with ritonavir substantially improves the pharmacokinetic profile of ABT-378, as a consequence of the inhibition of the CYP3A-mediated lopinavir metabolism by ritonavir. Ritonavir is a very potent inhibitor of Abbott-157378 metabolism with IC_{50} values of 0.035 and 0.073 μM in rat and human microsomes, respectively. Additionally, co-administration of ABT-378 and ritonavir produces sustained suppression of HIV replication.

Drug Activity Related to Proposed Indication

The EC_{50} of lopinavir for wild type HIV in 50% human serum is 0.1 μM. Ritonavir serves as a pharmacokinetic enhancer of lopinavir.

Ancillary Pharmacology Studies

1. **Study of ABT-378 in various receptor binding and ion transport assays. CEREP Report: RAP-860020 S 810/830/500. [redacted] Abbott Laboratories, [redacted], 1997.**

ABT-378 was evaluated in a battery of receptor binding and ion transport assays for possible ancillary pharmacological activity. ABT-378, in the absence of serum proteins, at concentrations of 10 μM reduced the binding of reference radio-ligands 47% to 54% of control at the L-type calcium channel, 62% of control at the sodium channel site 2 and 47% of control at the chloride ionophore. Additionally, inhibitions occurred in all potassium channels assays and at the muscarinic M_3 receptor site (<15%).

2. Effects of ABT-378 on K channels (Pharmaceutical Products Division, Drug Discovery, Abbott Laboratories, Scientific Report R&D/97/729, 1997)

ABT-378 was evaluated in a battery of membrane potential and K^+ channels assays, and receptor binding and ion transport assays for possible ancillary pharmacologic activity ABT-378 (0.1 nM to 1 μM) did not affect either ATP-sensitive or calcium-activated K^+ channels or the membrane potential in the rat smooth muscle A10 cell line. ABT-378 (10 pM to 10 μM) had no effect on the voltage-gated K^+ channels or calcium-activated maxi-K^+ channels labeled by [^{125}I]charybdotoxin in a [^{125}I]charybdotoxin binding assay. No displacement of [^{125}I]charybdotoxin binding to rat brain-derived membranes was observed. No effect on calcium-activated maxi-K^+ channel function of ABT-378 (1 nM to 10 μM) was seen. ABT-378 did not inhibit ionomycin-stimulated $^{86}Rb^+$ influx into C6 glioma cells and did not alter the basal levels of $^{86}Rb^+$ influx into rat C6 glioma cells.

SAFETY PHARMACOLOGY

Neurological Effects – in Mice and Rats

3. ABT-538 and ABT-378 CNS general pharmacology profile in the mouse and the rat after P.O. co-administration (I.T.E.M.-LABO Report No D28.1697/1. Pharmaceutical Products Division, Drug Discovery, Abbott Laboratories, Scientific Report R&D/97/079, 1997)

The combined administration of ABT-378 and ritonavir was evaluated for potential central nervous system (CNS) effects on rats and mice using a variety of tests. Locomotor stimulation or depression, motor coordination, hypnotic potentiation, pro-convulsant and anticonvulsant activity, and nociception were assessed in these animals.

Methods and Results

Mice Studies. In the preliminary test, groups of four mice were administered orally with a single dose of 10/5, 30/15, 100/50 or 300/150 mg/kg ABT-378 and ritonavir. Loss of traction, stereotypes (chewing) and ptosis were seen in one of four mice at 30/15 and in two of four mice at 100/50 mg/kg. Additionally, groups of ten mice were administered orally with the same dosages. Loss of traction, stereotypes (chewing), ptosis, slight sedation, and hypothermia were seen in two of four mice at 300/150 mg/kg. No effects on **motor coordination** and **hot plate test** (Nociception) were seen in mice at all dosages. However, an increase in **barbital-induced sleep** was seen in mice at 300/150 mg/kg. Increases in **ethanol-induced sleep** in mice were seen at 10/5 and 30/15 mg/kg, but not at the larger dose combinations. A non-dose related decrease in **electroshock threshold** was seen in mice at 10/5 mg/kg (↓50%), 100/50 mg/kg (↓25%), and 300/150 mg/kg (↓27%). Increases in the number of **tonic convulsions** occurred in mice at 300/150 mg/kg/day. Note that no changes in the latency and number of **convulsions induced by pentylenetetrazol** occurred in mice at all dosages.

Rat Studies. Groups of four rats were administered orally with a single dose of 10/5, 30/15, 100/50 or 300/150 mg/kg ABT-378 and ritonavir. No test article-related effects on **locomotor activity** and **tail-flick test** (Nociception) were seen in rats at all dosages.

Cardiovascular Effects-Rats and Dogs

4. **Burke SE, Cox BF, Polakowski JS, Preusser LC. Cardiovascular profile of ABT-378/ABT-538 in conscious rats, anesthetized dogs, and conscious dogs. Pharmaceutical Products Division, Drug Discovery, Abbott Laboratories, Scientific Report R&D/96/445, 1996.**

The cardiovascular profile of ABT-378 co-administered with ritonavir was evaluated in rats instrumented with telemetry transmitters.

Methods and Results
Conscious male rats (10/group) were dosed by oral gavage with a single dose of either vehicle (5% ethanol: 95% propylene glycol) or a fixed combination (2:1) of ABT-378 (Lot 16-269-AL) and ritonavir (Lot 91-792-AL) of 10/5, 30/15, or 100/50 mg/kg, respectively (dose volume: 2 mL ABT-378 and 4 mL ritonavir/kg/day) The dose combination of 10/5 mg/kg had no effect on heart rate or blood pressure, but dose combinations of 30/15 and 100/50 mg/kg produced mild, sustained **decreases in heart rate**. In the vehicle treated animals, heart rate was elevated by 6-7% at 6 hours after dosing whereas heart rate was reduced by 8% in animals receiving the 100:50 mg/kg dose combination. These **negative chronotropic effects** were observed at peak plasma concentrations of 6.08 ± 0.73 µg/mL of ABT-378 and 2.34 ± 0.75 µg/mL of ritonavir (6 hour time point).

Dog Studies. In a cardiovascular study, pentobarbital-anesthetized male beagle dogs were instrumented to measure both myocardial function and hemodynamic parameters.

Methods and Results
Pentobarbital-anesthetized dog Study. ABT-378 (2, 6 and 20 mg/kg) and ritonavir (1, 3 and 10 mg/kg) were intravenously infused at a fixed 2:1 ratio in one group while vehicle was infused in a second group of pentobarbital-anesthetized dogs (6/group). Each of the 3 dosing combinations was administered over a 30 minute time period for a total infusion protocol time of 90 minutes. Intravenous infusion of the low dose combination (2/1 mg/kg, respectively) produced no cardiovascular effect. After administration of the 6/3 mg/kg dose combination, there were statistically significant reductions in blood pressure and heart rate. These changes occurred at plasma concentrations of 11.95 and 3.68 µg/mL, respectively and were modest. Administration of the 20:10 mg/kg dose combination produced marked and sustained decreases in blood pressure (-12% systolic and -32% diastolic), heart rate (↓43%), and left ventricular contractility (dP/dt max, -30%). The decrease in contractility was accompanied by increases in central venous and left ventricular end-diastolic pressures. These negative chronotropic and inotropic responses were observed at peak plasma concentrations of 33.37 and 17.45 µg/ml, respectively. The responses lasted for 60-minutes (final plasma concentrations of 21.75 and 8.33 µg/mL). Note that plasma levels of sodium pentobarbital were rising in parallel with administration of ABT-378/ritonavir, possibly due to inhibition of CYP3A4 mediated metabolism by Ritonavir. The plasma concentrations of sodium pentobarbital were elevated from a control value of 35.6 to 49.1 µg/mL at the end of the final infusion period.

Methods
Conscious Beagle Dog Study. To address the possible contribution of anesthesia and/or increased pentobarbital levels to the cardiovascular responses noted above, a third cardiovascular study was performed in seven conscious beagle dogs chronically instrumented with telemetry transmitters for measurement of systemic arterial pressure and heart rate. The experimental design was a randomized, 7-way crossover using oral administration of 2:1 fixed dose combinations of ABT-378 and ritonavir. The seven oral treatment groups were 3/1.5, 10/5, 30/15, 100/50, 0/50, 100/0, or 0/0 mg/kg (vehicle), respectively.

Results
In this study, neither mean arterial pressure nor heart rate was altered at oral doses up to and including 100/50 mg/kg. Following administration of the highest dose combination, plasma concentrations of ABT-378 and ritonavir reached a C_{max} of 23.47 and 23.0 (μg/mL at a T_{max} of 4.0 and 3.7 hours, respectively. Thus, the combined administration of ABT-378 and ritonavir produced no cardiovascular effects in conscious beagles at plasma concentrations in excess of those achieved by the end of the protocol in the anesthetized dog study.

Comments
Administration of the 30/15 mg/kg produced negative chronotropic effects in conscious male rats. Administration of the 20/10 mg/kg in pentobarbital-anesthetized male dogs produced marked and sustained decreases in blood pressure (-12% systolic and -32% diastolic), heart rate (↓43%), and left ventricular contractility. Elevated plasma levels of pentobarbital in dogs could have been a factor in the etiology of these responses.

5. **Burke SE, Nelson RA, Cox BF. Effect of ABT-378/ABT-538 on electrocardiographic end-points in pentobarbital-anesthetized dogs. Pharmaceutical Products Division, Drug Discovery, Abbott Laboratories, Scientific Report R&D/97/660, 1997.**

Methods
Because one incident of ventricular tachycardia was observed in a follow-up test in a phase I clinical study (M97-650), a cardiovascular study was performed in **pentobarbital-anesthetized beagle dogs** (5/group) instrumented to measure blood pressure, heart rate and electrocardiographic indices.

Results
Intravenous infusion of ABT-378 and ritonavir at 10/1.5 mg/kg over 60 minutes produced modest decreases of 6% and 16% in mean arterial pressure and heart rate, respectively. The PR interval was increased by 13% at the end of the infusion. These modest hemodynamic and electrocardiographic effects were associated with plasma concentrations of 10.17 mg/mL and 0.93 μg/mL of ABT-378 and ritonavir, respectively. When the infusion rate was increased to deliver additional ABT-378 and ritonavir at a dosing ratio of 20/1.5 mg/kg over a subsequent 60 minutes, mean arterial pressure and heart rate were significantly decreased by -42% and -37%, respectively. These effects were accompanied by a significant increase in PR interval of 37%, while QT interval was unchanged (plasma concentrations of ABT-378 and ritonavir at the end of dosing were 22.4 μg/mL and 1.30 μg/mL, respectively).

NON-CLINICAL TOXICOLOGY

6. **Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Mice (Study No. TD96-220, R&D/96/458, 1996)**

This study was reviewed by Dr. Steve Kunder at HFD-590 (Attachment 1: Pharmacologist's Review-Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Mice). Lot Number: ABT-157378 lot no.16-276-AL; ABT-84538, lot no. 86-701-AL, 1996

The combination of ABT-157378/ABT-84538 was evaluated for acute oral toxicity in male and female Crl: CD-1 mice (4-5-week old, ([redacted]). A single dose of ABT-157378/ABT-84538 was administered to groups of one male and one female mouse in single doses of 20/10, 39/20, 78/39, 156/78, 313/156, 625/313 or 1250/625 mg/kg, followed by 14 days of observation. Mice were weighed weekly. During the first three days following dosing, decreased activity, ataxia, dyspnea, and squinting, ruffled coat were observed. Body weights were unaffected by treatment. All mice survived until necropsy. At necropsy mice were examined for gross pathology; no treatment related clinical signs were seen. The NOEL for this study was less than 20/10 mg/kg.

7. Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-218, R&D/96/456, 1996)

Dr. Steve Kunder at HFD-590 reviewed this study (Attachment 2: Pharmacologist's Review). Lot number: ABT-157378 lot no.16-276-AL, ABT-84538, lot no. 86-701-AL; 1996

The combination of ABT-157378/ABT-84538 was evaluated for acute oral toxicity in male and female Crl:CD(SD)BR rats (5-week old, [redacted]). A single dose of ABT-157378/ABT-84538 was administered to groups of one male and one female rat in single doses of 20/10, 39/20, 78/39, 156/78, 313/156, 625/313 or 1250/625 mg/kg, followed by 14 days of observation. Rats were weighed weekly. Following dosing, observations included alopecia (39/20 mg/kg female); decreased activity/ataxia (78/39 mg/kg and higher doses, males and females), dyspnea (156/78 and higher mg/kg males); salivation (313/156 and 625/313 mg/kg males); squinting (156/78 and higher mg/kg males); the above observations occurred on the day of dosing. On days 2-5 one female rat receiving 1250/625 mg/kg had no stool. Body weight gain was reduced in the male receiving 625/313 mg/kg and in males and females receiving 1250/625 mg/kg. At necropsy rats were examined for gross pathology; no treatment related clinical signs were seen. The NOEL for this study was 39/20 mg/kg.

8. Acute Intravenous Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in mice (Study No. TA96-221, R&D/96/459, 1996)

Vol. 3; **Pages:** 60-88; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 7/29, 1996; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, lot 16-276-AL; Abbott-84538.0, lot 86-701-AL **Formulation:** ABT-157378 stock solution (125 mg/mL) was prepared in a vehicle of propylene glycol/ethyl alcohol (95:5, v/v) while ABT-84538 stock solution (62.5 mg/mL) was prepared in p-toluene sulfonic acid monohydrate (2M)/ethyl alcohol (5%)/propylene glycol (50%). Abbott-84538 dosing solution was prepared using the ABT-84538 stock solution and a vehicle of propylene glycol and ethyl alcohol (95:5, v/v).

Methods

Twelve male and twelve female Crl:CD-1(ICR)BR mice ([redacted] about 4-5 weeks old (18-22g), were treated with ABT-157378/ABT-84538 at doses of 1.0/0.5, 2.0/1.0, 3.9/2.0, 7.8/3.9, 15.6/7.8, 31.3/15.6, or 62.5/31.3 mg/kg dose combinations by a single intravenous administration in a volume of 0.5 ml each drug. Mice were observed daily for 14 days post dosing, and weighed weekly.

Results

None of the mice died in this study. No gross morphological changes were seen in mice euthanized and necropsied at the end of the study. Red colored urine was present in females at 3.9/2.0 mg/kg and greater, and in males at doses of 7.8/3.9 mg/kg and greater. Bruised tail was noted in one male and in four female mice during the observations following treatment. Squinting occurred in males at doses of 7.8/3.9 and lower. No drug related changes in body weight were observed.

Comments

The acute intravenous NOEL for males was < 1.0/0.5 mg/kg, and 2.0/1.0 mg/kg for females. The acute intravenous approximate lethal dose (ALD) for mice was > 62.5/31.3 mg/kg.

9. Acute Intravenous Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-219, R&D/96/457, 1996)

Dr. Steve Kunder at DSPDP, HFD-590, reviewed this study (Attachment 3: Pharmacologist's Review). Lot number: Abbott-157378.0, lot 16-276-AL; Abbott-84538.0, lot 86-701-AL

Twelve male and female Crl:CD (SD)BR rats ([redacted] about five weeks old, were treated with ABT-157378/ABT-84538 at doses of 1.0/0.5, 2.0/1.0, 3.9/2.0, 7.8/3.9, 15.6/7.8, 31.3/15.6, and 62.5/31.3 mg/kg dose combinations by a single intravenous administration in a volume of 0.5 ml

each drug. ABT-157378 was prepared in a propylene glycol/ethylene glycol/ethyl alcohol vehicle while ABT-84538 was prepared in p-toluene sulfonic acid monohydrate/ethyl alcohol/propylene glycol. Rats were observed daily for 13 days following dosing. Rats were weighed weekly. Animals that died prior to the end of the study were necropsied as soon as possible following death. Both males and females at 62.5/31.3 and 31.3/15.6 mg/kg died on the day of dosing. Red colored urine was present in females at 2.0/1.0 mg/kg and greater, and in males at doses of 15.6/7.8 mg/kg and greater. Ataxia occurred in males at doses of 7.8/3.9 and 15.6/7.8 mg/kg. No drug related changes in body weight were observed. The NOEL for males was 3.9/2.0 mg/kg, and 1.0/0.5 mg/kg for females.

10. Single-Dose Oral Toxicity Study of Abbott-157378 in Rats (Study No. TA96-315, R&D/96/669)

Dr. Steve Kunder at DSPDP, HFD-590, reviewed this study (Attachment 4: Pharmacologist's Review). Drug Lot No: 18-306-AL

ABT-157378 was evaluated for acute oral toxicity in male and female Sprague-Dawley rats ([redacted] at single doses of 0, 20, 100, 500 or 2500 mg/kg. Drug was dissolved in propylene glycol/ethanol (95:5, v/v). Mice were distributed into groups of 9 rats /dose/sex. Three rats per group were sacrificed and necropsied on day 14 of the study, three rats per group were sacrificed and necropsied on day 2 of the study and three rats per group were used for plasma drug determinations. Blood was collected at 0.5, 1, 3, 6, 9, 12 and 24 h after dosing. These rats were then sacrificed without necropsy. Rats sacrificed on days 2 and 14 were bled prior to necropsy for hematology and clinical chemistry assays. Body weights were decreased on days -2, 0, 2, 7 and 14. At necropsy, a panel of organs was removed for gross and microscopic pathology. All rats survived until necropsy. Body weights, hematology and clinical chemistry did not appear affected by treatment. Rales and urine stained fur occurred in females receiving 2500 mg/kg, red discharge (male, 500 mg/kg), labored breathing (male and female, 500, 2500 mg/kg), tremors (male, 20 mg/kg), all occurring on the day of dosing. No drug related pathology signs were seen at necropsy or in microscopic examination. The NOEL of this study appears to be 100 mg/kg.

Repeat Dose Studies

11. Three-Month Oral Maximum-Tolerated Dosage Study with Abbott-157378 in Combination with Abbott-84538 in mice (Study No. TA97-029, R&D/97/501)

Vol. No.: 4; Pages: 8-324; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation: 4/22/1996; GLP Compliance: Yes (X); Drug Lot:** Abbott-157378.0, Lot No. 22-363-NI-00; Abbott-84538.0, lot No. 24-215-AL; **Formulation:** Abbott-157378 SEC formulations containing 20% (w/w) ABT-157378.0. Abbott-84538 soft elastic capsule (SEC) formulations containing 20% (w/w) ABT-84538.0. Excipients include oleic acid, ethanol, polyoxyl 135 castor oil and butylated hydroxytoluene (BHT). Formulations for Abbott-84538 and Abbott-157378 were combined into a single dosing formulation for the study.

Methods

Groups of ten male and ten female Crl: CD-1 (ICR) BR mice (about 7 weeks old, 22-35g; ([redacted] were orally treated with ABT-157378/ABT-84538 by gavage at doses of 0/0 (placebo formulation), 20/10, 60/30, or 200/100 mg/kg/day dose combinations for 90 consecutive days (dose volume: 2 ml/kg/day). Mice were observed daily during the pre-treatment and treatment for survival and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. Food consumption was recorded weekly. All animals were subjected to a gross necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, testes** (male only), **heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. Test article-related mortality was seen at 200/100 mg/kg/day (3/20). Decreased activity, distended abdomen, emaciation, dehydration, hunched posture, tremors and cold to touch were seen in some animals at 200/100 mg/kg/day. Dose-related distended abdomen and matted hair coat and urine stained hair occurred in all drug-treated groups.

Body weights and food consumption. Increases in mean body weights and body weight gain were seen in male and female mice at 200 mg/kg/day, and seen in male mice at 60/30 mg/kg/day. The increased body weight and body weight gain was related to distention of the gastrointestinal tract seen these mice. At the end of the dosing period, mean body weights of males and females at 200/100mg/kg were 109 and 117% of control, respectively; mean body weights of males at 60/30 mg/kg were 106% of control. No consistent changes in feed consumption were seen at any dose level.

Hematology. Reductions in RBC count (-10%), and hemoglobin (-13%) and hematocrit (-16%) values were seen in the female mice at 200/100 mg/kg/day.

Clinical Chemistry. Elevations of ALT (6-fold), AST (2-fold) and GGT (5-fold) activities along with increases in mean values of cholesterol (1.5-fold) and triglycerides (1.7-fold) were seen in at 200/100 mg/kg/day. Mild increases in mean cholesterol (1.3-fold) and triglycerides (1.3-fold) values were also seen in mice at 60/30 mg/kg/day.

Histopathology. Gas-filled small intestines and stomach as well as accentuated lobular pattern in the liver and hepatic discoloration were seen in mice at 200/100 mg/kg/day. One female mouse at 60/30 mg/kg/day also showed gas-filled small intestines and cecum at necropsy. Increase in liver weight was seen in mice at 60/30-mg/kg/day and 200/100-mg/kg/day. Hepatic cytoplasmic vacuolation, necrosis and subacute inflammation, moderate to marked microvesicular cytoplasmic vacuolation of the renal cortex, as well as lipid accumulation and electron dense inclusions in the liver and myeloid bodies in renal cortex were also observed in mice at 200/100 mg/kg/day.

Comments

The NOAEL was 60/30 mg/kg/day for this study (AUC values: 121/12 µg•hr/mL).

Toxicokinetics

Methods

An additional 5 mice/sex in the control group and three drug-treated satellite groups (25/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo formulation), 20/10, 60/30, or 200/100 mg/kg/day dose combinations for 63 days. From each animal, 0.5 mL venous blood samples at all dose levels were collected into heparinized tubes by cardiac puncture at 1.5, 3, 7, 12 and 24 hours postdose on Day 62. Plasma levels of Abbott-157378 and Abbott-84538 were measured by [redacted]'-MS/MS method.

Results

Pleak plasma concentration (Cmax) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Day 62 are summarized in Table 1.

Comments

Plasma levels of Abbott-157378 and Abbott-84538 were increased in a dose-dependent manner. No consistent differences were seen in plasma drug levels between sexes.

Table 1. Plasma concentration of Abbott-157378 and Abbott-84538 in mice after 63 days oral doses of Abbott-157378 and Abbott-84538

Dose (mg/kg/day)	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
20/10	[redacted]							
60/30								
200/100								

12. Two-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Rats (Study No. TA96-079, R&D/96/300, 1996)

Vol. No.: 5; Pages: 1-288; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 2/27/1996; GLP Compliance: Yes (X); Drug Lot: Abbott-157378.0, Lot No. 328306-0-AX; Abbott-84538.0, lot No. 95-852-AL; Formulation: ABT-157378 stock solution (125 mg/mL) was prepared in a vehicle of propylene glycol/ethyl alcohol (95:5, v/v) while ABT-84538 stock solution (62.5 mg/mL) was prepared in p-toluene sulfonic acid monohydrate (2M)/ethyl alcohol (5%)/propylene glycol (50%). Abbott-84538 dosing solution was prepared using the ABT-84538 stock solution and a vehicle of propylene glycol and ethyl alcohol (95:5, v/v).

Methods

Groups of ten male and ten female Sprague-Dawley rats (Crl:CD®(SD)BR, 6 weeks old, 160-250 g; [redacted] were orally treated with ABT-157378 and ABT-84538 by gavage at doses of 0/0 (placebo formulation), 10/5, 30/15, or 100/50 mg/kg/day dose combinations for 14 consecutive days. At the time of treatment, each rat received Abbott-84538 first, with the Abbott-157378 dose being administered promptly afterwards. A dose volume of each drug was used to achieve a combined daily dose of 2 mL/kg. Prior to and at the end of the study (Day 13), rats were given a physical and Ophthalmoscopic examination, respectively. Rats were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. All animals were subjected to a gross necropsy on Day 13, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1). Electron microscopic examination was conducted for liver from 4 rats in the control group and 4 rats in the 100/50-mg/kg/day group.

Results

Clinical signs and mortality. No drug-related deaths were seen. One female rat in the vehicle control group was found dead on Day 11. No overt signs of toxicity were seen in rats at all doses.

Body weights and food consumption. No mean body weights and body weight gain changes were seen in rats at all doses. A transient change in food consumption was seen at 100/50 mg/kg/day.

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

Hematology. Dose-dependent increases in mean reticulocyte counts, mean platelet counts, and mean partial thromboplastin time were observed at 30/15 mg/kg/day or higher. Mean reticulocyte counts (+125%), mean platelet counts (+114%) and mean partial thromboplastin time (+118%) were increased in rats at 100/50 mg/kg/day.

Clinical Chemistry. Elevation of albumin was seen in male rats (+108%) at 100/50 mg/kg/day. Increases in globulin (+110%) were seen in both sexes at 100/50 mg/kg/day. Decreases in mean sodium (-4 mM) and chloride (-3.4 mM) values were seen in both sexes at 100/50 mg/kg/day. Dose-dependent decreases in serum T4 and increases in TSH were observed at 10/5 mg/kg/day or higher. Serum T4 was decreased from the control levels of 5.5 µg/dL (male) or 3.0 µg/dL (female) to 3.9 µg/dL (male) or 1.4 µg/dL (female) at 100 mg/kg/day, respectively. TSH was increased from the control level of 2.1 ng/dL (male) or 1.4 ng/dL (female) to 4.6 ng/dL (male) or 3.8 ng/dL at 100 mg/kg/day, respectively.

Histopathology. Increases in mean absolute and relative liver weights (relative to body weight) were seen in rats at 30/15 mg/kg/day or higher. Hepatocytomegaly was seen in rats at 100/50 mg/kg (10/10 males and 8/10 females). Increases in mean absolute thyroid weights were seen in male and female rats at 100/50 mg/kg/day. Increases in relative thyroid weights (relative to body weight) were seen in male rats at 100/50 mg/kg/day and in female rats at 30/15 mg/kg. Increases in mean relative spleen weights were seen in female rats at 30/15 mg/kg/day or higher. Increases in mean absolute spleen weights were seen in male rats at 100/50 mg/kg/day (Tables 2 and 3). Microscopically, hypertrophy of follicular

epithelium and reduced follicular diameter of the thyroid follicle was seen in rats at 100/50 mg/kg/day (9/10 males and 7/10 females). Splenic erythropoiesis was seen in females at 30/15-mg/kg/day (2/10) and 100/50-mg/kg/day (5/10), respectively. A test article treatment-related poikilocytosis was seen in rats at all doses. Additionally, a slight increase in number of lysosomes and peroxisomes was observed in centrolobular hepatocytes in livers from the 100/50 mg/kg/day group.

Comments

The NOAEL for two weeks of exposure was 30/15 mg/kg/day. Test article-related histological changes were seen in rats at 30/15 mg/kg/day or higher, including blood (reticulocytosis), liver (hepatocytomegaly), spleen (extra-medullary erythropoiesis) and thyroid (hypertrophy). Increase in TSH that was secondary to the decreased plasma T4 correlated with thyroid follicular hypertrophy. The highest Abbott-84538 plasma exposure in the present study was 16.4 µg•hr/mL at 100/50 mg/kg/day, which is below the threshold level previously observed. Note that Abbott-84538 caused mild to moderate follicular cell hypertrophy at 50 mg/kg/day in a previous one-month rat study (AUC of 26 µg•hr/mL). Therefore, it is possible that Abbott-157378 may also contribute to the decreased T4 and thyroid pathology.

Table 2. Changes in Organ Weights in Male Rats with 14-days oral administration of Abbott-157378 and Abbott-84538 (Day 13)

Treatment (mg/kg)	Body Weight	Liver (g)		Thyroid (g)		Spleen (g)	
	g	g	BW%	g	BW%	g	BW%
0/0	308.6	11.1	3.6	0.016	0.005	0.72	0.23
10/5	307.9	11.2	3.6	0.019	0.006	0.74	0.24
30/15	311.0	12.3	3.5	0.019	0.006	0.81	0.26
100/50	300.8	15.0	6.0	0.021	0.007	0.81	0.27

*Abbott-157378/Abbott-84538

Table 3. Changes in Organ Weights in female Rats with 14-days oral administration of Abbott-157378 and Abbott-84538

Treatment* (mg/kg)	BW**	Liver (g)		Thyroid (g)		Spleen (g)	
	g	g	BW%	g	BW%	g	BW%
Vehicle	211.5	7.2	3.5	0.015	0.007	0.55	0.26
10/5	214.8	7.5	3.5	0.017	0.008	0.54	0.25
30/15	220.2	9.1	4.1	0.018	0.008	0.67	0.30
100/50	209.3	11.8	5.6	0.020	0.009	0.70	0.33

* Abbott-157378/Abbott-84538

Toxicokinetics

Methods

An additional 5 rats/sex in the control group and three drug-treated satellite groups (5/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (vehicle), 10/5, 30/15, or 100/50 mg/kg/day dose combinations for 14 days. Blood samples (0.3-0.4 mL/sample) were collected from the orbital plexus at 1, 2, 4, 8, 12, 15 and 24 h after dosing on Days 1 and 9. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted]-MS/MS method.

Results

Pleak plasma concentration (Cmax) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Day 9 are summarized in Table 4.

Comments

Plasma levels of Abbott-157378 were increased in less than a dosage proportional manner with increasing dosage on Day 9. For Abbott-84543, AUC values increased in a greater than dosage proportional manner on Day 1. No consistent differences were seen in plasma drug levels between sexes.

Table 4. Pharmacokinetics parameters of Abbott-157378 and Abbott-84538 in rats following oral administration of Abbott-157378 and Abbott-84538 (Day 9)

Dosage* (mg/kg/day)	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
10/5	2.0	2.1	16.7	24.5	0.2	0.2	0.7	1.7
30/15	4.0	6.0	31.8	57.2	0.3	1.2	1.4	5.2
100/50	10.5	9.1	107.2	134.2	1.0	2.0	6.9	13.3

* Abbott-157378/Abbott-84538

13. Three-Month Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Rats (with a One-Month Recovery Period), Study No. TA96-156, R&D/96/574

Vol. No.: 8; **Pages:** 1-264; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date Initiation:** 5/28/1996; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 16-276-AL and 18-306-AL; Abbott-84538.0, lot No. 86-701-AL; **Formulation:** ABT-157378 dosing solutions (10, 50 and 150mg/mL) were prepared in a vehicle of propylene glycol/ethyl alcohol (95:5, v/v) while ABT-84538 dosing solutions (5, 25 and 75 mg/mL) were prepared in p-toluene sulfonic acid monohydrate (2M)/ethyl alcohol (5%)/propylene glycol (50%).

Methods

Groups of fifteen male and fifteen female Sprague-Dawley rats (Crl:CD®(SD)BR, 6 weeks old, 125-225 g; [redacted] were orally treated with ABT-157378 and ABT-84538 by gavage at doses of 0/0 (vehicle), 10/5, 50/25, or 150/75 mg/kg/day dose combinations for 90 consecutive days. At the time of treatment, each rat received Abbott-84538 first, with the Abbott-157378 dose being administered promptly afterwards. All rats received a volume of 1 mL/kg/day for each drug at each treatment. At the end of the treatment period, 5 rats/sex/group were maintained without treatment for 34 or 35 days to determine the reversibility of adverse effects. All animals were observed at least twice daily during the pretreatment, treatment and recovery periods for survival, physical condition and behavior. Food consumption and body weights were measured once pretreatment and weekly during the treatment and recovery period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. A complete ophthalmoscopic examination was performed on rats at pretreatment, treatment and recovery periods. All animals were subjected to a gross necropsy at the end of the treatment and recovery periods, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on Days 28, 32, 33, 34, 56, or at the terminal necropsy. Wet tissue weights were obtained from the following organs: **brain, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined microscopically by a pathologist (Appendix 1). Electron microscopic examination was conducted for liver, kidney, and eyes from 4 rats in the control group and 4 rats in each dosing group at the end of the treatment and recovery period.

Results

Clinical signs and mortality. No drug-related deaths were seen. Two rats in the vehicle control group were found dead on Day 77 and on Day 69. Gavage-related trauma was the reason for these deaths. Drug-related clinical signs including dehydration, emaciation and weakness were seen in rats at 150/75 mg/kg/day. Matted hair and hunched posture were observed in female rats at 50/25 mg/kg or greater.

Body weights and food consumption. Mean body weights were statistically significantly deceased in rats at 150/75 mg/kg/day (Table 5). Food consumption was decreased in males at 150/75 mg/kg/day for five of the twelve intervals. Food consumption was decreased in males at 150/75 mg/kg/day for the first two weeks of the study. Increases in body-weight gain and food consumption were seen in rats at 150/75 mg/day.

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

Hematology. Dose-dependent increases in mean reticulocyte numbers and mean partial thromboplastin time were observed at 150/75 mg/kg/day. Mean reticulocyte counts was increased in rats at 50/25 mg/kg/day and greater. Poikilocytosis in erythrocyte morphology were seen in rats at all drug dosages.

Related but minimal reductions in Hb and RBC were seen in male rats at 150/75 mg/kg/day. Increases in RDW were seen in females at 50/25 mg/kg/day or greater (Table 6).

Clinical Chemistry. An increase in the hepatic glutamyl transferase (GGT) was seen in rats at 150/75 mg/kg (40-fold). Elevation of albumin was seen in male rats at 50/25 mg/kg/day (+110%) and 150/75 mg/kg/day. An increase in cholesterol (1.5 to 2-fold) was seen in rats at 50/25 mg/kg/day (+140%) and 150/75 mg/kg/day (200%). Decreases in mean triglyceride were seen in males at 50/25 mg/kg/day (-130%) or higher (-200%). Dose-dependent decreases in serum T4 and increases in TSH were observed at 50/25 mg/kg/day or higher. Serum T4 was decreased from the control levels of 4.4-5.1 µg/mL (male) or 2.5-2.9 µg/mL (female) to 2.1-2.5 µg/mL (male) or 0.8-1.0 µg/mL (female) at 150/75 mg/kg/day, respectively. TSH was increased from the control level of 2.4-3.0 ng/mL (male) or 1.3-1.5 ng/mL (female) to 4.5-6.3 ng/mL (male) or 5.6-7.0 ng/mL at 150/75 mg/kg/day, respectively. Following the one-month recovery period all toxicologic changes were reversed with exception of the cholesterol increase in the females at 150/75 mg/kg/day.

Histopathology. Increases in mean absolute and relative liver and thyroid weights (relative to body weight) were seen in rats at 50/25 mg/kg/day or higher (Tables 7 and 8). Hepatocytomegaly was observed in male (1/10) and female rats (2/10) at 50/25 mg/kg and 100/75 mg/kg/day (8/10/sex). Multinucleation of hepatocytes was seen microscopically in rats at 50/25 mg/kg/day (three-month males: 7/10; recovery males: 4/5) and 150/75 mg/kg/day (three-month males: 4/10; recovery males: 5/5; three-month females: 2/10; recovery females: 1/5). Increases in mean absolute spleen weights were seen in male rats at 50/25mg/kg/day and 100/75 mg/kg/day (Tables 7 and 8). Microscopically, mild hypertrophy of follicular epithelium was seen in rats at all doses, which was drug-related and was returned to normal during the recovery period. Additionally, slight increases in number of lysosomes, peroxisomes, and smooth endoplasmic reticulum (SER) were observed in centrilobular hepatocytes in livers from the 150/75mg/kg/day group. Following the one-month recovery period all toxicologic changes were reversed with exceptions of erythrocyte morphological changes and multinucleated hepatocytes and hepatic lysosomal inclusions.

Table 5 Changes in body weights and body weight gain in rats with three-month oral administration (with a one-month recovery period) of Abbott-157378 and Abbott-84538

Treatment *mg/kg/day**	Day 0 Body Wt (g)		Day 7 Body Wt (g)		Day 28 Body Wt (g)		Day 90 Body Wt (g)		Day 118*** Body Wt (g)		Body Wt Change *Days 0-90*		Body Wt Change *Days 90-118*	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0/0	197	165	261	194	424	265	628	343	663	367	431	177	54	20
10/5	198	165	257	190	412	262	623	354	666	391	427	201	51	21
50/25	198	164	261	186	412	256	595	338	687	345	397	162	65	22
150/75**	197	165	245	177	367	232	509	280	585	330	310	92	82	72

*Abbott-157378/Abbott-84538; M: male; F: female. ** Statistical significant decreases in mean body weight and body weight gain in rats on Day 7, 28, 90, or 118 were seen (P<0.01). *** Five rats per sex in each of these dosing groups were held for an additional one-month period without treatment.

Table 6 Hematological changes in rats with three-month (with a one-month recovery period) oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg)*	APTT (s)		Retics (10^3/µL)		RBC (10^6/µL)		HB (g/dL)	
	M	F	M	F	M	F	M	F
0/0	18.5	16.2	208.1	156.5	9.1	7.7	15.2	14.4
10/5	19.3	15.4	179.9	164.8	8.8	8.0	14.7	14.8
50/25	22.9	15.7	245.2**	238.5**	8.7	7.4	14.4	13.4
150/75	28.9**	19.1**	339.7**	319.7**	8.6	8.1	14.4	13.9

* Abbott-157378/Abbott-84538; ** P<0.01

Table 7 Organ weight changes in male rats with three-month (with a one-month recovery period) oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg)*	Body Weight	Liver		Thyroid		Spleen	
	g	g	BW%	g	BW%	g	BW%
0/0	615.2	20.1	3.3	0.024	0.003	1.0	0.16
10/5	604.7	20.4	3.4	0.025	0.004	1.0	0.16
50/25	559.9	22.1**	3.9**	0.030**	0.005**	1.1**	0.20**
150/75	481.2**	27.1**	5.6**	0.030**	0.006**	1.2**	0.24**

*Abbott-157378/Abbott-84538; ** P<0.01

Table 8 Organ weight changes in female rats with three-month (with a one-month recovery period) oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg)*	Body Weight	Liver		Thyroid		Spleen	
	g	g	BW%	g	BW%	g	BW%
0/0	326.7	8.7	2.7	0.019	0.006	0.57	0.18
10/5	326.6	9.6	2.9	0.019	0.006	0.61	0.19
50/25	328.2	15.7**	4.8**	0.024**	0.007**	0.83**	0.25**
150/75	266.6**	17.8**	6.7**	0.029**	0.01**	0.75**	0.28**

* Abbott-157378/Abbott-84538; ** P<0.01

Comments

The NOAEL for this study was 50/25 mg/kg/day. Test article-related histological changes were seen in rats at 50/25 mg/kg/day or higher, including thyroid (hypertrophy), blood (reticulocytosis), and liver (hepatocytomegaly). An increase in TSH that was secondary to the decreased plasma T4 correlated with thyroid follicular hypertrophy. The AUC of Abbott-84538 in the present study was 12.8 (males) and 12.2 µg•hr/mL (females) at 150/75 mg/kg/day on Day 83, which is below the threshold level previously observed. Note that Abbott-84538 caused mild to moderate follicular cell hypertrophy at 50 mg/kg/day in a previous one-month rat study (AUC of 26 µg•hr/mL). Therefore, it is possible that Abbott-157378 may also contribute to the decreased T4 and thyroid pathology.

Toxicokinetics

Methods

An additional 5 rats/sex in the control group and three drug-treated satellite groups (5/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378 and ABT-84538 at doses of 0/0 (vehicle), 10/5, 50/25, or 150/75 mg/kg/day dose combinations for 90 days. Blood samples (0.3 mL/sample) were collected from the orbital plexus at 1, 2, 4, 8, 12, 15 and 24 h after dosing on day 28 and 84. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted] MS/MS method. The lower quantifiable limits (LQL) of the method as established were [redacted] for Abbott-157378 and [redacted] for ritonavir based on 1 mL of plasma.

Results

Pleak plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Day 84 are summarized in Table 9. Maximal plasma Abbott-157378 levels were observed in males at 3-5 hours and in females at 4-10 hours following treatment on Day 84. The Abbott-157378 AUC values did not indicate dose-proportional increase in either sex on Day 84. Maximal plasma Abbott-84538 levels were observed in males and females at 2-6 hours following treatment on Day 84. The Abbott-84538 AUC values did not indicate a dose-proportional increase in either sex on Day 84. The mean Abbott-157378 AUC values were about 9 to 17 times greater than the mean ritonavir AUC values for each respective dosage group on Day 84.

Comments

Females appear to have higher Abbott-157378 AUCs than males at all dosages.

Table 9 Plasma concentration of Abbott-157378 and Abbott-84538 in neonatal rats after 3-months oral administration of Abbott-157378 and Abbott-84538 (Day 84)

Dosage (mg/kg/day)*	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
10/5								
50/25								
150/75								

*Abbott-157378/Abbott-84538

14. Six-Month Oral Toxicity Study with Abbott-157378 in Combination with Abbott-84538 (Ritonavir) in Rats, Study No. TA97-002, R&D/97/720

Vol. No.: 10; **Pages:** 1-360; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 2/24/97; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 22-363-NI-00; Lot No. 25-378-AR-XX; Abbott-84538.0, Lot No. 24-215-AL, Lot No. 25-377-AR-XX; **Formulation:** 20% of ABT-157378 (w/w); 20% Abbott-84538 (w/w). The vehicle solution used for dilution of stock formulations consisted of

Methods

Groups of twenty male and twenty female Sprague-Dawley rats (Crl:CD®(SD)BR, 8 weeks old, 180 to 367 g; were orally treated with ABT-157378 and ABT-84538 by gavage at doses of 0/0 (vehicle), 10/5, 50/25, or 150/75 mg/kg/day dose combinations for 6-months (dose volume: 2 mL/kg). Note that dosage in the 150/75 mg/kg/day groups were reduced to 100/50 mg/kg/day on Days 11 (in females) and 99 (in males). All animals were observed at least twice daily during the pretreatment, treatment and recovery periods for survival, physical condition and behavior. Food consumption and body weights were measured three to four times pretreatment and weekly during the treatment period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. A complete ophthalmoscopic examination was performed on rats pretreatment and at the end of treatment period. Hematology and clinical chemistry were evaluated in all animals on Day 85 and at the terminal necropsy. All animals were subjected to a gross necropsy at the end of the treatment period, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Wet tissue weights were obtained from the following organs: **brain, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined microscopically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No drug-related deaths were seen. Two rats in the vehicle control group (Days 133 and 142), three rats in the 10/5 mg/kg/day (Days 125 and 167), and three rats in the 150/75 mg/kg/day were found dead (Days 70, 94 and 104). Gavage-related trauma was the reason for these deaths. One male rat death in the 50/25 mg/day group (Day 119) and six male rats deaths in the 150/75 mg/kg/day (Days 40-94) were related to the drug treatment. On Day 99 the dosage for males in this group was reduced to 100/50 mg/kg/day as a result of cumulative deaths. Drug-related clinical signs including dehydration, emaciation and weakness were seen in rats at 50/25 mg/kg/day. Matted hair and hunched posture were observed in female rats at 10/5 mg/kg or greater.

Body weights and food consumption. Mean body weights were statistically significantly deceased in male rats at 150/75 mg/kg/day during the first week of treatment and thereafter. Note that the dosage for males in this group was reduced to 100/50 mg/kg/day on Day 99. At the end of the treatment period, body weight gain for 100/50 mg/kg/day males was 76% of control levels. Mean body weights were deceased in female rats at 150/75 mg/kg/day (-78%) during the first week of treatment and thereafter. Note that the dosage for males in this group was reduced to 100/50 mg/kg/day on Day 11. At the end of the treatment period, body weight gain for 100/50 mg/kg/day males was 76% of control levels. Food consumption was decreased in males (-30%) and in females (-61%) at 150/75 mg/kg/day during the first two weeks of

treatment, which returned to control levels during the third week of treatment. A less profound decrease in food consumption was also seen in males and in females at 50/25 mg/kg/day during the first weeks of treatment, which returned to control levels during the second week of treatment (Table 10).

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

Hematology. Dose-dependent increases in mean reticulocyte numbers and mean partial thromboplastin time were observed at 150/75 mg/kg/day. Mean reticulocyte counts was increased in rats at 50/25 mg/kg/day and greater. Poikilocytosis and anisocytosis in erythrocyte morphology were seen in rats at 50/25 mg/kg/day or greater. Related reductions in Hb, MCV, MCH, and RBC were seen in male rats at 50/25 mg/kg/day or greater. Increase in RDW and reticulocytes were seen in females at 50/25 mg/kg/day or greater. Direct Coomb's test on RBCs from rats at 100/50 mg/kg/day was negative, which did not support the immune-mediated damage of RBC. Note that the sponsor proposed that drug-related alterations in RBCs might be associated with the imbalances in lipid/cholesterol in the cell membrane. Increases in platelets and WBC (specifically lymphocytes and monocytes) were seen in rats at 50/20 mg/kg/day or greater. Prolonged APTT and PT were seen in male rats at 50/25 mg/kg or greater (Table 11). Note that the sponsor has attribute the prolonged APTT and PT to an impaired coagulating factor synthesis caused by the test articles.

Clinical Chemistry. Elevation of cholesterol, total protein and globulin levels were seen in rats at 50/25 mg/kg/day or greater. An increase in albumin and a decrease in triglycerides were seen in male rats at 50/25-mg/kg/day or greater. These changes in lipid and proteins were related to alter hepatic lipid/protein synthesis (Table 12). Increases in BUN values were seen in three male rats and four female rats at 150/75 mg/kg/day (20-30 mg/dL). An increase in the hepatic glutamyl transferees (GGT) was seen in rats at 50/25 mg/kg or higher. Increases in AP were seen in rats at 100/50 mg/kg/day. Increases in ALT were seen in male rats at 10/5 mg/kg/day (6/18), 50/25 mg/kg/day (8/19), and 100/50 mg/kg/day (8/12). Decreases in AST were seen in female rats at 50/25 mg/kg/day or greater. Except for lower AST in females, changes in serum enzymes in rats at 50/25 mg/kg/day or greater were consistent with drug-related hepatic/biliary injury (Table 13). Decreases in serum sodium levels were seen in rats at 100/50mg/kg/day. Decreases in serum chloride levels were seen in rats at 50/25 mg/kg/day or greater. Dose-dependent increases in TSH were observed in female rats at all doses. Dose-dependent decreases in serum T4 were observed in rats at 50 mg/kg/day or greater (Table 14). Note that lower T4 and higher TSH have been attributed to increased glucuronidation and elimination of T4 by the liver in Abbott-84538 treated rats. Serum T4 was decreased from the control levels of 3.3 µg/mL (male) or 2.2 µg/mL (female) to 2.1µg/mL (male) or 1.3 µg/mL (female) at 150/75 mg/kg/day, respectively. TSH was increased from the control level of 2.8 ng/mL (male) or 1.1 ng/mL (female) to 4.1 ng/mL (male) or 4.5 ng/mL at 150/75 mg/kg/day, respectively.

Histopathology. Increases in mean absolute liver, spleen, and thyroid weights (relative to body weight) were seen in females at ≥ 10/5 mg/kg/day. Increases in mean absolute liver and spleen weights were seen in males at ≥ 50/25 mg/kg/day (Tables 15 and 16). Multinucleation of hepatocytes was observed in male and female rats at ≥ 50/25 mg/kg. Hepatocytomegaly, single cell necrosis, histiocytosis, karyomegaly, and hematopoiesis were also seen in rats at 50/25 mg/kg/day or higher. Typically, livers from the highest dose groups had varying numbers of multinucleated hepatocytes, and hypereosinophilic hepatocytes with pyknotic nuclei (single-cell necrosis) scattered randomly within acini; more severely affected livers also had prominent hepatocytes with very large nuclei (karyomegaly). Multinucleated hepatocytes contained greater than two nuclei and variable quantities of eosinophilic cytoplasm. To a lesser extent, sinusoids had rare to occasional Kupffer cells with copious yellow to light brown heterogeneous material (histiocytosis). Three rats that died spontaneously had diffuse coagulative centrilobular necrosis with congestion and a minimal inflammatory, infiltrate of neutrophils. Erythroid hematopoiesis was scattered within livers of more affected rats, usually males. Localized centrilobular hepatocytomegaly was clearly present in only two rats. An eosinophilic hepatocellular focus was found in the liver of a single high-dose female, which was likely an incidental age-related lesion, but it could also be reflective of increased proliferative activity in the liver. Increases in mean absolute thyroid weights were seen in male and female rats at 150/75 mg/kg/day. Increases in relative thyroid weights (relative to body weight) were

seen in male rats at 150/75 mg/kg/day and in female rats at 50/25 mg/kg. Thyroids in females from the ≥50/25 mg/kg/day groups had clearly dose-related morphologic follicular epithelial hypertrophy and/or hyperplasia; the change was less dramatic in males, but the incidence was clearly related to dose and compatible with higher TSH. Increases in mean absolute spleen weights were seen in mid- and high-dose groups. Spleens were morphologically normal, except for histiocytosis; widely scattered clusters of macrophages were filled with yellow to light brown pigment and were most easily identified in splenic white pulp. Histiocytosis was not considered responsible for the increase in splenic weights. There was no additional evidence of erythrocyte destruction (e.g. hemosiderin was not increased). Note that histiocytosis in spleen was previously seen in rats treated with ritonavir, which was compatible with phospholipidosis. Additionally, increased incidence of histiocytosis in mesenteric lymph nodes was seen in high-dose groups. Note that the sponsor considered that the observation was not toxicologically significant and did not examine the intermediate dose groups. Lymphoid depletion in the thymus was seen in spontaneous deaths in 100/50 mg/kg/day males, a 10/5 mg/kg/day female and in a control male that died spontaneously. Adrenal hypertrophy in one 100/50 mg/kg/day male and one control female was also seen. Myocardial degeneration and inflammation, multifocal nephropathy and mineralization, sporadic pulmonary inflammation and foam cell accumulation, multi focal microgranulomas in the liver, pancreatic islet cell hyperplasia, pancreatic inflammation and exocrine atrophy, cysts of the pituitary and thyroid gland, inflammation of the prostate, minimal degeneration in the testis were seen in one high-dose male. A solitary astrocytoma in the cerebrum was also found in one high-dose male.

Table 10 Changes in body weights and body weight gain in rats with six-months oral administration of Abbott-157378 and Abbott-84538

Treatment (*mg/kg/day*)[a]	Day 0 Body Wt (g)		Day 9 Body Wt (g)		Day 27 Body Wt (g)		Day 90 Body Wt (g)		Day 181 Body Wt (g)		Body Wt Change *Days 0-181*	
	M	F	M	F	M	F	M	F	M	F	M	F
0/0	330	212	378	237	445	270	566	317	638	358	308	146
10/5	331	212	382	236	457	274	586	327	644	388	310	176
50/25	333	212	376	236	447	283	570	333	618	367	284	155
150/75[b]	332	209	354[c]	185[c]	418[c]	264[c]	506[c]	307[c]	569[c]	321[c]	236[c]	111[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 11 Hematological changes in rats with six-months oral administration of Abbott-157378 and Abbott-84538 (on Day 182)

Treatment (*mg/kg/day*)[a]	APTT (s)		Retics (10^3/μL)		Platelet (10^3/μL)		WBC (g/dL)	
	M	F	M	F	M	F	M	F
0/0	19.4	18.3	173.2	156.5	1117	1014	8.5	5.6
10/5	20.9	18.3	165.4	164.8	1028	1004	8.4	5.5
50/25	22.9	18.3	215.4[c]	238.5[c]	1232[c]	1211[c]	11.3	7.1
150/75[b]	35.4[c]	18.0	260.3[c]	319.7[c]	1259[c]	1211[c]	11.4[c]	9.3[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 12 Clinical chemistry in rats with six-months oral administration of Abbott-157378 and Abbott-84538 (on Days 85 and 182)

Treatment (*mg/kg/day*)[a]	Cholesterol (mg/dL)				Glucose (mg/dL)			
	M		F		M		F	
	D85	D182	D85	D182	D85	D182	D85	D182
0/0	65.1	74.5	87.9	83.2	95.3	144.8	87.6	143.1
10/5	72.4	77.1	93.2	98.6	132.0	151.7	100.5	163.7
50/25	91.6[c]	98.2[c]	214.4[c]	173.8[c]	120.4	131.9	103.3	151.9
150/75[b]	171.8[c]	120.3[c]	155.1[c]	213.3[c]	106.1	126.4	99.8	133.7

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 13 Clinical chemistry in rats with six-months oral administration of Abbott-157378 and Abbott-84538 (on Days 85 and 182)

Treatment (mg/kg/day)[a]	ALP (IU/L)				ALT (IU/L)				GGT (IU/L)			
	M		F		M		F		M		F	
	D85	D182	D85	D182	D85	D182	D85	D182	D85	D182	D85	D182
0/0	81.5	87.1	43.3	40.1	38.3	32.5	53.1	64.7	1.8	0.1	0.5	0.5
10/5	94.7[c]	85.6	52.0[c]	39.5	59.9	68.9	57.6	84.1	0.4	0.3	1.0	0.9
50/25	94.9[c]	109.9[c]	85.2[c]	49.8[c]	51.1	76.2	38.6	46.7	0.6	2.2	10.6[c]	3.3[c]
150/75[b]	162.0[c]	142.1[c]	68.0[c]	65.3[c]	79.7[c]	79.9[c]	91.9[c]	50.3	14.6	9.0	0.6	14.1[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 14 Clinical chemistry in rats with six-months oral administration of Abbott-157378 and Abbott-84538 (on Day 182)

Treatment (mg/kg/day)[a]	BUN (mg/dL)		Na (mmol/L)		Bilirubin (mg/dL)		TP (g/L)	
	M	F	M	F	M	F	M	F
0/0	12.2	15.3	146.8	144.5	0.3	0.2	7.7	8.5
10/5	13.8	14.4	146.7	144.6	0.2	0.2	7.5	8.6
50/25	14.9	14.7	146.2	143.4	0.2	0.2	8.3	9.2
150/75[b]	19.8[c]	18.2[c]	144.6[c]	142.4	0.4[c]	0.3[c]	8.6[c]	9.3[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 15 Organ weight changes in male rats with six-months oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	Body Weight	Liver		Thyroid		Spleen	
	g	g	BW%	g	BW%	g	BW%
0/0	607.3	16.5	2.7	0.023	0.004	0.8	0.14
10/5	613.5	16.4	2.7	0.024	0.004	0.9	0.14
50/25	585.9	21.0[c]	3.6[c]	0.027	0.005	1.0[c]	0.18[c]
150/75[b]	514.1	23.5[c]	4.6[c]	0.031[c]	0.006[c]	1.1[c]	0.22[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Table 16 Organ weight changes in male rats with six-months oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	Body Weight	Liver		Thyroid		Spleen	
	g	g	BW%	g	BW%	g	BW%
0/0	336.9	8.6	2.6	0.018	0.005	0.6	0.17
10/5	367.7	10.1[c]	2.7[c]	0.023	0.006	0.6	0.16
50/25	340.2	15.2[c]	4.5[c]	0.025[c]	0.007[c]	0.7[c]	0.21[c]
150/75[b]	283.4	16.7[c]	5.7[c]	0.027[c]	0.009[c]	0.7[c]	0.26[c]

[a]Abbott-157378/Abbott-84538; [b]on Days 11 for the female and 99 for the males the dosage level of the 150/75 mg/kg/day group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day. [c] P<0.01; M: male; F: female.

Comments

The NOAEL for this study was 50/25 mg/kg/day. This study demonstrated that oral administration of 150 mg/kg/day Abbott-157378 and 75 mg/kg/day Abbott-84538 for six months was not well tolerated by rats. Due to toxicity, dosages in the high-dosage group were reduced to 100 mg/kg/day Abbott-157378 and 50 mg/kg/day Abbott-84538 on Day 11 for females and Day 99 for males. The primary target organs were liver, thyroid, blood cells and spleen. Clinical chemistry and/or morphologic changes were compatible with hepatocellular injury, cholestasis, hepatocellular hypertrophy and proliferation, and imbalances in lipid/protein synthesis. Changes in the surface morphology of erythrocytes (acanthocytosis) were related to imbalances in hepatic lipid metabolism. An increase in TSH that was secondary to the decreased plasma T4 correlated with thyroid follicular hypertrophy. Increases in circulating lymphocytes and platelets were likely a drug-related phenomenon. Histiocytosis in spleen and lymph nodes was related to phospholipidosis, a finding previously observed with Abbott-84538.

Toxicokinetics

Methods

An additional 5 rats/sex in the control group (vehicle) and three drug-treated satellite groups (5/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (vehicle), 10/5, 50/25, or 150/75 mg/kg/day dose combinations for 175 days. Blood samples (0.3 mL/sample) were collected from the orbital plexus at 1, 2, 4, 8, 12, 15 and 24 hours after dosing on Days 28, 98, and 174. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted] MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 on Days 28, 98 and 174 are summarized in Table 17. The AUC values of Abbott-157378 in rats at 10/5 to 100/50 mg/kg/day Abbott-157378 and Abbott-84538 for 6 months ranged from 13 to 185 µg•hr/ml. The corresponding Abbott-84538 mean AUC values were 0 – 15 µg•hr/ml. Thus, the AUCs for Abbott-157378 was 10-15 times those of Abbott-84538. Females appear to have higher AUCs than males at 10/5 mg/kg/day and 50/25 mg/kg/day for both Abbott-15378 and Abbott-84538 on Day 28 and 98. This sex difference, however, was not observed for the 100/50 mg/kg/day group on Day 174. The T_{max} vales of Abbott-157378 on Days 28, 98 ranged from 4 to 8 hours. The CL/F of Abbott-157378 on Days 28, 98 and 174 ranged from 0.53 to 0.97 L/h/kg in females and 0.57 to 1.75 L/h/kg in males, respectively. The T_{max} vales of Abbott-84538 on Days 28 and 98 ranged from 2 to 6 hours. The CL/F of Abbott-84538 on Days 28, 98 and 174 ranged from 1.9 to 8.3 L/h/kg in females and 4.6 to 15.4 L/h/kg in males, respectively.

Table 17. Pharmacokinetics of Abbott-157378 and Abbott-84538 in neonatal rats after 6-months oral administration of Abbott-157378 and Abbott-84538

Dosage (mg/kg/day)[a]	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
10/5								
Day 28	1.4	1.7	14.4	19.7	0	0.2	0	0.9
Day 98	1.8	1.8	16.6	19.1	0	0.3	0.1	0.8
Day 174	1.6	1.7	18.4	19.3	0	0.2	0	1.2
50/25								
Day 28	6.4	8.7	53.6	83.5	0.8	1.6	4.6	7.5
Day 98	5.8	8.1	50.6	90.5	1.3	1.7	5.9	9.0
Day 174	6.7	9.2	63.3	97.9	1.0	2.6	5.2	14.1
150/75[b]								
Day 28	9.3	9.1	93.7	120.3	1.8	1.7	12.8	12.2
Day 98	9.5	10.2	126.7	124.2	1.3	2.5	8.6	10.8
Day 174	11.2	10.3	139.2	113.7	1.1	2.1	9.1	8.6

[a]Abbott-157378/Abbott-84538; [b]The dosage for the 150/75 mg/kg/day group was reduced from 150/75 mg/kg/day to 100/50/day on Day 11 for females and 99 days for males because of toxicity.

15. Two-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Neonatal Rats (Study No. TA98-069, R&D/98/307)

Vol. No.: 6; **Pages:** 1-268; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 4/28/98; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 328306-0-AX; Abbott-84538.0, lot No. 95-852-AL; **Formulation:** A pediatric formulation contains 10% (w/w) Abbott-157378, and another contains 5% (w/w) Abbott-84538 (ABT-157378: 100 mg/mL; ABT-84538, 50 mg/mL) in a vehicle solution. The placebo formulation (vehicle solution) contains [redacted]

Methods

Groups of ten male and ten female neonatal Sprague-Dawley rat pups (Crl:CD(SD)BR, 3-4 days old, 8.2-12.1 g; [redacted] were orally treated with ABT-157378 and ABT-84538 pediatric formulations by gavage at doses of 0/0 (placebo formulation), 10/5, 20/10, or 40/20 mg/kg/day dose combinations for 14 consecutive days. At the time of treatment, each rat received Abbott-84538 first,

with the Abbott-157378 dose being administered promptly afterwards (dose volume: 2 mL/kg/day). Control animals received placebo formulation by the oral administration. Prior to and at the end of the study, rats were given a physical and opthalmoscopic examination, respectively. Rats were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. All animals were subjected to a gross necropsy on Day 13, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No drug-related deaths were seen. One male rat in the 10/5 mg/kg/day group and two male rats in the 40/20 mg/kg/day were found dead on Day 9 and Day 12, respectively. The deaths were due to dosing injury or test article aspiration into the lungs. No drug-related signs of toxicity were seen in rats at all doses.

Body weights and food consumption. No mean body weights and body weight gain changes were seen in rats at all doses (Table 18). A transient change in food consumption was seen at 100/50 mg/kg/day from Day 5 to 13.

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

Hematology. Slight decreases in hematocrit and hemoglobin were seen in females at all doses, which is not considered toxicologically meaningful. No drug-related changes were seen in males.

Clinical Chemistry. No test article-related toxicological changes were observed.

Histopathology. Test article-related increases in mean absolute and relative liver weights (relative to body weight) were seen in rats at all doses. No microscopic changes were seen to explain these changes. There were no gross and microscopic findings attributable to drug treatment. Two drug-treated pups that died at 10/5 and 40/2 mg/kg/day, respectively, were caused by dosing error and were not drug-related.

Table 18. Changes in organ weights in neonatal rats with 14-days oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg)*	Body Wt (g)	Liver Wt (g)	Body Wt (g)	Liver Wt (g)
	Male	Female	Male	Female
0/0	40.2	1.32	43.3	1.66
10/5	41.1	1.41	45.2	1.82
20/10	38.9	1.49	40.9	1.73
40/20	40.4	1.66	44.9	2.21

*Abbott-157378/Abbott-84538

Comments

The NOAEL for two weeks of exposure in neonatal rats was 40/20 mg/kg/day. Although mean liver weights were increased for drug-treated groups, there were no microscopic alterations to explain the weight changes. The AUC values for Abbott-157378 and Abbott-84538 at 40/20 mg/kg/day for 14 days were 140 and 13 µg•hr/mL, respectively.

Toxicokinetics

Methods

An additional 5 rats/sex in the control group and three drug-treated satellite groups (15/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo formulation), 10/5, 30/15, or 100/50 mg/kg/day dose combinations for 14 days. Blood samples (1 mL/sample) were collected by cardiac puncture of CO2 anesthetized drug-treated satellite pups (3/sex/group/timepoint) at 2, 5, 15, and 24 hour after dosing on day 13. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted]MS/MS method.

Results

Pleak plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Day 13 are summarized in Table 19.

Table 19. Plasma concentration of Abbott-157378 and Abbott-84538 in neonatal rats after 14 days oral administration of Abbott-157378 and Abbott-84538

Dosage (mg/kg/day)*	Abbott-157378		Abbott-84538	
	C_{max} (µg/mL)	AUC (µg•hr/ml)	C_{max} (µg/mL)	AUC (µg•hr/ml)

Comments

The C_{max} for both Abbott-157378 and Abbott-84538 in neonatal rats treated with 10/5 mg/kg/day were about 3-fold higher than those found in adult rats on Day 9 of the two-week study. Similarly, the AUCs for Abbott-157378 and Abbott-84538 in neonatal rats treated with 10/5 mg/kg/day were also much higher than those found in adult rats treated with the same dosage (2 to 8-fold). No differences were seen in plasma drug levels between sexes.

16. Four-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Juvenile Rats (Study No. TA98-022, R&D/98/375)

Vol. No.: 7; **Pages:** 1-340; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 3/24/98; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 328306-0-AX; Abbott-84538.0, lot No. 95-852-AL **Formulation:** A pediatric formulation contains Abbott-157378 (96.8 mg/mL) and Abbott-84538 (59.5 mg/mL) in a vehicle solution. The placebo formulation (vehicle solution) contains ethyl alcohol (312.5 mg/g), propylene glycol (104.2 mg/g), glycerin (270.8 mg/g), menthol, povidone, and other inactive ingredients.

Methods

Groups of ten male and ten female neonatal Sprague-Dawley juvenile rats (Crl:CD(SD)BR, 16 days old, 25-45 g; [redacted] were orally treated with ABT-157378 and ABT-84538 pediatric formulations by gavage at doses of 0/0 (placebo formulation), 10/5, 30/15, or 100/50 mg/kg/day(equivalent to 50/25, 150/75, and 500/250 mg/m^2/day Abbott-157378/ritonavir, respectively) for four weeks (dose volume: 2 mL/kg/day). Prior to and at the end of the study, rats were given a physical and opthalmoscopic examination, respectively. Rats were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded at baseline, prior to each dose administration, and at study termination. All animals were subjected to a gross necropsy on Day 28, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, spleen, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No drug-related deaths were seen. Two male rats in the 100/50 mg/kg/day group were found dead during the first week of the study, which was due to gavage dosing error. Noisy respiration was seen in all drug-treated groups, which was not dosage-related and at a low incidence.

Body weights and food consumption. Decreases in mean body weights (10-15%) and body weight gain (7-8 %) were seen in rats at 100/50 mg/kg during the treatment period (Table 20). Reductions (< -10%) in mean food consumption were seen during the post-weaning period for the 100/50 mg/kg/day animals

when compared with control (P<0.01).

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

Hematology. Increases in mean reticulocyte counts were seen in female and male rats at 100/50 mg/kg/day. An increase in mean RBC count was also seen in female rats. However, these changes (< 10%) were small in magnitude and were not considered to be biologically or toxicologically meaningful.

Clinical Chemistry. Increases in serum cholesterols were seen in female rats at 30/15 mg/kg/day or greater. Increases in serum GGT (10-fold in males and 8-fold in females) and ALT (+130% in females only) were seen in rats at 100/50 mg/kg/day. Dose-dependent decreases in serum T4 and increases in TSH were observed at 10/5 mg/kg/day or higher. At 100 mg/kg/day, serum T4 level was dropped from the control level of 5.8 μg/dL (male) or 4.3 μg/dL (female) to 4.2 μg/dL (male) or 3.6 μg/dL (female), respectively. Serum TSH level was increased from the control level of 2.5 ng/dL to 5.3 ng/dL in females at 100 mg/kg/day. Note that serum TSH level was not increased in males at 100 mg/kg/day.

Histopathology. Test article-related increases in mean absolute and relative liver weights (relative to the body weight) were seen in rats at 30/15 mg/kg/day or greater (Table 21). Multifocal centrilobular hepatocytomegaly was seen in male rats (2/10) and female rats (1/10) at 30/15 mg/kg/day. At 100/50 mg/kg, the incidence of hepatocytomegaly was increased (7/10 in males and 8/10 in females). These changes were consistent with hepatic enzyme induction that has previously reported for Abbott-84538. Coagulative necrosis was found in one male rat at 100/50 mg/kg. Decreases in mean absolute and relative kidney weight (relative to the body weight) were seen in rats at 100/50 mg/kg/day. However, no related morphologic renal changes were seen in these animals. Thyroid follicular cell hypertrophy and hyperplasia were also seen at 30/15 mg/kg/day (in 9/10 males and 1/10 females). Decreases in mean absolute brain weights were seen in rats at 100/50 mg/kg/day, which were associated with the decreased body weight gain in these animals. The mean relative brain weights for the 100/50-mg/kg/day groups were greater than control.

Table 20 Changes in body weights and body weight gain in juvenile rats with 4-weeks oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	Day 0 Body Wt (g)		Day 3 Body Wt (g)		Day9 Body Wt (g)		Day16 Body Wt (g)		Day 23 Body Wt (g)		Day 27 Body Wt (g)		Body Wt Change *Day 0-27*	
	M[b]	F[b]	M	F	M	F	M	F	M	F	M	F	M	F
0/0	34.8	33.9	41.6	40.5	71.2	67.5	128.5	117.4	197.6	167.7	240.6	188.2	205.8	154.3
10/5	39.6	39.4	47.6	46.5	77.2	73.1	133.7	122.5	203.4	191.0	243.7	191.0	204.1	151.6
30/50	34.1	32.8	40.7	39.3	68.2	64.7	122.2	112.6	189.5	179.8	227.7	179.8	193.6	147.0
100/50	35.8	35.8	38.5	38.4	63.2[c]	59.3[c]	113.6[c]	103.7[c]	174.9[c]	177.1	210.9[c]	177.1[c]	174.5[c]	141.2[c]

[a] Abbott-157378/Abbott-84538; [b]M: male; F: female. [c] P<0.05.

Table 21 Changes in liver weights in juvenile rats with 4-weeks oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg)[a]	Body Wt (g) Male	Liver Wt (g) Male	Body Wt (g) Female	Liver Wt (9g) Female
0/0	222.8	9.4	175.8	7.1
10/5	229.5	9.9	179.4	7.7
30/15	214.7	9.9	166.4	7.8
100/50	200.9[b]	11.5[b]	162.8[b]	9.6[b]

[a]Abbott-157378/Abbott-84538; [b] P< 0.05.

Comments

The NOAEL for four weeks of exposure in juvenile rats (16 days old) was 30/15 mg/kg/day. Increases in liver weights, microscopic alterations in the liver (hepatocytomegaly) and thyroid (follicular cell hypertrophy and hyperplasia) in juvenile rats were seen, which were similar to those previously found in adult rats at the same dosage. Note that such changes were not observed in a 14-day neonatal rat study at 40/20 mg/kg/day. Decreases in body weight, body weight gain, food consumption as well as clinical chemistry alterations (increases in cholesterol, GGT, ALT, TSH and decrease in T4) were seen in rats at 100/50 mg/kg/day.

Toxicokinetics

Methods

An additional 3 rats/sex in the control group and three drug-treated satellite groups (15/sex/group) were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo formulation), 10/5, 30/15, or 100/50 mg/kg/day dose combinations for 14 days. Blood samples (1 mL/sample) were collected by cardiac puncture of CO_2 anesthetized drug-treated satelite pups (3/sex/group/timepoint) at 2, 5, 15, and 24 hour after dosing on day 13. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted]-MS/MS method.

Results

Pleak plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Day 27 are summarized in Table 22.

Comments

The AUC values for Abbott-157378 in juvenile rats at 10/5 mg/kg/day were similar to those found in the two-week toxicity study in adult rats at the same dosage. Note that Abbott-84538 AUCs at 10/5 mg/kg group were significantly lower than those found at the same dosage in the two-week toxicity study in adult rats. Additionally, differences were seen in plasma drug levels between sexes, and the AUCs were greater in females than males for both drugs.

Table 22. Plasma concentration of Abbott-157378 and Abbott-84538 in juvenile rats after four weeks oral administration of Abbott-157378 and Abbott-84538

Dosage (mg/kg/day)*	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/mL)		C_{max} (µg/mL)		AUC_{24h} (µg•hr/ml)	
	M	F	M	F	M	F	M	F
10/5	[redacted]							
30/15								
100/50								

* Abbott-157378/Abbott-84538

17. Two-Week Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (Study No. TB96-067, R&D/96/243)

Vol. No.: 12; **Pages:** 133-323; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 2/27/1996; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 328306-0-AX; Abbott-84538.0, lot No. 95-852-AL **Formulation:** ABT-157378 and ABT-84538 semisolid formulations contained 13% (w/w) of either ABT-157378 or ABT-84538. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Methods

Groups of three male and three female beagle dogs (7 months old, 8-12 kg; [redacted] were orally treated with ABT-157378 and ABT-84538 by capsules at doses of 0/0 (placebo capsules), 5/2.5, 15/7.5, or 50/25 mg/kg/day dose combinations for 14 consecutive days. Dogs were observed twice daily during the pre-treatment and treatment for physical condition, behavior and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded twice weekly during pretreatment and treatment period. Prior to and at the end of the study (Day 13), dogs were given an opthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. Additionally, non-fasted blood samples were collected from dogs on Day 2 for evaluation of liver enzyme activities. All animals were subjected to a gross necropsy on Day 13, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney and liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No drug-related deaths were seen. Emesis, excessive salivation, diarrhea and abnormal stools were seen in dogs at 15/7.5 and 50/25 mg/kg/day during the treatment period. A low incidence of salivation and diarrhea and abnormal stools was seen in females at 5/2.5 mg/kg/day. No overt signs of toxicity were seen in rats at all doses.

Body weights and food consumption. No mean body weights and body weight gain changes were seen in dogs at all dosages, except for the female dogs at 5/2.5 mg/kg/day. A transient decrease in mean body weights and body weight gain occurred in females at 5/2.5 mg/kg/day.

Ophthalmology. No ocular abnormalities were detected in dogs at all dosages.

ECG. No ECG abnormalities were seen in dogs at all dosages.

Hematology. No hematologic abnormalities were detected in dogs at all dosages.

Clinical Chemistry. No abnormalities were detected in dogs at all dosages.

Histopathology. No gross tissue abnormalities and microscopic abnormalities were detected in dogs at all dosages.

Comments

The NOAEL for two weeks of exposure was 50/25 mg/kg/day (AUCs: 195/94 µg/ml). Increased incidences of emesis, salivation and diarrhea or abnormal stools were seen in dogs at all doses.

Toxicokinetics

Methods

Groups of three male and three female dogs were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo), 5/2.5, 15/7.5 or 50/25 mg/kg/day dose combinations for 14 days. Blood samples (3 mL/sample) were collected at 2, 4, 8, 12, 15 and 24 h after dosing on Days 1 and 14. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted] MS/MS method.

Results

Pleak plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) values for Abbott-157378 and Abbott-84538 on Days 1 and 13 are summarized in Tables 23 and 24.

Table 23 Pharmacokinetic parameters of Abbott-157378 in dogs following oral administration of Abbott-157378 and Abbott-84538 (Days 1 and 13)

Dosage (mg/kg/day)*	Abbott-157378 (Day 1)				Abbott-157378 (Day 13)			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
5/2.5	4.3	6.8	23.6	35.0	3.8	4.8	21.8	19.8
15/7.5	8.6	10.5	78.7	92.7	10.3	13.0	79.1	114.6
50/25	10.8	13.4	108.5	118.7	14.8	18.5	172.3	220.6

* Abbott-157378/Abbott-84538

Table 24. Pharmacokinetic parameters of Abbott-84538 in dogs following oral administration of Abbott-157378 and Abbott-84538 (Days 1 and 13)

Dosage (mg/kg/day)*	Abbott-8453 (Day 1)				Abbott-84538 (Day 13)			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
5/2.5	1.9	2.2	4.8	4.8	0.9	0.3	2.5	0.6
15/7.5	6.9	10.6	23.6	33.1	5.5	17.2	22.6	27.8
50/25	11.0	10.0	39.2	38.0	5.8	20.1	98.1	90.0

*Abbott-157378/Abbott-84538

Comments

Peak plasma levels and AUCs of Abbott-157378 or Abbott-84538 were increased with increasing dosage on Days 1 and 13. No consistent differences were seen in plasma drug levels between sexes.

18. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (With a One –Month Recovery Period), Study No. TB96-157, R&D/96/675

Vol. No.: 13; Pages: 1-444; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 5/29/96; GLP Compliance: Yes (X); Drug Lot: Abbott-157378.0, Lot No. 16-276-AL; Abbott-84538.0, Lot No. 86-701-AL; Formulation: ABT-157378 and ABT-84538 semisolid formulations contained 13% (w/w) of either ABT-157378 or ABT-84538. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Methods

Groups of four male and four female beagle dogs (6 months old, 8-11 kg; [redacted] were orally treated with ABT-157378 and ABT-84538 by capsules at doses of 0/0 (placebo capsules), 10/5, 30/15 or 100/50 mg/kg/day dose combinations for up to 93 consecutive days. Groups of two male and two female dogs were orally treated with 0/0 or 100/50 mg/kg/day for three months with an additional one-month recovery period. Note that the high dosage level was lowered from 100/50 mg/kg/day to 75/35 mg/kg/day on Day 30. Dogs were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded twice weekly during pretreatment and treatment period. During the pretreatment and treatment period, and at the end of the recovery period, dogs were given an opthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. All animals were subjected to a scheduled necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. Two male and two female dogs at in the 100/50 mg/kg/day were found dead on Days 12-25 before lowering the high dose level from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30. One male dog at 70/35 mg/kg/day was found dead on Day 72. The cause of deaths was related to hypokalemia and associated cardiac arrhythmias seen in these animals. Ataxia, tremor, jerks, prostration, dehydration, emaciation, weakness, pale eyes, and labored and deep respiration were observed in dogs prior to death. Dose-related emesis, diarrhea and loose stools were observed in all groups including the placebo controls. Increased incidences of diarrhea (2 to 4-fold) and loose stools (6 to 12-fold) were seen in dogs at ≥ 30/15 mg/kg/day. Increased salivation and decreased activity were also seen in dogs at ≥ 30/15 mg/kg/day. Weakness and emaciation were observed in one male and one female at 70/35 mg/kg/day.

Body weights and food consumption. Mean body weights and body weight gains were statistically significantly decreased in male dogs at 100/50 mg/kg/day during the fourth week of treatment and thereafter. By contrast, mean body weights and body weight gains were statistically significantly decreased in female dogs at 100/50 mg/kg/day during the third month of treatment. Dogs that died in the high dosage group had experience 9 to 23% weight loss prior to deaths (Table 25). At the end of the recovery period, body weight gain for 100/50 mg/kg/day dogs was still lower than controls. Decreased food consumption was seen in dogs at 100/50 mg/kg/day. Note that moistened regular chow or dietary supplementation included BIO-SERVE treats (liquid and solid) and oral administrations of PEDIALYTE were provided to the high dosage dogs.

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

ECG. Abnormalities in ECG including increased U wave amplitude, fusion of QT and U waves, first

degree of A-V block and triggered ventricular extrasystoles were seen in six males (#3001, #3003, #3005, #3007, #3009, #3011) and one female (#3006) at 100/50 mg/kg on days 19/20, 54/55, and 82/83.
Hematology. Reductions in HB, Hct, and RBC were seen in male rats at 100/50 mg/kg/day. One male dog at 100/50 mg/kg (#3003) showed nongenerative anemia on Day 86. By the end of the recovery period, these changes were normalized. Decreases in platelet volumes, unaccompanied by thrombocytopenia, were seen in dogs at 100/50 mg/kg (8/12) during the treatment period, but not after the recovery period. Decreases in WBC were seen in dogs at 100/50 mg/kg/day (#3009, #3006, #3007, and #3001). Lower mean APTT was seen in male dogs at 10/5 and 100/50 mg/kg on Day 27.
Clinical Chemistry. Increases in serum ALT and AST were seen in dogs at 30/15 mg/kg/day (#2006) or greater (#3001, #3005, #3008, #3012), indicating a hepatocellular injury (and leakage) in these dogs. Additionally, increases in serum ALP were seen in dogs at 10/5 mg/kg/day (#1002) or greater (#2002, #2003, #2005, #2006, #3010, #3012), indicating drug-related cholestastic and hepatocellular lesions in these dogs (#3001, #3005, #3008, #3012, and #2006). Increases in serum AST were also seen in dogs at 30/15 mg/kg/day or greater (#3005 and #2006). Abnormalities in serum electrolyte levels, acid-base balance and blood-gas value were seen in dogs at 100/50 mg/kg/day. Decreases in serum potassium levels were seen in dogs at 30/15 mg/kg/day or greater (#3006 on Day 23; #3001 on Day 63). Factors possibly contributing to hypokalemia include loss of gastrointestinal fluids (vomiting and diarrhea), alkalosis (causing intracellular potassium shift), and decreased alimentation (anorexia, vomiting and diarrhea). Decreases in serum sodium and chloride were also seen in dogs at 100/50 mg/kg (#3001, #3008, #3004) during the treatment period. Hyponatremia, hypokalemia and hypochloridemia, and metabolic alkalosis and acidosis were seen in dogs at 100/50 mg/kg/day (#3001, #3006, #3006, #3009). A decrease in serum albumin was seen in dogs at 100/50 mg/kg/day. Elevation of triglycerides and glucose were seen dogs at 30/15-mg/kg/day or greater. These changes in lipid and proteins were related to altered hepatic lipid/protein synthesis. Increases in creatine values were seen in dogs at 10/5 and 30/15 mg/kg/. An increase in the hepatic glutamyl transferase (GGT) was seen in dogs at 30/15 mg/kg or higher on Day 19 (Tables 26 and 27). After the recovery period, abnormalities in serum chemistry parameters that were present during treatment in dogs at 100/50 mg/kg/day were either absent or had largely normalized.
Histopathology. Increases in mean absolute liver (relative to body weight) were seen in males at ≥ 10/5 mg/kg/day and in females at 100/50 mg/kg (Tables 28). Decreases in relative testis weights (relative to body weight) were seen in dogs at 10/5 and 30/15 mg/kg. In addition, two dogs at 100/50 mg/kg/day that died had testis weight decreases (-10%). Testicular atrophy was seen in these animals. At the end of the recovery period, these liver and testis changes weights were absent. Marked thymic atrophy was seen in 4/5 dogs that died at 100/50 mg/kg/day. One 100/50 mg/kg/day dog at the end of recovery period had a very heavy thymus (#3003), representing a compensatory hyperplastic response following the prolonged clinical debilitation of the animal during the treatment with test article. No gross tissue findings that were directly attributable to treatment with test articles was seen. Yellow discoloration of the aorta was seen in three dogs that died at 100/50 mg/kg/day, which was possibly related to bilirubinemia (serum levels were > 2 mg/dl in all three dogs). Thin and autolysed carcasses, rectal vascular congestion, altered gastrointestinal contents, discoloration of small intestine, small prostates and testes, and pulmonary congestion were seen in animals at 100/50 mg/kg/day. Hepatocellular vacuolar degeneration (#3001, #3002, #3007, #3009), perivasculitis (#3006) in the liver, inflammation, diffuse (#3001) or pericholangiolar (#3007), and canalicular bile casts (#3001) were observed in animals at 100/50 mg/kg/day. At the end of the recovery period, no drug related changes were found in the livers of 100/50 mg/kg/day dogs. Short and few microvilli were observed in affected plugged canaliculi in one high-dosage dog (#3008).

Comments

This study demonstrated that oral administration of 100 mg/kg/day Abbott-157378 and 50 mg/kg/day Abbott-84538 for three months with a one-month recovery period was not well tolerated by dogs. Due to the high mortality in dogs at 100/50 mg/kg/day during the treatment period, dosages were reduced to 70

mg/kg/day Abbott-157378 and 35 mg/kg/day Abbott-84538 on Day 30. The primary target organs appear to be gastrointestinal, biliary and hepatic systems. Clinical chemistry changes were compatible with severe gastrointestinal disturbances (emesis, diarrhea and anorexia). Toxicological meaningful changes in serum activities of hepatic enzymes and cholestatic markers occurred in dogs at ≥ 30/15 mg/kg/day. Hepatocellular injury occurred in dogs at 100/50 mg/kg/day. These changes were reversed at the end of one-month recovery period. The NOAEL for this study was 30/15 mg/kg/day (AUC values: 95/23 µg•hr/ml).

Table 25 Changes in body weights and body weight gain in dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Treatment (*mg/kg/day*)[a]	Day -1 Body Wt (kg)		Day 22 Body Wt (kg)		Day 29 Body Wt (kg)		Day 61 Body Wt (kg)		Day 89 Body Wt (kg)		Body Wt Change *Days -1-89*	
	M	F	M	F	M	F	M	F	M	F	M	F
0/0	10.1	8.9	10.8	9.5	11.1	9.5	11.8	10.2	11.9	10.6	1.95	1.77
[redacted]	10.1	9.0	11.0	9.6	10.9	9.6	11.6	10.6	11.9	11.0	1.85	2.02
[redacted]	9.9	8.9	10.6	9.4	10.5	9.4	11.4	10.1	11.5	10.0	1.57[c]	1.15[c]
	10.1	9.3	10.1	9.0	10.1	9.6	9.7	10.0	9.9	9.4	0.05[c]	0.15[c]

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 100/50 mg/kg/day group was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30; [c] P<0.01. M: male; F: female.

Table 26 Clinical chemistry in dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Treatment (*mg/kg/day*)[a]	ALP (IU/L)				Triglyceride (mg/dL)			
	M		F		M		F	
	Day 54	Day 86	D85	D182	Day 27	Day 86	D27	D86
0/0	126.8	108.3	109.7	105.3	45.2	35.8	58.7	47.0
[redacted]	119.0	111.3	157.5	158.8	49.5	39.5	61.3	46.0
[redacted]	129.8	133.0	183.0	178.3	52.5	37.5	54.5	41.3
	274.5[c]	330.3[c]	192.3[c]	290.0[c]	59.3[c]	47.0[c]	61.0	48.3

[a]Abbott-157378/Abbott-84538; due to the toxicity, the dosage level of the 100/50 mg/kg/day group was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30. **: P<0.01. M: male; F: female.

Table 27 Clinical chemistry data related to hepatocellular and cholestatic lesions in dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Treatment (mg/kg/day)	Day	Cholestatic Markers			Liver Enzymes		Day of Death
		ALP IU/L	Tbil mg/dL	Bile Acids µg/dL	ALT IU/L	AST IU/L	
0/0		59-296	0.1-0.5	0.7-7.3	23-51	21-80	
[redacted]							
[redacted]	20	743	0.6	33.0	157	30	23
	22	234	6.4	41.8	81	50	22
[redacted]	23	3063	2.5	56.1	198	110	23
[redacted]	24	420	2.7	32.8	44	46	25
	54	245	0.3	11.7	402	46	72
	63	831	0.8	38.0	189	53	72
[redacted]	54	440	0.5	4.5	56	41	120
[redacted]	86	535	1.1	14.3	30	36	120
[redacted]	86	427	0.5	28.0	94	41	120

* Abbott-157378/Abbott-84538; due to the toxicity, the dosage level of the 100/50 mg/kg/day group was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30.

Table 28 Organ weight changes in male dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Treatment (mg/kg/day)[a]	n	Body Weight	Liver		Testis	
		kg	g	BW%	g	BW%
0/0	4	11.9	325.2	2.7	15.8	0.13
[redacted]	4	12.1	363.4	3.0	13.6	0.11
[redacted]	4	11.5	374.0[c]	3.27[c]	12.7[c]	0.11[c]
[redacted]	1	12.2	408.6	3.36	12.9	0.11

[a]Abbott-157378/Abbott-845438; [b]due to the toxicity, the dosage level of the 100/50 mg/kg/day group was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30. [c] P<0.01

Table 29 Organ weight changes in female dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Treatment (mg/kg/day)[a]	n	Body Weight	Liver		Ovary	
		kg	g	BW%	g	BW%
0/0	4	10.6	329.5	3.1	1.11	0.01
[redacted]	4	11.1	329.9	3.0	1.63	0.01
[redacted]	4	10.0	315.7	3.2	0.80	0.008
[redacted]	2	9.4	372.5	4.0[c]	0.76[c]	0.008

[a]Abbott-157378/Abbott-845438; [b]due to the toxicity, the dosage level of the 100/50 mg/kg/day group was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30. [c] P<0.01

Toxicokinetics

Methods

Groups of 4 dogs per sex/group were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules), 10/5, 30/15, or 100/50 mg/kg/day dose combinations for 90 days. Additional control and drug treated groups (2/sex/group) were administered with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules) and 100/50, respectively for 90 days with a one-month treatment free period. Note that the high dosage level was lowered from 100/50 mg/kg/day to 75/35 mg/kg/day on Day 30. Blood samples (3 mL/sample) were collected from the jugular or cephalic vein at 2, 4, 6, 9, 12, 15 and 24 hours after dosing on days 1, 29 and 82. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted] MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 30. Mean AUC and C_{max} values of Abbott-157378 and Abbott-84538 generally increased with increasing dosages. Mean AUC and C_{max} values of Abbott-157378 for the 100/50-mg/kg/day group on Day 29 and 82 were higher than corresponding values on Day 1. However, mean AUC and C_{max} values of Abbott-84538 for all dosage groups except for the 100/50-mg/kg/day females on Day 29 and 82 were lower than corresponding values on Day 1. Note that no such changes were seen in a previous one-year dog study with Abbott-84538. Sex differences in the plasma drug level were not observed. Note that plasma drug levels of both drugs were quite variable between dogs of the same sex and dosage group.

Table 30. Pharmacokinetics of Abbott-157378 and Abbott-84538 in female dogs with three-month oral administration of Abbott-157378 and Abbott-84538 (with a one-month recovery period)

Dosage (mg/kg/day)[a]	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	Male	Female	Male	Female	Male	Female	Male	Female
10/5								
Day 1	7.9	8.0	60.1	63.8	3.4	4.7	13.9	19.8
Day 29	7.3	7.7	50.0	52.4	2.7	1.9	8.0	5.6
Day 82	7.8	8.9	65.8	63.2	2.3	3.0	9.0	8.5
30/15								
Day 1	11.8	12.0	97.0	92.9	10.7	12.4	42.4	51.1
Day 29	14.1	8.4	106.6	79.0	5.5	7.1	18.7	27.6
Day 82	11.6	10.5	104.3	95.1	9.0	6.1	28.7	22.0
100/50[b]								
Day 1	11.4	15.6	108.9	141.7	20.3	11.4	98.4	49.5
Day 29	15.9	25.3	144.2	261.0	15.6	13.3	54.3	71.5
Day 82	16.9	26.3	233.6	245.2	6.9	14.1	54.9	61.4

[a] Abbott-157378/Abbott-84538; [b] the dosage for the 100/50 mg/kg/day group was reduced to 70/35 mg/kg/day on Day 30 because of toxicity.

Comments

Note that a reduction in drug exposures of Abbott-84538 was seen, which may be related to liver enzyme (P450) induction in dogs. No such changes were seen in a previous one-year dog study with Abbott-84538 (Re.: Drug Safety Evaluation Division, Abbott Laboratories, Study No TB94-311. Scientific Report No R&D/96/220, 1996)

19. Six-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (Study No. TB97-003, R&D/97/752)

Vol. No.: 14; **Pages:** 1-480; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 2/17/97; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 22-363-NI-00; Abbott-84538.0, Lot No. 24-215-AL; **Formulation:** ABT-157378 and ABT-84538 semisolid formulations contained 20% (w/w) of either ABT-157378 or ABT-84538 (the lot numbers for formulations: 25-378-AR-XX for Abbott-157378 SEC; 25-377-AR-XX for Abbott-84538 SEC; 25-379-AR-XX and 29-404-AR-XX for placebo). The placebo formulation consists of oleic acid, ethanol, polyoxyl 35 castor oil and butylated hydroxytoluene. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Methods

Groups of four male and four female beagle dogs (6 months old, 6-10 kg; . [redacted] were orally treated with ABT-157378 and ABT-84538 by capsules at doses of 0/0 (placebo capsules), 10/3, 25/8 or 60/20 mg/kg/day dose combinations in divided dosages twice daily for up to 180 consecutive days. Note that the high dosage level was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91. Dogs were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured once pretreatment and weekly during the treatment period. Body weights were recorded twice weekly during the pretreatment and treatment period. During the pretreatment and treatment period, and at the end of the recovery period, dogs were given an ophthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. All animals were subjected to a scheduled necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No death was seen in the study. Dose-related emesis, diarrhea and loose stools were seen in dogs in all groups including the placebo controls. Increased incidence of emesis (6-

fold) was seen in dogs at 25/8 mg/kg/day. Increased incidences of emesis (27-fold), diarrhea and loose stools (4-fold) were seen in dogs at 60/20 (45/15) mg/kg/day. Increased salivation, decreased activity, emaciation, dehydration and weakness were observed in dogs at 60/20 (45/15) mg/kg/day.

Body weights and food consumption. Mean body weights and body weight gains were deceased in male dogs at 60/20 mg/kg/day during the first week of treatment and throughout the most treatment period (Table 31). Note that additional dietary supplementation including moistened regular chow or dietary supplementation included BIO-SERVE treats (liquid and solid) and oral administrations of PEDIALYTE were provided to all high dosage dogs during the treatment period.

Ophthalmology. No ocular abnormalities were detected in rats at all doses.

ECG. No drug-related abnormalities in ECG were seen in all dogs. The data from this present study indicated that Abbott-157378/ritonavir combination at 60/20 mg/kg/day did not produce any ECG changes. Note that increased U wave amplitude, fusion of QT and U waves, first degree of A-V block and triggered ventricular extrasystoles were seen in six males and one female dogs at 100/50 mg/kg in a previous three-month toxicology study.

Hematology. Reductions in HB (Day 175 for males), Hct (Day 175 for females), and RBC (Days 8, 84 and 175 for males) were seen in dogs at 60 (45)/20(15) mg/kg/day. Two female dogs at 60/20 mg/kg (#3002, #3004) showed a mild neutropenia (1790 and 2240 cells/μL, respectively). One of these dogs (#3004) had thrombocytopenia and nongenerative anemia with spherocytosis (Days 84 and 86). One male dog (#3001) had borderline anemia (Hct: 35.7%) and a low reticulocyte count (Table 32).

Clinical Chemistry. Increases in serum ALT and AST were seen in three dogs at 25/8 mg/kg/day (#2002) or greater (#3001, #3004). Additionally, increases in serum ALP were seen in dogs at 25/8 mg/kg/day or greater on Day 28 and Day 84, indicating drug-related cholestastic and hepatocellular lesions in these dogs (Table 33). Mild to moderate hypoalbuminemia was seen in a male dog at 60/20 mg/kg (#3004).

Histopathology. Increases in mean absolute liver weight (relative to body weight) were seen in dogs at ≥25/8 mg/kg. Decreases in mean absolute prostate weight (relative to body weight) were seen in dogs at 60/20 mg/kg. (Tables 34 and 35). Drug-related microscopic changes were found in liver and testis. Note that decreases in relative testis weights (relative to body weight) were seen in dogs at 10/5 and 30/15 mg/kg in the previous 3-month dog study. Hepatic cell swelling was seen in one control, one 10/3 mg/kg/day dogs, four 25/8 mg/kg/day dogs, and four 45/15 mg/kg/day dogs. Cytoplasmic vacuolation, single cell necrosis and bile accumulation were seen in dogs at 60/20 mg/kg/day (#3001 and #3004). Testicular degeneration was seen in males treated with the test article (2 at low dosage, 3 at mid dosage and 2 at high dosage). Loss of germ cells, germ cell degeneration, and tubular vacuolization were observed in these animals. Prostate atrophy was noted in a dog at 60/20 mg/kg/day. Crystalline inclusion was seen in enlarged mitochondria from one dog at 60/20 mg/kg/day Crystalline inclusion without enlarged mitochondria was also seen in one dog at 10/3 mg/kg/day.

Comments

This study demonstrated that oral administration of 60/20 - 45/15 mg/kg/day Abbott-157378 and Abbott-84538 for six months produce overt toxicity. Due to the toxicity, dosages were reduced to from 60/20 mg/kg/day to 70 mg/kg/day on Day 91 in the high dosage dogs. The primary target organs appear to be gastrointestinal, biliary, testis, and hepatic systems. Clinical chemistry changes were compatible with severe gastrointestinal disturbances (emesis, diarrhea and anorexia). Toxicological meaningful changes in serum activities of hepatic enzymes and cholestatic markers, and testicular degeneration occurred in dogs at ≥25/8 mg/kg/day. Hepatocellular injury occurred in dogs at 60/20 mg/kg/day. The NOAEL for this study was 25/8 mg/kg/day.

Table 31 Changes in body weights and body weight gain in dogs with six-month oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	Day -1 Body Wt (kg)		Day 29 Body Wt (kg)		Day 89 Body Wt (kg)		Day 92 Body Wt (kg)		Day 180 Body Wt (kg)		Body Wt Change *Days -1-180*	
	M[d]	F[d]	M	F	M	F	M	F	M	F	M	F
0/0	8.5	7.3	9.1	7.8	10.1	8.6	10.3	8.8	10.6	8.9	2.18	1.54
10/3	8.6	7.3	9.3	7.7	10.3	8.2	10.3	8.2	11.0	8.7	2.36	1.44
25/8	8.8	7.3	9.4	7.4	9.9	8.0	10.2	8.2	10.6	8.3	1.80	0.98
60/20[b]	8.7	7.3	8.6	7.3	8.5[c]	7.4	8.7[c]	7.2	9.9	8.6	1.26	0.77

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 60/20mg/kg/day group was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91; [c] P<0.01. [d]M: male; F: female.

Table 32 Clinical chemistry in dogs with six-month oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	RBC (10^6/μL)				Retic (10^3/μL)				APTT (sec)			
	Male		Female		Male		Female		Male		Female	
	D 8	D 175	D 28	D 175	D 8	D 175	D 8	D175	D8	D175	D8	D175
0/0	7.3	7.4	6.4	6.6	45.2	35.8	75.4	62.4	14.8	13.7	15.2	13.8
10/3	6.6	7.3	6.6	6.8	49.5	39.5	57.4	42.0	14.7	13.5	15.1	13.7
25/8	6.8	6.4	7.0	6.3	52.5	37.5	61.5	37.1	13.9[c]	12.9[c]	14.2[c]	13.2
60/20[b]	6.4[c]	6.1[c]	6.0	6.0	59.3[c]	47.0[c]	47.2	32.1[c]	13.9[c]	12.9[c]	14.0[c]	12.9[c]

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 60/20mg/kg/day group was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91; [c] P<0.01.

Table 33 Clinical chemistry in dogs with six-month oral administration of Abbott-157378 and Abbott-84538

Treatment (mg/kg/day)[a]	ALP (IU/L)				Triglyceride (mg/dL)			
	Male		Female		Male		Female	
	D84	D175	D84	D175	D27	D 86	D28	D175
0/0	73.0	64.0	63.8	45.3	40.0	43.5	61.8	60.6
10/3	88.8	72.5	72.5	59.0	46.5	46.0	53.5	51.5
25/8	91.8	79.8	179.8[c]	189.3[c]	40.3	42.5	47.5[c]	44.5
60/20[b]	171.8[c]	178.8[c]	263.8[c]	169.5[c]	50.8	48.5	49.5	53.5

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 60/20mg/kg/day group was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91; [c] P<0.01.

Table 34 Organ weight changes in male dogs with six-months oral administration of Abbott-157378 and Abbott-84538 (at necropsy)

Treatment (mg/kg/day)[a]	n	Body Weight (BW)	Liver		Thymus		Prostate	
		kg	g	BW%	g	BW%	g	BW%
0/0	4	10.6	270.4	2.6	4.3	0.03	8.6	0.08
10/3	4	10.9	286.5	2.6	5.9	0.05	7.7	0.07
25/8	4	10.5	328.1	3.1[c]	4.8	0.05	6.3	0.06
60/20[b]	4	9.8	317.5	3.3[c]	7.3[c]	0.07[c]	3.6[c]	0.04[c]

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 60/20mg/kg/day group was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91; [c] P<0.01.

Table 35 Organ weight changes in female dogs with six-month oral administration of Abbott-157378 and Abbott-84538 (at necropsy)

Treatment (mg/kg/day)[a]	n	Body Weight (BW)	Liver	
		kg	g	Body Weight %
0/0	4	8.7	225.7	2.6
10/3	4	8.6	249.8	2.9
25/8	4	8.2	274.6	3.4[c]
60/20[b]	4	8.4	258.8	3.1[c]

[a]Abbott-157378/Abbott-84538; [b] due to the toxicity, the dosage level of the 60/20mg/kg/day group was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91; [c] P<0.01.

Toxicokinetics

Methods

Groups of 4 dogs per sex/group were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules), 10/3, 25/8, or 60/20 mg/kg/day dose combinations for 90 days. Note that the high dosage level was lowered from 60/20 mg/kg/day to

45/15 mg/kg/day on Day 90. Blood samples (3 mL/sample) were collected from the jugular or cephalic vein at 2, 4, 6, 9, 12, 15 and 24 hours after dosing on days 1, 90 and 167. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted]-MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 36. Mean AUC and C_{max} values of Abbott-157378 and Abbott-84538 generally increased with increasing dosages. Mean AUC and C_{max} values of Abbott-84538 for all dosage groups on Day 90 and 167 were lower than corresponding values on Day 1. Note that no such changes were seen in a previous one-year dog study with Abbott-84538 alone. Sex differences in the plasma drug level were not observed. Note that plasma drug levels of both drugs were quite variable between dogs of the same sex and dosage group.

Table 36. Pharmacokinetics of Abbott-157378 and Abbott-84538 in dogs with six-month oral administration of Abbott-157378 and Abbott-84538

Dosage (mg/kg/day)[a]	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	M	F	M	F	M	F	M	F
10/3								
Day 1	6.4	5.9	32.5	41.2	1.2	1.5	5.0	6.8
Day 90	4.8	4.8	17.4	22.0	0.2	0.6	0.9	2.6
Day 167	2.6	6.0	11.0	25.4	0.2	0.2	0.5	1.5
25/8								
Day 1	13.5	9.0	83.4	72.3	3.0	4.6	15.1	18.9
Day 90	10.4	9.2	62.1	45.6	1.4	0.8	7.7	4.7
Day 167	12.3	10.9	88.7	104.9	1.5	2.5	8.8	13.2
60/20[b]								
Day 1	19.6	21.3	219.0	217.1	12.3	17.0	58.8	76.8
Day 90	20.9	19.3	242.0	286.4	4.7	6.8	40.7	58.9
Day 167	13.8	12.2	154.1	116.5	4.9	7.6	35.8	44.7

[a]Abbott-157378/Abbott-84538; [b]the dosage for the 60/20 mg/kg/day group was reduced from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91.

20. Nine-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (Study No. TB98-020, R&D/99/124)

Vol. No.: 15; **Pages:** 1-375; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 5/8/98; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0, Lot No. 36-537-NI-00; Abbott-84538.0, Lot No. 24-215-TL; **Formulation:** ABT-157378 semisolid formulation contained 197 mg ABT-157378. ABT-84538 semisolid formulation contained 98.7mg ABT-84538. The placebo formulation consists of oleic acid, ethanol, polyoxyl 35 castor oil and butylated hydroxytoluene. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Methods

Groups of four male and four female beagle dogs (6-6.5 months old, 6.8-11 kg; [redacted]) were orally treated with ABT-157378 and ABT-84538 by capsules at doses of 0/0 (placebo capsules), 10/5, 25/12.5 or 50/25 mg/kg/day dose combinations in divided dosages twice daily for 271 consecutive days. Dose volumes were 0.26, 0.05, 0.13 and 0.26 mL/kg/dose for groups 0/0, 10/5, 25/12.5 and 50/25 mg/kg/day, respectively. Dogs were observed twice daily during the pre-treatment and treatment periods for physical condition, behavior, and clinical signs. Food consumption was measured semi-quantitatively twice pretreatment and twice weekly during the treatment period. Body weights were recorded twice weekly during the pretreatment and treatment period. During the pretreatment and treatment period, dogs were given an opthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. All animals were subjected to a scheduled necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No death was seen in the study. Dose-related emesis, diarrhea and loose stools were seen in dogs in all groups including the placebo controls. Increased incidence of emesis was seen in dogs at 50/25 mg/kg/day. Drug-related increases in salivation and diarrhea or loose stools were seen in dogs at all three doses.

Body weights and food consumption. Mean body weights were deceased in male and female dogs in all three drug treated groups (5-10%) at the end of the treatment period, but no statistically significant differences were seen between the test article treated and control group mean body weights throughout the study. Mean body weight gains from Day -2 to Day 271 were lowered in dogs at ≥ 10/5 mg/kg/day. Mean food consumption values for the 50/25 mg/kg/day dogs tended to be lower than the control values. Note that all these changes were not dosage-related and were not statistically significant.

Ophthalmology. No drug-related ocular abnormalities were detected.

ECG. No drug-related abnormalities in ECG were seen in all dogs.

Hematology. No drug-related meaningful differences were seen between the control and drug-treated groups in all the hematological parameters examined.

Clinical Chemistry. Increases in serum ALP were seen in dogs at all doses. The statistical significance was achieved for the low and high dosage groups. There were no accompanying increases in related serum enzymes including ALT and AST and GGT. A control dog (#0005), three dogs at 10/5 mg/kg/day (#1003, 31004, #1005) and one dog at 25/12.5 mg/kg/day (#2007) had a large quantity of bilirubin in their urine at the end of the study. A high-dosage dog (#3005) had a large quantity of bilirubin, urobilinogen (4 EU/dl) and protein (100 mg/dl) in its urine at the end of the study. However, it did not have concurrent rise in any other parameters expected to increase with bilirubinuria, including ALT, AST, GGT and bile acids. No abnormal renal serum chemistry profile was found in this animal.

Histopathology. Increases in mean absolute and relative liver weight (relative to body weight) were seen in dogs at ≥25/12.5 mg/kg. No additional drug-related organ weight changes were seen in all animals (Table 38). No drug-related microscopic changes were found in liver.

Table 37 Changes in body weights and body weight gain in dogs with nine-month oral administration of Abbott-157378 and Abbott-84538

Treatment *mg/kg/day*[a]	Day -2 Body Wt (kg)		Day 33 Body Wt (kg)		Day 92 Body Wt (kg)		Day 183 Body Wt (kg)		Day 271 Body Wt (kg)		Body Wt Change *Days -2 to 271*	
	M[b]	F[b]	M	F	M	F	M	F	M	F	M	F
0/0	9.1	7.4	10.2	8.0	11.5	9.1	12.6	10.2	13.8	11.0	4.7	3.6
10/5	9.1	7.7	10.0	8.1	11.0	8.7	11.9	9.2	12.7	9.8	3.6	2.1
25/12.5	9.2	7.8	10.1	8.0	11.7	9.0	12.4	9.1	13.2	10.1	4.0	2.3
50/25	9.2	7.5	9.8	7.9	10.8	9.0	11.2	8.9	12.4	10.1	3.2	2.6

[a]Abbott-157378/Abbott-84538; [b]M: male; F: female.

Table 38 Organ weight changes in male dogs with nine-months oral administration of Abbott-157378 and Abbott-84538

Treatment *mg/kg/day*[a]	n	Liver (Male)		Liver (Female)	
		g	Body Weight %	g	Body Weight %
0/0	4	329.3	2.39	263.4	2.4
10/5	4	323.0	2.55	262.2	2.6
25/12.5	4	352.7	2.70	334.5	3.3[b]
50/25	4	364.0[b]	2.88[b]	331.5	3.2[b]

[a]Abbott-157378/Abbott-84538; [b]P<0.01.

Comments

This study demonstrated that oral administration of ≥ 25/12.5mg/kg/day Abbott-157378 and Abbott-84538 for nine months produce mild toxicity including gastrointestinal disturbance (emesis, salivation, abnormal stools and diarrhea and decreased food consumption), decreases in body weight gain, elevation in ALP, bilirubinuria and increased absolute and relative liver weights. The NOAEL for this study was 25/12.5 mg/kg/day.

Toxicokinetics

Methods

Groups of 4 dogs per sex/group were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules), 10/5, 25/12.5, or 50/25 mg/kg/day dose combinations for 267 days. Blood samples (3 mL/sample) were collected from the jugular or cephalic vein at 2, 4, 6, 9, 12, 15 and 24 hours after dosing on days 1, 182 and 267. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted]-MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 39. Mean AUC and C_{max} values of Abbott-157378 and Abbott-84538 generally increased with increasing dosages. Plasma Abbott-157378 AUCs for dogs treated with 10/5 and 25/12.5 mg/kg/day approximated those found in dogs treated with Abbott-157378 and Abbott-84538 at 10/3 and 25/8 mg/kg/day in the previous six-month dog study. Sex differences in the plasma drug level were not observed. Note that plasma drug levels of both drugs were quite variable between dogs of the same sex and dosage group.

Table 39. Pharmacokinetics of Abbott-157378 and Abbott-84538 in dogs with nine-month oral administration of Abbott-157378 and Abbott-84538

Dosage (mg/kg/day)*	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	Male	Female	Male	Female	Male	Female	Male	Female
10/5								
Day 1	4.4	3.1	25.0	21.3	1.9	1.7	4.6	5.1
Day 182	5.6	3.3	39.3	19.7	2.3	1.1	8.3	3.9
Day 267	6.0	3.5	43.4	23.6	3.0	2.3	10.6	8.6
25/12.5								
Day 1	7.4	8.1	92.5	54.3	7.3	5.6	25.8	14.6
Day 182	6.7	11.3	55.8	97.6	4.3	6.7	15.2	24.2
Day 267	6.9	9.2	53.6	55.6	5.5	4.2	19.4	13.3
50/25								
Day 1	6.1	7.0	63.6	59.6	8.2	9.6	29.3	31.9
Day 182	9.4	9.8	108.8	84.5	11.8	9.1	60.9	34.7
Day 267	8.0	7.7	84.5	65.5	11.2	5.9	55.3	20.7

* Abbott-157378/Abbott-84538

Comments

Note that the AUCs at 50/25 mg/kg/day in the present nine-month study were lower than expected from results of the previous six-month dog study. Note that doses of Abbott-84538 were administered twice daily in the six-month dog study, which may attribute to an increase in Abbott-84538 and thereby increase Abbott-157378 exposures.

21. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with Impurities in Beagle Dogs, Study No. TB98-013, R&D/98/371

Vol. No.: 16; **Pages:** 1-258; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 2/26/98; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378 and Abbott-84538; **Formulation:** ABT-157378 SEC formulation contained 20% (w/w) ABT-157378. ABT-84538 SEC formulation contained 20% (w/w) ABT-84538. The placebo formulation (lot 54713-157) consists of 65.5% (w/w) oleic acid, 12.5% (w/w) ethanol, 12.5% polyoxyl 35 castor oil, 9.4% (w/w) propylene glycol and 0.13% butylated hydroxytoluene. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Drug Lot No	Impurity (%)
Abbott-157378.0	
Lot 36-537-NI-00	[redacted]
Lot 37-324-ZW-00	
Abbott-84538.0	
Lot 24-215-TL	

Methods

Groups of four male and four female beagle dogs (6 months old, 7-10 kg; . [redacted] were orally treated with ABT-157378 and ABT-84538 by capsules at doses of 0/0 (placebo capsules), [redacted] (normal impurity), [redacted] (high impurity) mg/kg/day once daily for 93 consecutive days. Dose volumes were 1.25 mL/kg/day. Dogs were observed twice daily during the pre-treatment and treatment for physical condition, behavior, and clinical signs. Food consumption was measured semi-quantitatively twice pretreatment and twice weekly during the treatment period. Body weights were recorded twice weekly during the pretreatment and treatment period. During the pretreatment and treatment period, dogs were given an ophthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. All animals were subjected to a scheduled necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined histologically by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No death was seen in the study. Dose-related emesis, diarrhea and loose stools were seen in dogs in all groups including the placebo controls. Increased incidence of emesis was seen in dogs at 30/15 mg/kg/day. No consistent difference was seen in the incidence of these adverse clinical signs between dogs in the 30/15 mg/kg/day with high impurity and 30/15 mg/kg/day with low impurity. Increases in salivation were seen in dogs at 30/15 mg/kg/day with high impurity.

Body weights and food consumption. Mean body weights gains were decreased in male and female dogs receiving two drug formulations (high and low impurities, respectively) at the end of the treatment period (Table 40). No drug-related food consumption changes were seen.

Ophthalmology. No drug-related ocular abnormalities were detected.

ECG. No drug-related abnormalities in ECG were seen in all dogs.

Hematology. No drug-related meaningful differences were seen between the control and drug-treated groups in all the hematological parameters examined.

Clinical Chemistry. No drug-related effects were seen in clinical chemistry.

Histopathology. Increases in mean absolute and relative liver weight (relative to body weight) were seen in dogs receiving two drug formulations with high and low impurities, respectively (Table 41). No additional drug-related organ weight changes were seen in all animals. No drug-related microscopic changes were found in liver.

Comments

This study demonstrated that oral administration of 30/15mg/kg/day Abbott-157378 (with up to [redacted] impurities or degradants) and Abbott-84538 (with up to [redacted] impurities or degradants) for three months produce mild toxicity including gastrointestinal disturbance (emesis, salivation, abnormal stools and diarrhea and decreased food consumption), decreases in body weight gain, and increased absolute and relative liver weights). Increased level of total impurities did not change the toxicity profile of the test articles at a dosage level of 30/15 mg/kg/day. The NOAEL for this study was 30/15 mg/kg/day.

Table 40 Changes in body weights and body weight gain in dogs with three-months oral administration of Abbott-157378 and Abbott-84538 with normal and high impurities

Treatment *mg/kg/dcy*[a]	Day -1 Body Wt (kg)		Day 8 Body Wt (kg)		Day 29 Body Wt (kg)		Day 61 Body Wt (kg)		Day 89 Body Wt (kg)		Body Wt Change (kg) *Days -1 to 89*	
	M	F	M	F	M	F	M	F	M	F	M	F
0/0	9.3	7.8	9.6	8.0	10.3	8.5	11.4	9.2	11.9	9.7	2.7	1.8
30/15 (normal)	9.2	7.5	9.3[b]	7.6	9.8	8.1	10.4[b]	8.6	10.9[b]	9.0	1.7[b]	1.5
30/15 (high)	9.3	7.4	9.8	7.7	10.2	8.1	11.2	8.7	11.4	9.0	2.1	1.6

[a] Abbott-157378 and Abbott-84538 with normal and high impurities; [b] P<0.05

Table 41 Organ weight changes in male dogs with three-month oral administration of Abbott-157378 and Abbott-84538 with normal and high impurities

Treatment mg/kg/day[a]	n	Liver (Male)		Liver (Female)	
		g	Body Weight %	g	Body Weight %
0/0	4				
(normal)	4				
(high)	4				

[a] Abbott-157378 and Abbott-84538; [b] P<0.05

Toxicokinetics

Methods

Groups of 4 dogs per sex/group were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules), 30/15 mg/kg/day with normal impurities, or 30/15 mg/kg/day with high impurities for 89 days. Blood samples (3 mL/sample) were collected from the jugular or cephalic vein at 2, 4, 6, 9, 12, 15 and 24 hours after dosing on days 89. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an -MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 42. Mean AUC values for Abbott-157378 in dogs that received the SEC formulations with or without increased levels of impurities in the present study were lower than the expected mean AUC value of about 100 µg•hr/ml for a dosage of 30 mg/kg/day. Sex differences in the plasma drug level were not observed.

Table 42. Pharmacokinetics of Abbott-157378 and Abbott-84538 in dogs with three-month oral administration of Abbott-157378 and Abbott-84538 with normal and high impurities

Dosage (mg/kg/day)*	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	Male	Female	Male	Female	Male	Female	Male	Female
(normal impurities)								
high impurities)								

* Abbott-157378/Abbott-84538

Comments

Mean AUC values for Abbott-157378 in dogs that received the SEC formulations with or without increased levels of impurities in the present study were lower than the expected mean AUC value of about 100 µg•hr/ml for a dosage of 30 mg/kg/day. Sex differences in the plasma drug level were not observed. The group mean AUC values for Abbott-84538 in the 30/15 mg/kg/day groups with normal and high impurities were comparable to the expected value of about •hr/ml.

22. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with New Impurities in Beagle Dogs, Study No. TB98-150, R&D/99/093

Vol. No.: 17; Pages: 1-384; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 9/29/98; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378 and Abbott-84538; **Formulation:** ABT-157378 SEC formulation contained 20% (w/w) ABT-157378. ABT-84538 SEC formulation contained 20% (w/w) ABT-84538. The placebo formulation (lot 62650-68) consists of 73.5% (w/w) oleic acid, 13.2% (w/w) ethanol, 12.2% cremophor EL, 1.1% water and 0.01% butylated hydroxytoluene. The drug formulations and the placebos were placed in gelatin capsules for oral administration to dogs.

Drug	Total Impurity	New Impurity
Abbott-157378.0		
Lot 40-979-TL		
Lot 58841-65		
Abbott-84538.0		
Lot 24-215-TL		

Methods

Groups of four male and four female beagle dogs (6 months old, 8-12 kg; [redacted] were orally treated with ABT-157378 and ABT-84538 in the SEC formulation by capsules at doses of 0/0 (placebo capsules), 30/15 (normal impurities; levels of impurities in Abbott-157378: 0.28% containing new impurities), and 30/15 (high impurities; levels of impurities in Abbott-157378: containing . new impurities) mg/kg/day once daily for 91-93 consecutive days. Dose volumes were 1.25 mL/kg/day. Dogs were observed twice daily during the pre-treatment and treatment periods for physical condition, behavior, and clinical signs. Food consumption was measured semi-quantitatively twice pretreatment and twice weekly during the treatment period. Body weights were recorded twice weekly during pretreatment and treatment periods. During pretreatment and treatment periods, dogs were given an opthalmoscopic examination, hematology and clinical chemistry tests, and ECG examination. All animals were subjected to a scheduled necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Hematology and clinical chemistry were evaluated on the day of necropsy. Wet tissue weights were obtained from the following organs: **brain, adrenals, thymus, thyroid, parathyroid, prostate, pituitary, gonads, heart, kidney** and **liver**. The following tissues were collected and examined by a pathologist (Appendix 1).

Results

Clinical signs and mortality. No death was seen in the study. Dose-related emesis, diarrhea and loose stools were seen in dogs in all groups including the placebo controls. Increased incidence of emesis was seen in dogs receiving two drug-formulations with high and low impurities, respectively. Increases in salivation were seen in dogs at 30/15 mg/kg/day with normal impurities.

Body weights and food consumption. Mean body weights gains were decreased in male and female dogs receiving two drug formulations with new impurities at the end of the treatment period (Table 43). Note that no statistical analyses were performed on food consumption data. No mean food consumption changes were seen in dogs receiving two drug formulations (high and low new impurities, respectively).

Ophthalmology. No drug-related ocular abnormalities were detected.

ECG. No drug-related abnormalities in ECG were seen in all dogs.

Hematology. No drug-related meaningful differences were seen between the control and drug-treated groups in all the hematological parameters examined.

Clinical Chemistry. No drug-related effects were seen in clinical chemistry with the exception of increased ALP values for dogs receiving two drug formulations with new impurities, respectively.

Histopathology. Slight increases in mean absolute and relative liver weight (relative to body weight) were seen in dogs receiving two drug formulations with new impurities (Table 44). No drug-related microscopic changes were found in liver.

Table 43 Changes in body weights and body weight gain in dogs with three-months oral administration of Abbott-157378 and Abbott-84538 with new impurities

Treatment (*mg/kg/day*)[a]	Day -1 Body Wt (kg)		Day 8 Body Wt (kg)		Day 29 Body Wt (kg)		Day 61 Body Wt (kg)		Day 89 Body Wt (kg)		Body Wt Change *Days -1 to 89*	
	M	F	M	F	M	F	M	F	M	F	M	F
0/0												
30/15												
30/15												

Table 44 Organ weight changes in male dogs with three-month oral administration of Abbott-157378 and Abbott-84538 with new impurities

Treatment (mg/kg/day)[a]	n	Liver (M)		Liver (F)	
		g	Body Weight %	g	Body Weight %
0/0	4				
30/15	4				
30/15	4				

Comments

This study demonstrated that oral administration of 30/15mg/kg/day Abbott-157378 (with up to 9% impurities or degradants, including 2-3% of each new impurities and Abbott-84538 (with up to 1% impurities or degradants) for three months did not change the toxicity profile of the Abbott-157378/Abbott-84538 combination. Mild toxicity seen in dogs receiving two drug formulations (high and normal impurities) at 30/15 mg/kg/day includes gastrointestinal disturbance (emesis, salivation, abnormal stools and diarrhea and decreased food consumption), decreases in body weight gain, increased serum ALP and absolute and relative liver weights. The NOAEL for this study was 30/15 mg/kg/day.

Toxicokinetics

Methods

Groups of 4 dogs per sex/group were used for plasma drug level determination. Animals were orally treated with ABT-157378/ABT-84538 at doses of 0/0 (placebo capsules), 30/15 mg/kg/day (normal new impurities) or 30/15 mg/kg/day (high new impurities) for 89 days. Blood samples (3 mL/sample) were collected from the jugular or cephalic vein at 2, 4, 6, 9, 12, 15 and 24 hours after dosing on days 89. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an -MS/MS method.

Results

Plasma C_{max} and AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 45. Sex differences in the plasma drug level were not observed. Both Abbott-157378 and Abbott-84538 obtained in the present study were within the expected range produced by dosages of 30/15 mg/kg/day.

Table 45. Pharmacokinetics of Abbott-157378 and Abbott-84538 in dogs with three-month oral administration of Abbott-157378 and Abbott-84538 with new impurities

Treatment (mg/kg/day)[a]	Abbott-157378				Abbott-84538			
	C_{max} (µg/mL)		AUC (µg•hr/ml)		C_{max} (µg/mL)		AUC (µg•hr/ml)	
	Male	Female	Male	Female	Male	Female	Male	Female
30/10								
30/15								

Comments

Mean AUC values for Abbott-157378 in dogs that received the SEC formulations with of impurities in the present study were similar to the expected mean AUC value of about •hr/ml for a dosage of 30 mg/kg/day. Sex differences in the plasma drug level were not observed.

23. Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with Related Substances In Beagle Dogs (Abbott R&D/00/030-Study No. TB99-127)

Vol. No.: 42; Pages: 117-323; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories
Date of Initiation: 8/18/1999; GLP Compliance: Yes (X); Drug Lot: Abbott-157378.0; Lot 27-441-NI-00; Abbott-84538.0; Lot 43-084-TL