Formulations: The ABT-378/ABT-538 liquid formulation or the placebo liquid formulation was placed in gelatin capsules. The ABT-378 or ABT-53; Liquid formulation contained 80 mg of ABT-378 and 40 mg of ABT-538, as well as excipients (w/w) which included 35.6% ethanol, 16.7% corn syrup, 15.3% propylene glycol, 5.9% glycerin, 3.1% povidone, 1% polyoxyl 40 hydrogenated castor oil and 6.9% distilled water. The placebo liquid formulation consists of all excipients used in the ABT-378/ABT-538 combination liquid formulation except for the drugs. The combination drug formulation (Lot 63964-178) designated for high levels of related substances contained 4.7% total related substances (relative to ABT-378) and 6.7% total related substances (relative to ABT-538). Another combination drug formulation (Lot 63964-177) designated for normal levels of related substances contained 0.9% total related substances (relative to ABT-378) and 0.3% related substances (relative to ABT-538).

Methods

Groups of four male and four female beagle dogs (9 months old, 7-11 kg; [redacted] were orally treated with ABT-157378 and ABT-84538 in liquid formulations by capsules at doses of 0/0 (placebo capsules), 50/25 (normal related substances), and 50/25 (high related substances) mg/kg/day once daily for 92 consecutive days (Table 46).

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 period. During the pretreatment and at the end of treatment period, dogs were given an opthalmoscopic examination (Day 84), hematology and clinical chemistry tests, and ECG examination (Day 84). 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).

Table 46. Treatment Groups and Dosages

Test Group (Liquid Formulation)	No. of Dogs		Levels of Related Substances in Combination Formulation		ABT-378/ABT-538 (mg/kg/day)
	Male	Female	Relative to ABT-378 (%)	Relative to ABT-538 (%)	
Placebo	4	4	0	0	0/0
Normal related substances	4	4	0.9-1.1	0.3-1.46	50/25
High related substances	4	4	4.5-4.7	6.7-7.58	50/25

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. Slightly increases in emesis were seen in dogs at 50/25 mg/kg/day with high levels of related substances.

Body weights and food consumption. Mean body weights gains were not altered in dogs receiving two drug formulations (with or without high levels of related substances) compared with controls. No mean food consumption changes were seen in dogs receiving two drug formulations compared with controls.

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. Increased serum ALP levels (1.5-2 fold) were seen in one male and one female dog at 50/25 mg/kg/day with normal levels of related substances (#1005, #1006). Increased serum ALP and ALT values (1.5-fold) were seen in one female dog at 50/25 mg/kg/day with high levels of related substances.

Histopathology. Decreased mean absolute and relative prostate weight (relative to body weight) were seen in dogs receiving two drug formulations with normal (-35%) and high levels of related substances (-18%), respectively. 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 50/25 mg/kg/day Abbott-157378 and Abbott-84538 liquid formulation with increased levels of related substances (0.4% glycerin adduct, 2% propylene glycol adduct, 3.3% ethanol adduct, [redacted]) for three months did not change the toxicity profile of the Abbott-157378/Abbott-84538 combination at the same dose. Mild toxicity seen in dogs receiving two drug formulations at 50/25 mg/kg/day includes gastrointestinal disturbance (emesis, salivation, abnormal stools and diarrhea), increased serum ALP and decreased absolute and relative prostate weights. The NOAEL for this study was 50/25 mg/kg/day.

Lot Number and levels of Major Related Substances (Relative to ABT-378) of the ABT-378/ABT-538 liquid formulation

Drug Lot No (Abbott-157378.0) Liquid Formulation Lot Number	Levels of Related Substances (%, w/w)			
	27-441-NI-00 63964-177 (Normal Related Substances)		27-441-NI-00 63964-178 (High Related Substances)	
	Initial	End of 3 months	Initial	End of 3 months
Bulk Potency (mg/g) Liquid Formulation Potency (mg Abbott-127378/ml)	[redacted]			
Total	0.91	1.05	4.67	4.54

Lot Number and levels of Major Related Substances (Relative to ABT-538) of the ABT-378/ABT-538 liquid formulation

Drug Lot No (Abbott-43-084-TL) Liquid Formulation Lot Number	Levels of Related Substances (%, w/w)			
	43-084-TL 63964-177 (Normal Related Substances)		43-084-TL 63964-178 (High Related Substances)	
	Initial	End of 3 months	Initial	End of 3 months
Bulk Potency (mg/g) Liquid Formulation Potency (mg Abbott-127378/ml)	[redacted]			
Total	0.27	1.46	6.68	7.58

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), 50/25 mg/kg/day (normal levels of related substances) or 50/25 mg/kg/day (high levels of related substances) 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 Day 89. 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 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 50/25mg/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 normal and high levels of related substances.

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
50/25 (normal)[b]	7.1	7.0	47.1	47.1	1.9	2.4	5.7	7.0
50/25 (high)[c]	12.4	12.8	102.6	150.6	7.3	5.1	21.5	27.0

[a]Abbott-157378/Abbott-84538; [b]Normal: normal levels of related substances; [c]High: High levels of related substances

Comments

Mean AUC values for Abbott-157378 in dogs that received the SEC formulations with high levels of related substances in the present study were similar to the expected mean AUC value of about 100 µg•hr/ml for a dosage of 50/25 mg/kg/day. However, the C_{max} and AUC values for both Abbott-84538 and Abbott-157378 in dogs receiving the formulation with normal levels of related substances were lower than expected values at the dosage used in this study. Sex differences in the plasma drug level were not observed.

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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)

Vol. No.: 17; Pages: 246-384; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation: 3/17/97; GLP Compliance: Yes (X); Drug Lot:** Abbott-157378.0; Lot 22-363--NI-00; Abbott-84538.0; Lot 24-215-TL **Formulations:** The stock 20% (w/w) ABT-378 and ABT-538 soft elastic capsule (SEC) formulations were diluted to achieve the test concentrations containing 12.5% ethanol, 75% oleic acid, 12.5% Cremophor, and 0.013% BHT. The placebo liquid formulation consists of all excipients used in the ABT-378/ABT-538 combination liquid formulation except for the drugs.

Methods

Sprague-Dawley rats (Crl:CD®(SD)BR; 28 rats/sex/group) were orally given Abbott-157378/Abbott-84538 SEC formulations once daily at doses of 0/0 (vehicle) 10/5, 30/15, or 100/50 mg/kg/day (dose volume: 2 mL/kg). Male rats were treated for 28 days premating, continuing through the mating period and post-mating period until euthanized. Female rats were dosed starting 14 days premating and continuing through mating until gestation day 7. Four rats per sex from each group were designed as satellite rats for plasma drug level determinations conducted at the end of the premating period. The animals were observed twice daily for survival. Animals were observed for physical and behavioral changes twice weekly throughout the study. Body weights of all rats were measured twice pretreatment. Males were weighed twice weekly during the treatment period. Food consumption was measured pretreatment and once weekly. Mated females (sperm positive) were weighed on gestation days 0, 6, 9, 12, 15,18 and 20. Daily vaginal smear were initiated on all females beginning thirteen days pretreatment, during the treatment period until a mating sign was observed. The phase of the cycle (proestrus, estrus, metestrus, and diestrus) was recorded. All surviving dams were euthanized on gestation day 13 and subjected to morphological examination. The uterus was examined for numbers and distribution of viable and nonviable embryos, and the total numbers of implantation. The number of corpora lutea on each ovary was recorded. Ovaries and uteri from all animals with macroscopic evidence of implants were subject to histological examination. Uteri with no macroscopic evidence of implants were subjected to the ammonium sulfite test. All males were necropsied after the last female was euthanized. Testes and epididymides were examined microscopically.

Results

Clinical signs and mortality. Two rats (#1031, #1041) died at 10/5 mg/kg. One female rat died at 30/15 mg/kg/day (#2030) on gestation 6. Dosing accidents were the causes of death for rats #1041 and #2030. Rales were seen in two male rats at 100/50 mg/kg/day and in all drug treated females. Salivation, rough and matted hair were seen in rats at ≥10/5 mg/kg/day.

Body weight and food consumption. There was a reduction in mean body weights and mean body weight gains in males at 100/50 mg/kg/day (Table 46). Mean body weight losses (-5%) were also seen in females at 100/50 mg/kg/day premating (Days 0-6). These responses were associated with statistically significant reductions (-35%) in food consumption (Days 0-7).

Fertility in Males. No test article-realted abnormalities were seen. There were no gross and microscopic abnormalities in the reproductive organs in male drug-treated groups. One male rat (#1041) at 10/5 mg/kg/day that died on Gestation Day 6 revealed bilateral discoloration of the lungs and fluid in the trachea, which was associated with dosing error.

Fertility and Early Embryonic Development in Females. There is no drug-related effect on estrous cycle during this study. One 10/5 mg/kg/day female and one 30/15 mg/kg/day females did not show mating signs. One 10/5 mg/kg/day female and three 100/50mg/kg/day females with positive mating signs were found not to be gravid. No drug treatment-related effects were seen in the copulatory, fertility or fecundity index or the copulatory interval of female rats. At gestation day 13, no drug treatment-related differences in the mean corpora lutea, implantation sites, viable and non-viable implants or resorptions,

and pre- or post-implantation losses were found between the drug treated groups and the control group. An increase in early resorptions (2-fold) was seen in female rats at 10/5 mg/kg/day. Enlarged liver was seen in the 100/50 mg/kg female groups. There were no other gross abnormalities in the reproductive organs in female drug-treated groups.

Table 46 Changes in body weights and body weight gain in male rats with oral administration of Abbott-157378 and Abbott-84538

Treatment *mg/kg/day*	Day 0 Body Wt (g)	Day 3 Body Wt (g)	Day 13 Body Wt (g)	Day 27 Body Wt (g)	Day 55 Body Wt (g)	Body Wt Change *Days 0-55*
0/0	291.7	309.1	371.9	436.6	523.8	233.0
10/5	291.3	308.0	372.3	438.7	524.7	233.1
30/15	289.6	307.7	362.8	424.9	506.3	216.7
100/50	292.7	301.2	355.5*	406.5*	485.6*	193.0*

*P<0.01

Comments

The NOAEL for male and female reproductive toxicity was 100/50 mg/kg/day. Salivation was seen in male rats at ≥10/5 mg/kg/day and in female rats at ≥ 30/15 mg/kg/day with transient reductions in food consumption. Decreases in body weight in males and increases in liver weights in females were observed at 100/50mg/kg/day. Based on these findings, the systemic NOAEL in the present study was 100/50 mg/kg/day.

Toxicokinetics

Methods

Satellite groups of 4 rats/sex/group were used for plasma drug level determination. Blood samples (0.3 mL/sample) were collected at 1,2, 4, 8, 12, 15 and 24 hours after dosing at the end of premating period. 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 47. Sex differences in the plasma drug level were not observed. Note that Abbott-84538 was not detected in the 10/5 mg/kg/day dosage group with the exception of the 4-hour time point for one female rat.

Table 47. Pharmacokinetics of Abbott-157378 and Abbott-84538 in male and female Rats 14-day oral administration of Abbott-157378 and Abbott-84538 at the end of premating period

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	1.2	1.0	12.4	13.3	--	0.02	--	--
30/15	3.8	4.3	34.4	40.5	0.2	0.7	1.0	3.2
100/50	7.1	8.6	94.4	134.2	1.5	1.5	7.3	8.7

* Abbott-157378/Abbott-84538

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)

Vol. No.: 18; Pages: 1-356; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 3/18/97; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0; Lot 22-363--NI-00; Abbott-84538.0; Lot 24-215-TL **Formulations and vehicle:** The stock 20% (w/w) ABT-378 and ABT-538 soft elastic capsule (SEC) formulations were dilated to achieve the test concentrations containing 12.5% ethanol, 75% oleic acid, 12.5% Cremophor, and 0.013% BHT. The placebo liquid formulation consists of all excipients used in the ABT-378/ABT-538 combination liquid formulation except for the drugs.

Methods

Mated Sprague-Dawley rats (Crl:CD®(SD)BR; 28 rats/sex/group) were orally given Abbott-157378 and Abbott-84538 SEC formulations once daily at doses of 0/0 (vehicle) 20/10/ 50/25, or 100/50 mg/kg/day

from Gestation Day 6 to 17 (dose volume: 2 mL/kg). Four rats per sex from each group were designed as satellite rats for plasma drug level determinations conducted at the end of the treatment period. The dams were observed twice daily for survival. Dams were observed for physical and behavioral changes twice weekly throughout the study. Body weights were measured on Gestation Day 6, 9, 12, 15, 18 and once again prior to laparotomy on Gestation Day 20. Food consumption was measured for Gestation Days 6-20. All surviving dams were euthanized on Gestation Day 20 and subjected to morphological examination. The uterus was examined externally and internally. All placentas were examined and any abnormal placentas were examined microscopically. All fetuses were sexed, weighed and examined for grossly visible external malformations and variations. Visceral and skeletal development was evaluated from fetuses in the 0/0, 50/25, or 100/50 mg/kg/day groups. The numbers and uterine positions of viable and dead fetuses, early resorptions, late resorptions and any fetal malformations and variations, and the number of corpora lutea on each ovary from gravid dams were recorded. Note that corpora lutea counts from dams with total litter resorptions were not included in statistic evaluation. Ovaries and uteri from all animals with macroscopic evidence of implants were subject to histological examination. Uteri with no macroscopic evidence of implants were subjected to the ammonium sulfite test.

Results

Clinical signs and mortality. No drug-related mortality occurred in this study. Tinted hair, decreased activity, hunched posture and discharge from eyes and nose, and emaciation were seen in rats at 100/50 mg/kg/day.

Maternal body weight and food consumption. There was a reduction in mean body weights in rats at ≥50/25 mg/kg/day on gestation days 9, 12, 15 and 18 (Table 48). These responses were associated with statistically significant reductions (-35%) in food consumption (Gestation Days 6-12). A compensatory increase in body weight change was seen in these dams after cessation of treatment (Gestation Day 18 through 20).

Embryo-fetal Development. The incidence of non-gravid dams was 4/24, 2/24, 2/24 and 5/24 in the vehicle control, 20/10, 50/25 and 100/50 mg/kg/day groups, respectively. Six dams at 100/50 mg/kg/day had complete resorption of all fetuses. Morphological examination of dams at cesarean section revealed no drug-related abnormalities. Fetal viability and body weights were statistically significantly decreased in litters from dams at 100/50 mg/kg/day. An increased incidence in skeletal variations (14th ribs and 27 presacral vertebrae) and reduction in skeletal ossification (sternebrae, hyoid, cervical centrum, vertebral arches) were seen in fetus from dams at 100/50 mg/kg/day (Table 49). A spontaneous malformation, omphalocele, was seen in one fetus (#4, Dam 3038) from a dam at 100/50 mg/kg/day. No other drug related abnormalities were seen in fetus at all doses.

Table 48 Changes in maternal body weights and body weight gain in rats with oral administration of Abbott-157378 and Abbott-84538 (On Gestation Day)

Treatment *(mg/kg/day)*[a]	Day 6 Body Wt (g)	Day 9 Body Wt (g)	Day 12 Body Wt (g)	Day 15 Body Wt (g)	Day 18 Body Wt (g)	Body Wt Change *Days 6-18*
0/0	244.8	262.2	282.3	306.3	344.2	99.4
20/10	244.2	263.0	287.5	315.5	356.7	113.8
50/25	245.2	261.6	285.7	312.5	355.0	109.8
100/50	243.8	219.6[b]	242.8[b]	273.8[b]	309.0[b]	65.2[b]

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

Comments

The NOAEL for maternal and developmental toxicity was 100/50 mg/kg/day. The NOEL for maternal and developmental toxicity was 50/25 mg/kg/day (AUC: 64/8.8 µg•hr/ml). Fetal viability and body weights were statistically significantly decreased in litters from dams at 100/50 mg/kg/day. An increased incidence in skeletal variations (14th ribs and 27 pre-sacral vertebrae) and reduction in skeletal ossification (sternebrae, hyoid, cervical centrum, vertebral arches) were seen in fetus from dams at 100/50 mg/kg/day (Table 49). A spontaneous malformation, omphalocele, was seen in one fetus (#4, Dam 3038) from a dam at 100/50 mg/kg/day.

Toxicokinetics

Methods

Satellite groups of 4 rats/sex/group were used for plasma drug level determination. Blood samples (0.3 mL/sample) were collected at 1,2, 4, 8, 12, 15 and 24 hours after dosing at the end of premating period. 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 49. The abbott-157378 AUC values were about 6-7 times greater than the Abbott-84538 AUC values for each respective dosage group after 12 days of treatment. Note that Abbott-157378 levels were observed at 4 – 9 hours. Abbott-84538 maximal plasma levels were seen at 3-4 hours post dose.

Table 49 Skeletal variations and ossifications (% of litters)

Treatment *Groups*	0/0 mg/kg/day	50/25 *mg/kg/day*	100/50 *mg/kg/day*
Variations			
14th rudimentary ribs	13.5	25.6	36.7*
14th full ribs	0.8	0	4.3
27 presacral vertebrae	0	0	6.2
Ossifications			
Sternebrae #1.2.3.4 unossified	0	0.6	6.4*
Sternebrae#5 and 6 unossified	11	7.8	21.4
Cervical centrum#1 ossified	12.7	26.8*	6.7
Reduced ossification vertebral arches	0	0	7.7
Hyoid unossified	0	1.4	5.1

*P<0.05

Table 50. Pharmacokinetics of Abbott-157378 and Abbott-84538 in rats with oral administration of Abbott-157378 and Abbott-84538 (Gestation Days 6-17)

Dosage* (mg/kg/day)	Abbott-157378		Abbott-84538	
	C_{max} (µg/mL) Dams	AUC (µg•hr/ml) Dams	C_{max} (µg/mL) Dams	AUC (µg•hr/ml) Dams
20/10	3.49	28.1	1.2	4.9
50/25	6.44	64.1	1.9	8.8
100/50	9.36	116.4	2.8	16.1

*Abbott-157378/Abbott-84538; M: male; F: female

Conclusion

Abbott-157378 and ritonavir were administered by oral gavage to gravid female rats at 20/10, 50/25 and 100/50 mg/kg/day on Gestation Days 6 through 17. Maternal toxicity (reduction in body weight and food consumption) was observed at 100/50 mg/kg/day. Embryo/fetal toxicity in rats was observed at a maternally toxic dosage (100/50 µg/kg/day, AUC 116/16 µg•hr/ml) and was characterized by early resorption, reduced fetal viability, reduced fetal weight, delayed skeletal ossification (sternebrae, hyoid, cervical centrum, vertebral arches) and an increased incidence in skeletal variations (14th ribs and 27 presacral vertebrae). Based on these results the maternal and developmental NOAEL was 100/50 mg/kg/day and the NOEL was 50/25 mg/kg/day (AUCs: 64/8.8 µg•hr/ml). Abbott-157378/ritonavir combination produced no effects on fertility in rats at 100/50 µg/kg/day. No teratogenesis was observed at 100/50 mg/kg/day.

26. 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; WIL-57014)

Vol. No.: 19; **Pages:** 1-365 **Conducting Laboratory:** WIL Laboratories, Inc; **Date of Initiation:** 6/5/97; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0; Lot 22-363–NI-00; Abbott-84538.0; Lot 24-215-TL; **Formulations and vehicle:** The stock 20% (w/w) ABT-378 and ABT-538 soft elastic capsule (SEC) formulations were diluted to achieve the test concentrations containing 12.5% ethanol, 75% oleic acid, 12.5% Cremophor, and 0.013% BHT. The placebo liquid formulation consists of all excipients used in the ABT-378/ABT-538 combination liquid formulation except for the drugs.

Methods

Mated Sprague-Dawley rats (Crl:CD®(SD)BR; 25 rats/sex/group) were orally given Abbott-157378 and Abbott-84538 SEC formulations once daily by gavage at doses of 0/0 (vehicle) 20/10, 40/20, or 80/40 mg/kg/day from Gestation Day 6 to lactation Day 20 (dose volume: 2 mL/kg). All maternal animals were observed twice daily for mortality, physical and behavioral changes throughout the study. Body weights were measured on gestation days 0, 6, 7, 8, 9, 12, 15, 18 and 20 and on lactation days 1, 4, 7, 14 and 21. Food consumption was measured for Gestation Days 0, 6-20, and on lactation days 1 and 4. All surviving maternal females were allowed to deliver and rear their offspring to lactation day 21. Females that did not deliver were euthanized on post-mating day 25. Females with total litter loss were euthanized within 24 hours. At necropsy, uterus and ovaries from animals were subject to histological examination. Uteri with no macroscopic evidence of implants were subjected to the ammonium sulfite test. The number of former implantation sites was recorded. Litters were examined daily for survival and all death were recorded. Intact offspring dying from PND 0 to 4 or thereafter were necropsied and detailed gross necropsies were performed. Eight pups per litter, four per sex were randomly selected on PND 4. Litters were examined daily for clinical signs and any abnormalities in nesting and nursing behavior. Physical examination was conducted on PND 1, 4, 7, 14, 21 and at weekly interval thereafter until necropsy. Body weights were measured on PND 1, 4, 7, 14 and 21, and at weekly interval thereafter until necropsy. Pups were sexed on PND 0, 4 and 21. Litter parameters (live litter size, stillbirth index, live birth index, postnatal survival between birth and PND 0 or PND 4) were calculated. When pups were between 9 and 13 days of age, 25 males and 25 females per group were randomly selected for the F1 generation. From these selected pups, 10 males and 10 females per group were selected for neurobehavioral (auditory startle response on lactation day 15, learning and memory assessment between PND 21 and 29, and motor activity on PND 35), physical and functional development assessment. All F1 animals were observed twice daily for clinical signs. Body weights were measured weekly from PND 28 until necropsy. When evidence of mating was established, female body weights were measured on gestation days 0, 6, 9, 12 and 13. Reproductive performances of the F1 animals were evaluated and reproductive performances indices (estrous cycles, female mating index, male mating index, female fertility index, male fertility index) were calculated. All F1 females with evidence of mating were necropsied on gestation day 13. F1 females without evidence of mating were necropsied on completion of the mating period. F1 males were necropsied following the last laparohysteretomy. At necropsy, uterus and ovaries from animals were subject to histological examination (the number of corpora lutea, the number and location of all embryos, early and late resorptions, total number of implantation sites). Uteri with no macroscopic evidence of implants were subjected to the ammonium sulfite test.

Results

F_0 Maternal Generation

Clinical signs and mortality. One F_0 female at 80/40 mg/kg/day died of a moribund condition on lactation day 8. This female had foamy contents in the lungs and all lobes of the lungs were mottled. No other drug-related clinical signs were seen in this study.

Pregnancy status. One female in each of the 20/10 and 80/40 mg/kg/day groups failed to deliver offspring (post-mating day 25).

Body weights and food consumption. No drug-related changes in mean body weight and mean body weight gain were seen in F_0 female rats during gestation and lactation periods. Food consumption during gestation and lactation in the treated F0 females was not affected by the test article administration.

Gestation length and parturition. No drug-related changes in the mean gestation lengths were seen in F_0 female rats at all doses. No signs of dystocia were seen in this study.

Terminal and necroscopic evaluations. One F_0 female in each of the 20/10 and 80/40 mg/kg/day groups failed to deliver and were euthanized on post-mating day 25. No macroscopic and microscopic abnormalities were seen in these animals. One F_0 female at 40/20 mg/kg/day had total litter loss on

lactation day 17. No macroscopic and microscopic abnormalities were seen in this animal. One F_0 female at 80/40 mg/kg/day had total litter loss on lactation day 2. Enlarged and discolored (yellow) adrenal glands were seen in this animal. No drug-related changes in the number of pups born and the number of implantation sites counted at the necropsy were seen in females on lactation day 21 at necropsy.

F_1 litters

Clinical signs and mortality. Pups that were found dead during the F1 postnatal period (PND 0-21) numbered 4, 3, 24 and 27 in the control, 20/10, 40/20 and 80/40 mg/kg/day groups, respectively. Twenty and 13 pups in the 40/20 and 80/40 mg/kg/day groups, respectively, were cool to touch. A decrease in postnatal pup survival (percent per litter) was seen in the 80/40 mg/kg/day group during the birth to PND 4 interval (91% compared to 99% in the control group). No drug-related changes in mean postimplantation survival indices, numbers of pups born per litter, percentages of males per litter, live litter sizes on PND 0, stillbirth indices and live birth indices were seen in this study.

Body weights and food consumption. No drug-related changes in mean body weight and mean body weight gain were seen in F_1 pups throughout lactation.

Terminal and necroscopic evaluations. The numbers of pups (litters) found dead during the F_1 postnatal period (PND 0-21) in the control, 20/10, 40/20 and 80/40 mg/kg/day groups were 4(3), 3(3), 24(8) and 27(9) pups, respectively. One F_1 pup at 80/40 mg/kg/day had dilated renal pelves. The numbers of surplus pups (litters) in the control, 20/10, 40/20 and 80/40 mg/kg/day groups were 3, 1, 5 and 4 pups, respectively. One F_1 pup at 20/10 mg/kg/day had distended ureter. Malformations were seen in the 20/10 mg/kg/day group (one pup had anury) and the 40/20/mg/kg/day (two pups had hydrocephalic and one pup had anury).

Post weaning developmental landmarks and neurobehavioral functions. No drug-related changes were seen in pups in Pinnal detachment (lactation day 7), surface righting response (lactation day 7) and eye opening (lactation day 17). Increases in total and ambulatory activities were seen in F_1 females at 80/40 mg/kg/day. No other changes in the indicators of functional behavior were seen. No drug-related changes in swimming ability (Biel maze), learning and memory were seen in animals at all doses.

F_1 Generation

Clinical signs and mortality. No other drug-related clinical signs and mortality were seen in this study.

Body weights and food consumption. A drug-related decrease (-6%) in mean body weight was seen in F_1 females at 80/40 mg/kg/days for week 7. No F_0 maternal treatment-related changes in gestation mean body weight and mean body weight gain were seen in F_1 females at all doses.

Reproductive performances. Reproductive performances in the F_1 generation were not affected by the F_0 maternal treatment. No F_0 maternal treatment-related changes in the estrous cycles were seen in all F_1 females. No F_0 maternal treatment-related changes in the male fertility indices, male mating indices and female mating indices were seen in all F_1 animals.

Gestation day 13 laparohysterectomy and uterine evaluation, and necroscopic evaluations. No treatment-related changes in viable embryos, pre- and postimplantation loss and numbers of corpora lutea and implantation sites were seen in F_1 females at all doses (gestation day 13). One, two and one female in the control, 20/10 and 40/20 mg/kg/day groups, respectively had a dilated renal pelvis. One female in the 80/40 mg/kg/day group had a cystic ovary. One female in the 40/20 mg/kg/day group had a subcutaneous mass.

Comments

Oral administration of Abbott-15738/ritonavir at 20/10, 40/20 and 80/40 mg/kg/day did not affect F_0 duration of gestation or parturition. Note that plasma drug levels were not measured in this study. No signs of maternal toxicity and body weight changes were seen at the 80/40 mg/kg/day in this study. F_1 neonatal toxicity (reduced postnatal pup survival) was seen in the ≥40/20 mg/kg/day group during the birth to PND 4 interval. No reproductive toxicity (F_0 parturition and F_1 reproductive performance) was

seen at all doses. No developmental toxicity was seen in the F_2 embryos. The NOAEL for pre-, peri- and post-natal toxicities was considered to be ≥ 40/20 mg/kg/day.

27. 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)

Vol. No.: 21; **Pages:** 1-336; **Conducting Laboratory:** Pharmaceutical Products Division Research and Development, Abbott Laboratories **Date of Initiation:** 3/19/97; **GLP Compliance:** Yes (X); **Drug Lot:** Abbott-157378.0: Lot 22-363--NI-00; Abbott-84538.0; Lot 24-215-TL **Formulations and vehicle:** The stock 20% (w/w) ABT-378 and ABT-538 soft elastic capsule (SEC) formulations were diluted to achieve the test concentrations containing 12.5% ethanol, 75% oleic acid, 12.5% Cremophor, and 0.013% BHT. The placebo liquid formulation consists of all excipients used in the ABT-378/ABT-538 combination liquid formulation except of the drugs.

Methods

Mated New Zealand White rabbits (24 rabbits/sex/group; age: 4-5 months, body weight: 3.0-4.1 kg) were orally given Abbott-157378 and Abbott-84538 SEC formulations once daily at doses of 0/0 (vehicle) 30/15, 50/25, or 80/40 mg/kg/day from Gestation Day 6 to 18 (dose volume: 4 mL/kg). Four rats per sex from each group were designated as satellite rats for plasma drug level determinations conducted at the end of the treatment period (gestation days 6-19). The dams were observed twice daily for survival. Dams were observed for physical and behavioral changes twice weekly throughout the study. Body weights were measured on gestation days 6, 9, 12, 15, 18 and once again prior to laparotomy on Gestation Day 20. Food consumption was measured for gestation days 6-20. All surviving dams were euthanized on Gestation Day 29 and subjected to morphological examination. The uterus was examined externally and internally. All placentas were examined and any abnormal placentas were examined microscopically. All fetuses were sexed, weighed and examined for grossly visible external malformations and variations. Visceral and skeletal development were evaluated from fetuses in the 0/0 and 80/40 mg/kg/day groups. The numbers and uterine positions of viable and dead fetuses, early resorptions, late resorptions and any fetal malformations and variations, and the number of corpora lutea on each ovary from gravid dams were recorded. Ovaries and uteri from all animals with macroscopic evidence of implants were subject to histological examination. Uteri with no macroscopic evidence of implants were subjected to the ammonium sulfite test.

Results

Clinical signs and mortality. No drug-related mortality occurred in this study. Increases in emaciation, loose stool and absent stool were seen at 80/40/mg/kg.

Maternal body weight and food consumption. There was a reduction (6%) in mean body weight gain in rats at 80/40 mg/kg/day during gestation period (days 0-18). These responses were associated with statistically significant reductions (-30%) in food consumption. A small reduction (12%) in food consumptions was seen in animals at 50/25 mg/kg/day. A compensatory increase in body weight change was seen in these dams after cessation of treatment (Gestation Day 18 through 20).

Embryo-fetal Development. No developmental toxicity, including teratogenicty was observed in fetus at all doses. Morphological examination of dams at cesarean section revealed no drug-related abnormalities.

Comments

The NOAEL for maternal developmental toxicity in this oral rabbit study was considered to be 80/40 mg/kg/day (AUC: 90/9 µg/ml).

Toxicokinetics

Methods

Satellite groups of 4 rabbits/sex/group were used for plasma drug level determination. Blood samples were collected at 1, 2, 4, 8, 12, 15 and 24 hours after dosing on gestation day 19. Plasma levels of Abbott-157378 and Abbott-84538 were measured by an [redacted] MS/MS method.

Results

Plasma AUC values for Abbott-157378 and Abbott-84538 are summarized in Table 50.

Table 50. Pharmacokinetics of Abbott-157378 and Abbott-84538 in rabbits with oral administration of Abbott-157378 and Abbott-84538 (Gestation Days 19)

Dosage* (mg/kg/day)	Abbott-157378 AUC (µg•hr/ml) Dams	Abbott-84538 AUC (µg•hr/ml) Dams
30/15	11.2	0.3
50/25	39.4	3.9
80/40	89.6	9.1

* Abbott-157378/Abbott-84538

GENETIC TOXICOLOGY

28. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli*) of Abbott-157378 (Study No. TX96-114, Scientific report no. R&D/96/439, 1996)

Vol. No.: 21; Pages: 1-30; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories
Date of Initiation: 5/28/96; GLP Compliance: Yes (X); Drug Lot: Abbott-157378.0; Lot 16-276-AL

Methods

Four strains of *Salmonella typhimurium* (TA-1535, TA-1537, TA-98 and TA-100) and one *Escerichia coli* strain (WP2uvrA⁻) were used in this study to detect mutations caused by either frame-shift or substitution mutations due to ABT-157378. Aroclor-1254 induced rat liver microsomes (S-9 fraction, Lot No. 39912) were used in activated tests. Positive controls used in the tests were N-methyl-N-nitro-nitrosoguanidine, quinacrine mustard (5 µg/plate), 2-nitrofluorene (10 µg/plate), and 2-aminoanthracene (2.5 µg/plate). [redacted] was used as the vehicle control. ABT-157378 was tested at concentrations of 100, 300, 1000, 2000, 5000 and 10,000µg/plate (2, 6, 20, 40, 100 and 200 mg/ml, respectively).

Results

Toxicity was observed only at 10,000 µg/plate (200mg/ml) in a single test (*Salmonella typhimurium TA-1537*), manifested by reduced colony counts. ABT-157378 did not show mutagenic activity with or without metabolic activation at maximum concentration; positive controls were effective.

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)

Vol. No.: 21; Pages: 31-69; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories
Date of Initiation: 4/15/98; GLP Compliance: Yes (X); Drug Lot: Abbott-157378.0; Lot 37-324-ZW-00. [redacted]

Methods

Four strains of *Salmonella typhimurium* (TA-1535, TA-1537, TA-98 and TA-100) and one *Escerichia coli* strain (WP2uvrA⁻) were used in this study to detect mutations caused by either frameshift or substitution mutations due to ABT-157378. Aroclor-1254 induced rat liver microsomes (S-9 fraction, Lot No. 39912) were used in activated tests. Positive controls used in the tests were N-methyl-N'-nitro-

nitrosoguanidine, quinacrine mustard (5 µg/plate), 2-nitrofluorene (10 µg/plate), and 2-aminoanthracene (2.5 µg/plate). [redacted] was used as the vehicle control. A specific lot of ABT-157378 with high impurity levels was tested at concentrations of [redacted]).

Results

No toxicity was seen at 5000 µg/plate (100 mg/ml). ABT-157378 did not show mutagenic activity with or without metabolic activation at maximum concentration; positive controls were effective.

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)

Vol. No.: 21; Pages: 70-105 Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 9/28/98; GLP Compliance: Yes (X) Drug Lot: Abbott-157378.0; Lot 58841-65. [redacted]

Methods

Four strains of *Salmonella typhimurium* (TA-1535, TA-1537, TA-98 and TA-100) and one *Escerichia coli* strain (WP2uvrA⁻) were used in this study to detect mutations caused by either frame-shift or substitution mutations due to ABT-157378. Aroclor-1254 induced rat liver microsomes (S-9 fraction, Lot No. 39912) were used in activated tests. Positive controls used in the tests were N-methyl-N-nitro-nitrosoguanidine, quinacrine mustard (5 µg/plate), 2-nitrofluorene (10 µg/plate), and 2-aminoanthracene (2.5 µg/plate). [redacted] was used as the vehicle control. A specific lot of ABT-157378 with [redacted]

Results

No toxicity was seen at 5000 µg/plate (100 mg/ml). ABT-157378 did not show mutagenic activity with or without metabolic activation at maximum concentration; positive controls were effective.

31. Bacterial Reverse Mutation Assay (Ames Test plus *E. coli*) of a Liquid Combination Formulation of Abbott-157378/ritonavir With Related Substances (Abbott Study No.TX99-137, R&D/99/447)

Vol. No.: 42; Pages: 324-361; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 9/21/99; GLP Compliance: Yes (X); Drug Lot: The Abbott-157378 (81 mg/ml) and ritonavir (40.8 mg/ml) combination liquid formulation (lot 63-964-178) contained 0.4-3% each of the following related substances: [redacted]

Methods

Four strains of *Salmonella typhimurium* (TA-1535, TA-1537, TA-98 and TA-100) and one *Escerichia coli* strain (WP2uvrA⁻) were used in this study to detect mutations caused by either frameshift or substitution mutations due to ABT-157378/ritonavir combination liquid formulation with a high level of impurities. Aroclor-1254 induced rat liver microsomes (S-9 fraction, Lot No. 39912) were used in activated tests. Positive controls used in the tests were N-methyl-N'-nitro-nitrosoguanidine, quinacrine mustard (5 µg/plate), 2-nitrofluorene (10 µg/plate), and 2-aminoanthracene (2.5 µg/plate. [redacted] was used as the vehicle control. A stock liquid formulation of ABT-157378/ritonavir was diluted by DMSO. The formulation was tested at concentrations of [redacted]

Methods

A specific lot of Abbott-157378 with an impurity level of [redacted] was evaluated for its ability to induce chromosome damage in human peripheral blood lymphocytes grown in culture. The assay was conducted in the absence and presence of an exogenous metabolic activation system. Concentrations of 1, 3, 10, 20, 30, 50, 100 and 300 µg/ml of Abbott-157378 (dissolved in DMSO) were used in both the non-activated and rat liver S-9 activated assay. Mitomycin C (0.125 µg/ml, dissolved in sterile water) and 1% DMSO were used as positive and negative controls, respectively. With concentrations of 10, 20 and 30 µg/ml (4 hour, non-activated), 3, 10, 20 and 30 µg/ml (24 hour, non-activated) and 20, 30 and 50 µg/ml (4 hour, rat liver S-9 activated), Abbott-157378 was evaluated for genotoxicity.

Results

Toxicity was seen at 20 µg/ml (4 hour, non-activated) and 10 µg/ml (24 hour, non-activated) and 30 µg/ml (4 hour, rat liver S-9 activated) and higher concentrations. Negative and positive controls met the criteria for a valid assay and were consistent with historical laboratory data. The results indicated that Abbot-157375 exhibited no clastogenic potential in this assay.

35. *In Vitro* Cytogenetics Human Lymphocyte Culture Assay of Abbott-157378 With High New Impurities (Study No. TX98-186, R&D/98/645)

Vol. No.: 21; Pages: 183-219; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 4/16/98; GLP Compliance: Yes (X); Drug Lot: Lot 588-41-65; [redacted]

Methods

A specific lot of Abbott-157378 with an impurity level of [redacted] was evaluated for its ability to induce chromosome damage in human peripheral blood lymphocytes grown in culture. The assay was conducted in the absence and presence of an exogenous metabolic activation system. Concentrations of 1, 3, 10, 20, 30, 50, 100 and 300 µg/ml of Abbott-157378 (dissolved in DMSO) were used in both the non-activated and rat liver S-9 activated assay. Mitomycin C (0.125 µg/ml, dissolved in sterile water) and 1% DMSO were used as positive and negative controls, respectively. With concentrations of 10, 20 and 30 µg/ml (4 hour, non-activated), 10, 20 and 30 µg/ml (24 hour, non-activated) and 20, 30 and 50 µg/ml (4 hour, rat liver S-9 activated), Abbott-157378 was evaluated for genotoxicity.

Results

Toxicity was seen at 20 µg/ml (4 hour, non-activated) and 20 µg/ml (24 hour, non-activated) and 30 µg/ml (4 hour, rat liver S-9 activated) and higher concentrations. Negative and positive controls met the criteria for a valid assay and were consistent with historical laboratory data. The results indicated that Abbot-157375 exhibited no clastogenic potential in this assay.

36. *In Vitro* Cytogenetics Assay in Human Lymphocytes of a Liquid Combination Formulation of Abbott-157378 and Abbott-84538 with Related Impurities (Study No. TX99-138, R&D/99-448)

Vol. No.: 42; Pages: 362-401; Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories Date of Initiation: 9/21/99; GLP Compliance: Yes (X); Drug Lot: The Abbott-157378 (81 mg/ml) and ritonavir (40.8 mg/ml) combination liquid formulation (lot 63-964-178) contained 0.4-3% each of the following related substances: 1.26% core-wing-B, 1.06% B-wing diacyl, 1.08% regioisomer, 2.04% propylene glycol adduct, 3.26% ethanol adduct and 0.41% glycerin adduct as well as lower concentrations of known and unknown substances.

Methods

A liquid formulation of Abbott-157378 and ritonavir with an impurity level of [redacted] was evaluated for its ability to induce chromosome damage in human peripheral blood lymphocytes grown in culture. The

assay was conducted in the absence and presence of an exogenous metabolic activation system. Concentrations of 1/0.5, 2/1, 3/1.5, 5/2.5, 10/5, 30/15, 50//25 and 100/50 µg/ml of Abbott-157378/ritonavir (dissolved in DMSO) were used in both the non-activated and rat liver S-9 activated assay. Mitomycin C (0.125 µg/ml, dissolved in sterile water) and 1% DMSO were used as positive and negative controls, respectively. With concentrations of 10/5, 30/15 and 50/25 µg/ml (4 hour, non-activated), 5/2.5, 10/5 and 30/15 µg/ml (4 hour, rat liver S-9 activated) and 2/1, 3/1.5 and 5/2.5µg/ml (24 hour, non-activated), Abbott-157378 was evaluated for genotoxicity.

Results

Toxicity was seen at 30/15 µg/ml (4 hour, non-activated) and 10/5 µg/ml (4 hour, activated) and 5/2.5 µg/ml (24 hour, non-activated) and higher concentrations. Negative and positive controls met the criteria for a valid assay and were consistent with historical laboratory data. The results indicated that Abbot-157375 exhibited no clastogenic potential in this assay.

37. *In Vitro* Cytogenetics Assay in Human Lymphocytes of Abbott-84538 Soft Elastic Capsule (SEC) Formulation (Study No. TX96-401, R&D/96/746)

Vol. No.: 42; Pages: 402-453 Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories **GLP Compliance:** Yes (X) **Drug Lot:** The Abbott-84538 soft elastic capsule (SEC) formulation (Lot 21-334-AR-XX). This formulation contained 2.89% (w/w) and 1.59% (w/w) of an acid hydrolysis degradation product and a base cyclized degradant product, respectively.

Methods

A SEC formulation of Abbott-84538 with high levels of impurities was evaluated for its ability to induce chromosome damage in human peripheral blood lymphocytes grown in culture. The assay was conducted in the absence and presence of an exogenous metabolic activation system. Concentrations of 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000 and 5000 µg/ml of Abbott-84538 (dissolved in DMSO) were used in both the non-activated and rat liver S-9 activated assay. Mitomycin C (0.125 µg/ml, dissolved in sterile water), cyclophosphamide (12.5 µg/ml, dissolved in DMSO) and 1% DMSO were used as positive and negative controls, respectively. With concentrations of 10, 30 and 100 µg/ml (24 hour, non-activated; 4 hour, rat liver S-9 activated), Abbott-84538 was evaluated for genotoxicity.

Results

Toxicity was seen at 30 µg/ml (24 hour, non-activated; 4 hour, activated) and higher concentrations. Negative and positive controls met the criteria for a valid assay and were consistent with historical laboratory data. The results indicated that Abbot-157375 exhibited no clastogenic potential in this assay.

38. Mouse Micronucleus Assay of Abbott-157378 Alone and in Combination with Abbott-84538 (Drug Safety Evaluation Division, Abbott Study No. TD96-211, Scientific Report No. R&D/96/250)

Conducting Laboratory: Pharmaceutical Products Division Research and Development, Abbott Laboratories; **GLP Compliance:** Yes (X); **Drug Lot:** lot 16-276-AL (Abbott-157378), 86-701-AL (Abbott-84538)

Methods

To evaluate its clastogenic potential, Abbott-157378 was tested alone and in combination with Abbott-84538 in the bone marrow micronucleus assay. Drugs were administered to male Crl:CD-1 mice (5 to 10 mice/group) by oral gavage in combinations of ABT 157378/ritonavir at concentrations of 0/0 (vehicle: propylene glycol, ethanol 95:5, v/v), 625/0, 1250/0, 2500/0, 78/35, 156/78, 313/156 mg/kg/day for two days. Positive control groups were treated with cyclophosphamide (25 mg/kg). Mice were sacrificed and bone marrow was harvested from the femur approximately 28 hours after the last dose and examined for polychromatic erythrocytes.

Results
Single doses or combinations of ABT 157378/ritonavir did not produce more micronucleated bone marrow polychromatic erythrocytes (PCEs) than that occurring in vehicle controls, while negative and positive controls produced met the criteria for a valid assay and were consistent with laboratory historical data.

39. L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay of Abbott-157378, Study No. TX96-230, R&D/96/773; Study No. G96BC78.702)

Vol. No.: 21; **Pages:** 256-299; **Conducting Laboratory:** Microbiological Associates, Inc; **GLP Compliance:** Yes (X); **Drug Lot:** lot 16-276-AL (Abbott-157378), 86-701-AL (Abbott-84538); **Formulation:** the test article (500 mg/ml) was dissolved in vehicle (DMSO)

Methods
To evaluate its genotoxicity, Abbott-157378 was tested for its potential to induce mutations at the thymidine kinase locus in L5178Y/TK$^{+/-}$ Mouse Lymphoma cell line in the presence or absence of a metabolic activation system. Abbott-157378 was at concentrations of 1 to 20 µg/ml and 25 to 100 µg/ml (initial assay) for the non activated and activated assays, respectively. A repeat (supplemental) assay was performed at 40 to 120 µg/ml in the presence of Aroclor 1254-induced rat liver S9 to attain toxicity. The positive controls were treated with 10 and 20 µg/ml methyl methane sulfonate (MMS), and 2.5 and 4.0 µg/ml 7,12-dimethyl-benzyanthracene (DMBA), respectively. The vehicle controls were treated with DMSO.

Results
Toxicity was seen at ≥50 µg/ml without activation and ≥ 501 µg/ml with S9activation. ABT 157378 was concluded to be negative under the conditions of the L5178Y/TK$^{+/-}$ Mouse Lymphoma mutagenesis assay.

NON-CLINICAL PHARMACOKINETIC STUDIES

Absorption and Pharmacokinetics

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

Vol. No.: 29; **Pages:** 183-342; **Conducting Laboratory:** Abbott; Non GLP; **Drug Lot:** lot 16-276-AL (Abbott-157378), 86-701-AL (Abbott-84538); **Formulation or vehicle:** For oral and intravenous administration Abbott-157378 was prepared as a 5 mg/mL solution in an ethanol:propylene: glycol:dextrose in water (20:30:50, v/v/v). For co-dosing experiments, Abbott-157378 and ritonavir were combined at concentrations appropriate for either a 2 mL/kg (rat, monkey) or 1 mL/kg (dog) dose volume in each study. Two molar equivalents of methane sulfonic acid were added to aid in the solubilization of ritonavir. An ethanol: propylene glycol (5:95, v/v) vehicle was utilized in studies that required higher concentrations of Abbott-157378 (10-250 mg/mL, 1 mL/kg dose volumes). The vehicle contained two molar equivalents of p-toluene sulfonic acid, was used to solubilize higher concentrations of ritonavir. Semi-solid capsules of both Abbott-157378 and ritonavir were prepared for oral administration in dog.

Methods
Rats. Groups of four male Sprague-Dawley rats received either a single 5 mg/kg i.v. dose (1mL/kg), or a 10 mg/kg oral dose Abbott-157378 by gavage (2 mL/kg). To evaluate the effect of dose and dose ratio on the pharmacokinetics of Abbott-157378 and ritonavir, rats (3-4 rats/group) were orally administered Abbott-157378/ritonavir at 10/1, 10/5, 10/10, 5/1, 5/2.5, 5/5, 3/3, 2.5/5 or 1/10 mg/kg, respectively. Sequential blood samples were obtained 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours after dosing. Plasma drug concentrations were determined by an [redacted] method. Additionally, groups of Sprague-Dawley rats (3 rats/sex/group) received a 20, 100, 500, or 2500 mg/kg single oral dose Abbott-157378 by gavage, plasma drug concentrations were determined at 0.5, 1.5, 3, 6, 9, 12 and 24 hours after dosing.
Dogs. Three fasted beagle dogs (male/female) received either a single 5 mg/kg i.v. dose of Abbott-

157378 (1 mL/kg) or an oral dose of Abbott-157378/ritonavir at 5/5, 5/1, or 5/2.5 mg/kg (2 ml/kg) in a sequential manner. A washout/recovery period of at least seven days separated each of the dosing periods. All dogs were fasted overnight prior to dosing and throughout the duration of the study. Blood samples were obtained from a jugular vein of each dog prior to dosing and 0.1 (i.v. only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after drug administration. Groups of dogs (3/group) received semi-solid capsule formulations at doses corresponding to 2.5/5, 5/5, 10/15, 15/15 or 30/15 mg/kg. Blood samples were obtained for each dog prior to dosing and 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15 and 24 hours postdosing.

Monkeys. The pharmacokinetics of Abbott-157378 following oral administration in the presence and absence of ritonavir was evaluated in three female cynomolgus monkeys. During the first dosing period, each animal received a 10 mg/kg oral dose of Abbott-157378. Following a washout period of three weeks, the same three fasted monkeys received 10/10 mg/kg oral dose of Abbott-157378 and ritonavir *via* a nasoesophageal tube. Blood samples were obtained prior to dosing and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9 and 12 hours after dosing. Plasma drug concentrations were determined by an [redacted] method.

Results

Rats. $T_{1/2}$ values of Abbott- 157378 in rats receiving a 5 mg/kg i.v dose was 1.08 hours, with volume of distribution values of 0.88 L/kg and 2.2 L/kg for Vc and V_β, respectively. The plasma clearance (CL_p) of Abbott-157378 was 1.37 L/hr•kg. The mean AUC value was 3.8 µg•hr/ml. After oral administration in rats, Abbott-157378 was rapidly absorbed, with T_{max} values of 0.25-2 hours. Peak plasma concentrations averaged 0.96 µg/mL. Abbott-157378 was rapidly absorbed from the solution formulation, with peak plasma concentrations averaging 0.96 µg/mL in the rat, corresponding to a bioavailability of 25.2%. Abbott-157378 bioavailability declined with increasing dose, averaging 18.8, 20.1, 12.3 and 2.3% following 20, 100, 500 or 2500 mg/kg in the single oral dose rats, respectively. The Abbott-157378 AUC value in rat was elevated 12-fold following administration of a 10 mg/kg oral dose in combination with a 10 mg/kg oral dose or ritonavir.

Dogs. Abbott-157378 was not detected in plasma samples obtained from dogs at 5 mg/kg oral dose. The plasma elimination half-life was 0.56 hours with a volume of distribution of 0.52 L/kg and 1.1 L/kg for Vc and V_β, respectively. Plasma clearance values (CLp) ranged from 1.21-1.47 L/hr•kg, with a mean value of 1.30 L/ hr•kg. Co-administration of Abbott- 157378 and ritonavir produced sustained plasma concentrations of Abbott-157378 in dogs. The plasma concentrations of Abbott-157378 remained constant 8 hours after dosing, while the concentrations of ritonavir were declining. Concentrations of Abbott- 157378 were not detected in plasma samples of dogs receiving a single 5 mg/kg oral dose of Abbott-157378. In contrast, peak plasma concentrations of Abbott-157378 averaged 2.50µg/mL with an AUC of 11.26µg•hr/mL following a 5/5mg/kg oral dose co-administration. The peak plasma concentrations of Abbott-157378 obtained from the 5/1 mg/kg oral dose declined proportionally to the decrease of the ritonavir dose. Ritonavir C_{max} and AUC values derived from a single 5 mg/kg oral dose of ritonavir averaged 3.78 and 12.21 µg•hr/mL, respectively. The co-administration of Abbott-157378 produced a significant decline in the plasma concentrations of ritonavir. The use of a formulated capsule for both compounds increased the plasma concentrations of ritonavir with a correspondingly large increase in the Abbott-157378 C_{max} and AUC values.

Monkeys. A single 10 mg/kg dose of Abbott-157378 to female cynomolgus monkeys did not result in detectable peak plasma concentrations of the drug. Co-administration of the same dose of Abbott-157378 with a 10 mg/kg dose of ritonavir provided an average C_{max} and AUC of 3.06 µg/mL and 14.72 µg•h/mL, respectively.

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 (Abbott laboratories Division 46 Report No. R&D/97/625, 1997)

Mice (25/sex/groups) were orally dosed with 20/10, 60/30, or 200/100 mg/kg/day Abbott-157378/ritonavir in a soft elastic capsule formulation for 92 days. Plasma drug levels were determined

(Toxicokinetics-Table 2; R&D/97/501, 1997). Mice displayed similar C_{max} Abbott-157378 and AUC values for ABT-378 at comparable doses. Mean C_{max} values for ABT-378 increased approximately 5-fold over the 10-fold increase in dose size and was therefore less than dose proportional. However, AUC increased in an approximately dose proportional manner. Ritonavir exhibited a somewhat different profile, with C_{max} and AUC values increasing in a greater than dose proportional manner with a 12- to 18-fold increase in C_{max} and a 19- to 21-fold increase in AUC over the 10-fold increase in ritonavir dose.

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)

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics-Table 9; R&D/97/574)

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)

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 17; R&D/97/720)

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)

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 49; R&D/97/335)

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)

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 22; R&D/98/375)

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);GLP

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 18; R&D/98/307)

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 R&D/96/740, 1997); GLP

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 30; R&D/96/675)

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); GLP

The mean pharmacokinetic parameters in male and female dogs for Abbott-157378 and ritonavir on Day 1, Day 90 and Day 167 are summarized in Table 36 (Toxicokinetics; R&D/97/752). The plasma concentrations of Abbott-157378 in dogs are substantially increased when co-administered with ritonavir. There were no gender differences in the pharmacokinetic parameters of Abbott-157378 or ritonavir in dogs. The steady-state pharmacokinetic parameters on Days 90 and 167 were similar with mean apparent clearances of Abbott-157378 ranging from 0.26 to 3.84 L/h/kg. The large mean clearance of Abbott-157378 and ritonavir observed for the lowest dose group on Day 167 was due to some dogs having very low exposures on the sampling day. In general, the plasma concentrations of Abbott-157378 increased with increasing dose. The highest exposures (AUC) obtained in dogs after the 60 mg/kg/day dose of Abbott-157378 with 20 mg/kg/day ritonavir dose was 264.2 µg•h/mL for Abbott-157378 and 49.8 µg•h/mL for ritonavir for 90 days. The *ex vivo* plasma protein binding of Abbott-157378 and ritonavir was greater than 98%; which are similar to that seen in humans.

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); GLP

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 42, R&D/98/371)

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)

Comments

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 45; R&D/99/093)

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)

The mean estimates of the non-compartmental pharmacokinetic parameters for Abbott-157378 and Abbott-84538 obtained in this study are summarized (Toxicokinetics -Table 39; R&D/99/124). For Abbott-157378, the increase in mean C_{max} and AUC values appeared to be less than dose-proportional for 50/25 mg/kg/day group, for all three PK days (Days 1,182 and 267). For ritonavir, mean C_{max} and AUC values increased relatively dose-proportionally over the dose range studied. The Abbott-157378 AUC and C_{max} values observed in the 50/25 dose group are 45 and 39% lower than those observed in the six-month toxicity study in dogs at 60/20 mg/kg/day for 90 days, followed by 45/15 mg/kg/day for the remainder of the study (135.3 µg•h/mL and 13.0 µg/mL for AUC and C_{max}, respectively). Note that Abbott-157378 and ritonavir were administered in a 2:1 ratio in the nine-month study, and a 3:1 ratio in the six-month study. Also, the ritonavir dose was not divided twice daily in the nine-month study as in the six-month study. Although the formulations are different between the 6-month and 9-month studies,

there were no differences between exposures (AUCs) of the two studies at the same dose.

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) (Abbott laboratories Division 46 Report No. R&D/96/305, 1996); non-GLP

The *in vitro* protein binding of [^{14}C]Abbott-157378 was determined *via* an equilibrium dialysis technique in mouse, rat, dog, monkey and human plasma at five different initial drug concentrations of 0.1, 1.0, 10, 30 and 100 µg/mL. The percent of [^{14}C]Abbott-157378 bound ranged from [redacted] in mouse, [redacted] in rat, [redacted] in dog, [redacted] in monkey and [redacted] in human plasma over a drug concentration range of 0.1-1 µg/mL. Monkey plasma had the highest free drug. Saturation of protein binding was evident at concentrations greater than 1 µg/mL. The extent of protein binding decreased with increasing drug concentration in all five species. The increase in free fraction was the highest for rat (16-fold) and the lowest for mouse (2-fold). In human plasma, binding did not change appreciably between 0.1 and 10 µg/mL of [^{14}C]Abbott-157378 (99.4%), but was lower at 30 and 100 µg/mL. Between 10 and 100 µg/mL, the free fraction of [^{14}C]Abbott-157378 increased approximately 5-fold in human plasma.

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) (Abbott Laboratories Division 46 Report No. R&D/97/392, August 1997); non-GLP

The *ex vivo* protein binding of Abbott- 157378 and Abbott-84538 in dog plasma obtained at 2 hours after dose on Day 76 from a 6-month oral toxicity study (Protocol TB97-003) was examined. The treatments for four groups were placebo 0/0, 10/3, 25/8, or 60/20 mg/kg/day of Abbott-157378 and Abbott-84538, respectively. In all cases, the protein binding of both Abbott-157378 and Abbott-84538 was extensive (>98%). A concentration-dependent decrease in the protein binding of Abbott-157378 was observed with 0.90, 1.32 and 1.51% as free fractions in the 10/3. 25/8 and 60/20 mg/kg/day, respectively. The free fraction of Abbott-84538 also increased in a concentration-dependent manner, averaging 0.92, 1.07 and 1.40% in the 10/3, 25/8 and 60/20 mg/kg/day, respectively. In addition, Abbott-157378 was found to decrease the protein binding of Abbott-84538.

Comments

Since higher levels of Abbott-157378 compared to Abbott-84538, possibly exists in humans, the potential for an effect of Abbott- 157378 on the protein binding of Abbott-84538 may exist.

Human

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); Non-GLP

The *in vitro* human plasma protein displacement of warfarin, imipramine and digoxin by the combination of Abbott-157378 (15 µg/mL) and ritonavir (1.5 µg/mL or 0.75 µg/mL) was studied using ultrafiltration techniques. The addition of Abbott-157378 and ritonavir in a 10:1 ratio affected warfarin plasma binding where a free fraction increase of 1.4-fold was seen. Abbott-157378 and ritonavir (10/1 and 20/1) produced an increase (up to 1.8-fold) in the plasma free fraction of imipramine. A trend towards increasing the free fraction in the presence of Abbott-157378/ritonavir was observed with digoxin.

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)

Vol: 37; Pages: 305-326; Non-GLP

The effects of saquinavir, amprenavir, nelfinavir and ibuprofen on the human plasma protein binding of Abbott-157378 was examined. Fresh human plasma (2 male, 2 female) was used to determine the percent of [^{14}C]Abbott-157378 (Lot162050-ST-01; 199 µCi/ml) displaced by each drug at clinically relevant concentrations using ultrafiltration techniques. The plasma binding of [^{14}C]Abbott-157378 (15 µg/mL) in the presence of ritonavir was 98% and was independent of ritonavir at 0.75 or 1.5µg/mL. The addition of saquinavir (5 µg/mL), amprenavir (5 µg/mL), nelfinavir (5 µg/mL) or ibuprofen (50 µg/mL) did not affect the binding of [^{14}C]Abbott-157378 at these concentrations.

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); Non-GLP

The *in vitro* binding of [^{14}C]Abbott-157378 in human serum albumin (HSA) and α_1-acid glycoprotein (AAG) was determined *via* an equilibrium dialysis technique at an initial concentration range of 0.1-30 µg/mL. [^{14}C]Abbott-157378 was bound to HSA and the binding ranged from 93.6-96.3% with a concentration-dependent decrease in binding. The binding in HSA was constant between 0.1 and 4 µg/mL, but was significantly lower at 10 and 30 µg/mL. [^{14}C]Abbott-157378 was extensively bound to AAG at lower concentrations of the drug and the binding ranged from 72.5-99.6%. In human plasma, a 4.6-fold increase in free fraction of [^{14}C]Abbott-157378 over a concentration range of 0.1-30 µg/mL was seen. Saturable binding of [^{14}C]Abbott-157378 in HSA and AAG is probably responsible for the concentration-dependent decrease in the binding of [14C]Abbott-157378 in human plasma.

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)

Vol. No.: 39; **Pages:** 81-101; **Conducting Laboratory:** Abbott Laboratories; **GLP:** No (x); **Drug Lot:** ^{14}C Abbott-157378 (lot 53572-JU-052, 88.2 µCi/mg, purity > 97%); ^{14}C Abbott-84538 (lot 57444-CR-20, 50.45 µCi/mg, purity > 96%) **Formulation:** 0.05 mg ^{14}C Abbott-157378/mL or 0.05 mg ^{14}C Abbott-84538 /mL

This study was conducted to determine the protein binding of ritonavir and Abbott-157378 ex *vivo* in human plasma obtained from normal subjects of a multiple dose Phase I study (Protocol M97-650). The plasma concentrations of Abbott-157378 and ritonavir ranged from 2.12 to 12.60 µg/mL and 0.06 to 0.95µg/mL, respectively. The protein binding of Abbott-157378 and ritonavir was extensive and was >98% and >97%, respectively. There was no significant difference in the binding of either Abbott-157378 or ritonavir between Day 1 predose plasma spiked with Abbott-157378 or ritonavir and Day 16 18-hour (steady-state) plasma. These data indicate that chronic dosing of this protease inhibitor combination does not alter the protein binding of either compound. The free fraction of Abbott-157378 and ritonavir ranged from 0.77 to 1.76% and 1.16 to 2.66%, respectively.

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)

Vol. No.: 39; **Pages:** 102-118; **Conducting Laboratory:** Abbott Laboratories; **GLP:** No (x); **Drug Lot:** ^{14}C Abbott-157378 (lot 62050-ST-01, 86 uCi/mg, purity > 97%); **Formulation:** 0.05 mg ^{14}C Abbott-157378/mL in ethanol

This study was conducted to determine the protein binding of Abbott-157378 *ex vivo* in human plasma obtained from HIV-infected subjects of a multiple dose Phase I study (Protocol M98-720). The protein binding of Abbott-157378, determined *via* an ultrafiltration technique, was extensive and ranged from [redacted] in the plasma of HIV-infected subjects. No significant difference in the plasma protein binding of Abbott-157378 between the two dose groups (200/100 mg and 400/100 mg of Abbott-157378

and ritonavir) was seen. The plasma protein binding of Abbott-157378 observed in HIV-infected patients was not different from that observed in healthy normal volunteers (98.2-99.3% bound).

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); Non-GLP

Vol. No.: 39; **Pages:** 119-135 **Conducting Laboratory:** Abbott Laboratories; **GLP:** No (x); **Drug Lot:** ^{14}C Abbott-157378 (lot 53572, 88.2 uCi/mg, purity > 97%); **Formulation:** 0.1 mg ^{14}C Abbott-157378/mL in ethanol

The *in vitro* protein binding of [^{14}C]Abbott-157378 was determined *via* equilibrium dialysis and ultrafiltration techniques in human plasma at four different drug concentrations of 0.1, 1, 10 and 30 µg/mL. Protein binding was high over the drug concentration range of 0.1-30 µg/mL (97.2-99.6%). A concentration-dependent decrease in protein binding of Abbott-157378 was observed, with a 4-fold increase in free fraction of Abbott-157378 over the 0.1-30 µg/mL concentration range.

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); Non-GLP

Methods

This study was conducted to examine the disposition and distribution of radioactivity after an oral dose of [^{14}C]Abbott-157378 (10 mg/kg; Lot 53572-JU-052, 88.2µCi/mg, purity: >93%) and Abbott-84538 (5 mg/kg) in Sprague-Dawley rats (2/sex/group). The distribution of radioactivity after an oral dose of [^{14}C]Abbott-157378 and Abbott-84538 in selected tissues in Long-Evans rats was also examined to study the potential for binding to pigment.

Results

A rapid and almost complete excretion of dose radioactivity was seen in Sprague-Dawley rats with 95.3% of the administered radioactivity recovered in excreta within three days of dosing. Only 0.13% of the radioactivity was recovered in tissues. The radioactivity was excreted predominantly in feces (94.2%), with <1% excreted in urine. A rapid decline in total plasma radioactivity was observed 4 hours after dosing. The highest radioactivity levels in tissues were seen at 4 hours postdose. Radioactivity was well distributed throughout the body with highest and lowest concentrations achieved in liver (52.3µg eq/g of tissue) and brain (0.046µg eq/g of tissue), respectively. The radioactivity recovered from liver during 1-7 hours after dosing was substantial (322.4µg eq/g of tissue; tissue to plasma ratio: 22.2), indicating that very high levels of [^{14}C]Abbott-157378 are achieved in this organ. [^{14}C]Abbott-157378 penetrated the blood-brain barrier poorly and the brain radioactivity levels (2% of the plasma radioactivity levels) are approximately equal to the free fraction of [^{14}C]Abbott-157378 in plasma. [^{14}C]Abbott-157378 readily distributed into the lymphatic system and the radioactivity levels were approximately 6-61% of those observed in plasma. The tissue to plasma radioactivity ratios at 4 hours after dosing were < 1 in bone marrow (0.13), eyes (0.16), perirenal fat (0.78), heart (0.52), kidneys (0.92), lungs (0.51), lumbar lymph nodes (0.15), submaxillary lymph node (0.61), pancreas (0.94), prostate gland (0.45), skeletal muscle (0.25), skin (0.29), spleen (0.41), testes (0.17), thymus (0.42) and urinary bladder (0.55). The tissue to plasma radioactivity ratios were 2.07 in adrenal and 1.90 in thyroid glands. No preferential binding of [^{14}C]Abbott-157378 to pigment in the skin was observed.

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); non-GLP

Methods

[^{14}C]Abbott-157378 (10 mg/kg) and ritonavir (5 mg/kg) were given orally to pregnant rats (2/groups, at approximately 18 days of gestation) and lactating rats (approximately 10 days postpartum). Maternal and fetal blood and several tissues were collected and analyzed for total radioactivity levels. Blood and milk of lactating rats were collected at several time points after dosing.

Results

The highest mean plasma radioactivity level (1.76 µg eq/mL) in maternal plasma was observed at 6 h after dosing, with no detectable levels at 72 h after dosing. The tissue to plasma (T/P) ratios were >1 for adrenals and liver, with minimal penetration of radioactivity into the brain. The T/P values in the placenta were 0.30 and 0.94 after 1 and 6 h after dosing, indicating a good distribution of radioactivity into the placenta. The T/P values in the uterus were 0.24, and 0.80 after 1 and 6 h after dosing, again indicating an adequate distribution of radioactivity into this organ. However, the T/P values for amniotic fluid were lower, 0.013 and 0.049 after 1 and 6 h postdosing, indicating a limited transport of radioactivity across the placental-fetal barrier. Accordingly, the fetal radioactivity levels were <0.14 µg eq/g. Detectable levels of radioactivity were observed fetal tissues only at 6 h which represented the peak plasma radioactivity level. Ratios of fetus to maternal plasma radioactivity were 0.045 (1 hr postdose) and 0.08 (6 hr postdose). The modest transport of [^{14}C]Abbott-157378 across the placenta (T/P >1) were seen in rats. [^{14}C]Abbott-157378 associated radioactivity is excreted into the milk of rats. The milk to plasma ratio of radioactivity ranged from 0.084 to 1.53 through 24 hours after dosing. The milk to plasma radioactivity ratios during 3-8 hours after dosing were 0.29 to 0.63.

<u>Enzyme Induction/Inhibition</u>

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)

The rate of metabolism of Abbott-157378 was determined in a panel of human liver microsomes examined. Abbott-157378 undergoes CYP-dependent biotransformation to three major metabolites (M-1, M-3 and M-4) as well as several minor metabolites in human liver microsomes. The metabolism of Abbott-157378 followed Michaelis-Menten kinetics with an apparent K_m of 6.81µM and a V_{max} of 9.38 nmol/mg protein/minute. Members of the CYP3A subfamily (CYP3A4 and CYP3A5) are the exclusive contributors to the human liver microsomal metabolism of Abbott-157378. Abbott-84538 was a potent inhibitor of Abbott-157378 metabolism with a low Ki value (0.013 µM), compared to ketoconazole (Ki: 0.2 µM). Abbott-157378 inhibited the human liver microsomal metabolism of Abbott-84538 (Ki of 130 µM). Abbott-157378 was a potent inhibitor of nifedipine oxidation (IC_{50}: 1.3µM).

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)

The study was to examine if ritonavir induces CYP1A2, CYP3A and planar phenol glucuronosyl-transferase activities in primary cultures of human hepatocytes. Human hepatocytes from five donors cultured in collagen-coated wells were treated with ritonavir (2, 10 and 30 µg/mL) or Abbott-157378 (2, 10 and 30 µg/mL), or positive controls rifampicin (50 µM)and 3-methylcholanthrene (1µM) for three days, After the treatment period, ethoxyresorufin O-deethylation (EROD, CYP1A2), testosterone 6β-hydroxylation (CYP3A) and p-nitrophenol glucuronidation activities were assayed. The levels of CYP1A and CYP3A proteins were also examined by SDS-get electrophoresis followed by Western blotting. Neither ritonavir nor Abbott-157378 had any effect on ethoxyresorufin O-deethylation (CYP 1A2) and p-

nitrophenol glucuronidation. Ritonavir almost completely inhibited CYP3A-dependent testosterone 6β-hydroxylation, but increased the immunoreactive CYP3A protein in three of the five hepatocyte cultures examined. Abbott-157378 decreased testosterone 6β-hydroxylation, albeit less potently than ritonavir, and did not have a marked effect on the immunoreactive CYP3A protein. Abbott- 157378 did not induce CYP3A, CYP1A2 or p-nitrophenol glucuronosyltransferase *in vitro*.

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); non-GLP; Vol 34, Pages 136-191

The biliary excretion in chronically bile duct cannulated and non- cannulated rats (4rats/group) and the metabolite profile in bile after intravenous (10mg/kg; 13.8-16.8 μCi/rat, 1mL/kg) or intraduodenal (10mg/kg; 14.4-19.2 μCi/rat) administration of [^{14}C]Abbott-157378 and the effect of Abbott-84538 (5 mg/kg) on the biliary excretion of [^{14}C]Abbott-157378 (10 mg/kg) were examined in this study.

Results

In cannulated rats after i.v. administration of [^{14}C]Abbott-157378, 69.5% of the total dose radioactivity was excreted in bile and 1.5% appeared in urine within the first 24 hours. After ritonavir co-administration, delayed excretion of radioactivity in bile were seen in rats (32.6% of the dose excreted from 4 to 6 hours after dosing). On intraduodenal administration, there was an 2-fold increase in the excretion of radioactivity in bile after dosing of the combination (24.7%) compared to dosing of [^{14}C] Abbott-157378 alone (11.7%), with less than 2% excreted in urine in both cases. A majority of the radioactivity was excreted in bile within the first hour after dosing [^{14}C]Abbott-157378 alone compared to a more sustained excretion after dosing the combination, with a major portion being excreted during 6-24 hours after dosing. Unchanged [^{14}C]Abbott-157378 accounted for less than 2% of the total dose in bile and it was extensively metabolized to three primary (M-3, M-4 and M-5), three secondary-(M-1, M-11 and M-12). two tertiary (M-9 and M-10) and several unknown polar metabolites. When ABT-378 was administered alone by the intravenous or intraduodenal route, greater than 64% of the biliary radioactivity was present as unknown polar metabolites, with primary secondary and tertiary metabolites accounting for the remaining radioactivity. When ABT-378 was administered in combination with Abbott-84538 by the intravenous or intraduodenal route, the content of the unknown polar metabolites was comparatively less (38-50% of the biliary radioactivity), with substantially higher amounts of the primary, secondary and tertiary metabolites. After intravenous dosing, Abbott-84538 did not affect the total dose excreted but delayed the excretion. In contrast, a two-fold increase in the total dose excreted as well as delayed excretion was observed after intraduodenal dosing, suggesting that Abbott-84538 significantly inhibited the first pass metabolism of [^{14}C]Abbott- 157378.

65. Abbott- 157378 Drug Metabolism Report No. 26 -Metabolism and disposition of [^{14}C]Abbott-15737 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); non-GLP

The metabolism and disposition of Abbott-157378 were studied in male and female beagle dogs after i.v. or oral administration of [^{14}C]Abbott-157378 (5 mg/kg) in combination with Abbott-84538 (2.5 mg/kg). The maximal plasma radioactivity achieved was 8.6-12.6 μg eq/mL and 3.1-3.8 μg eq/mL after intravenous and oral dosing, respectively. After an initial distribution (intravenous) or absorption (oral) phase, steady plasma levels of Abbott-157378 were observed for up to 4 hours, followed by a rapid decline to undetectable levels by 24 hours after dosing. After intravenous dosing, 90% of the dose of radioactivity was excreted in feces within the first 48 hours of dosing, with less than 1.5% appearing in

urine, suggesting that Abbott-157378 is predominantly excreted *via* the hepatic route. After intravenous dosing, the unchanged parent drug in feces or bile accounted for <8% of the fecal or biliary radioactivity. The remaining radioactivity in feces and bile was present as several metabolites including six primary, several secondary and tertiary metabolites (Figures 1 and 2). The primary metabolites were M-3 and M-4 (an epimeric pair of 3-hydroxylated derivatives of Abbott- 157378), M-5 (a hydroxylated derivative of Abbott-157378 with the hydroxyl group located on the dimethylphenoxy moiety) and metabolites M-6, M-7 and M-8 (monohydroxylated derivatives of Abbott 157378 with the hydroxyl group located on the dibenzyl core moiety). The secondary metabolites were M-1 and the dihydroxylated metabolites M-11 to M-15, which have a hydroxyl group located on the carbon-3 position and another hydroxyl group located on the dibenzyl core moiety. The tertiary metabolites were M-9 and M-10, products of hydroxylation of M-1. A quaternary metabolite M-16 (a dihydroxylated derivative of M-1) was also observed. Even though Abbott-157378 was extensively metabolized in dog, the circulating radioactivity in plasma was present mainly as the unchanged parent drug, with only trace amounts of metabolites M-3, M-4 and M-5 present.

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), non-GLP

[^{14}C] Abbott-157378 was incubated with liver microsomes from mouse, rat, dog, monkey and humans, and human liver slices in an NADPH-dependent assay system. Abbott- 157378 was metabolized to twelve metabolites (M-1 to M-12) which were characterized by mass and NMR spectroscopy. 95.5% of parent drug was converted to three major metabolites M-1, M-3 and M4 plus several other minor metabolites including M-2, M-5 through M- 12 and other unknowns. The metabolite profile of Abbott-157378 in liver microsomes from all five species was similar, except that the mouse liver microsomes did not form M-9, a minor secondary metabolite. The rates of NADPH-dependent metabolism of Abbott-157378 ranged from 2.39-9.80 nmol/mg microsomal protein/minute, with monkey liver microsomes exhibiting the highest rates of metabolism.

The proposed *in vitro* metabolic pathway for Abbott-157378 in human liver preparations and animal liver microsomes is presented in Appendix 2. M-2 (5-hydroxy-Abbott-157378) to M-8 (the 3-hydroxy-Abbott-157378 derivatives), M-1(a secondary metabolite with the 3-hydroxy group of metabolites M-3/M-4 further oxidized to an oxo- group), M-9 and M-10 (the hydroxylated products of an oxo- derivative of Abbott-157378), M-11 and M-12 (the dihydroxylated products of Abbott-157378) were identified in this study. In all five species, the major metabolites were M-1, M-3 and M-4. Rat microsomes formed M-2 at a faster rate than dog, monkey and human liver microsomes. M-4 was the major metabolite accounting for about 40% of the metabolites formed. Monkey liver microsomes formed M-4 at a 3-fold higher rate than microsomes from the other species. The rate of formation of M-1 was lowest by mouse and highest by monkey (0.17 *vs* 1.43 nmol/mg/minute) liver microsomes. The rate of formation of M-2 was lowest by dog and highest by mouse (0.05 *vs* 0.31 nmol/mg/minute) liver microsomes. M-3 was formed at approximately similar rates in mouse, rat, dog and human liver microsomes, but was formed at a 4-fold higher rate by monkey liver microsomes. The metabolite profile obtained with human liver slices and hepatocytes after short incubation periods was similar to that obtained with human liver microsomes. On prolonged incubation, both liver slices and hepatocytes produced several secondary metabolites and other polar unknown metabolites. This type of metabolite profile was similar to that obtained in rat bile after intravenous administration of [^{14}C]Abbott-157378. Abbott-84538 potently inhibited the metabolism of [^{14}C]Abbott- 157378 in both human liver microsomes (IC50: 0.12 μM) and liver slices (IC50: 0.95μM).

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)

An *in vitro* permeability assay was performed using Caco-2 cells grown on [redacted] polycarbonate filters. Radiolabeled and unlabeled Abbott-157378 ([^{14}C]ABT-378; Lot 62050-ST-01, Lot 357159-O-AX, respectively) or unlabeled Abbott-84538 (ABT-538; Lot 79-594-AL) were dissolved at room temperature into DMSO (final concentration 1%) to produce stock solutions. The absorptive permeability values seen *in vitro* with ABT-378 and ABT-538 predict that they will be moderately absorbed in the human gut, respectively. The prediction of high absorption for ABT-378 and moderate absorption for ABT-538 is maintained at apical pH values of 6.4, 6.8, or 7.4. The absorptive Papp values of ABT-378 are approximately equivalent to the secretive Papp values, indicating the ABT-378 is not a substrate for P-glycoprotein mediated transport. ABT-538 exhibits a large degree of polarized transport across Caco-2 cells, consistent with it being a Pgp-substrate. ABT-378 and ABT-538 can inhibit the active transport of vinblastine to the same degree as Cyclosporin A, a known Pgp inhibitor. ABT-378 and ABT-538 increase the absorptive transport of, and decreases the secretive transport of vinblastine.

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); non-GLP

The metabolism and disposition of Abbott-157378 were studied in rats after intravenous or oral administration with [^{14}C]Abbott-157378 (5 mg/kg), with or without co-administration of ritonavior (2.5 mg/kg). Abbott-157378 was cleared in rats very rapidly after i.v. administration with only traces of plasma radioactivity present after 12 hours of dosing. The decline in plasma radioactivity in female rats was slower than in male rats. Female rats produced a higher $AUC_{0\text{-}72h}$ (1.8-fold) and C_{max} (1.5-fold) than male rats. The $AUC_{0\text{-}72h}$ values after oral dosing were comparable between sexes (1.25 and 1.46 μg eq.h/mL in male and female rats, respectively), with male rats (0.26 μg eq/mL) producing slightly higher C_{max} values than female rats (0.18 μg eq/mL). The bioavailability of Abbott-157378 was 27.4 and 17.4% in male and female rats, respectively. A majority of the radioactivity was excreted in feces within the first 24 hours after dosing, with less than 1% of the total dose radioactivity appearing in urine. After intravenous or oral dosing, the unchanged parent drug in feces accounted for less than 2 and 24% of the fecal radioactivity, respectively. Three primary (M-5, M-3 and M-4), three secondary (M-1, M-11 and M-12), two tertiary (M-9 and M-10) and several unknown polar metabolites were found in the remaining radioactivity in feces. The structures of these metabolites are presented in Sponsor's Figure 1. After both intravenous and oral dosing, a majority (>53%) of the fecal radioactivity was present as unknown polar metabolites of Abbott-157378.

On co-administration of Abbott- 157378 and Abbott-84538, the plasma radioactivity (C_{max}: 5.41-6.69 μg eq/mL after intravenous dosing and 1.22-1.74 μg eq/mL after oral dosing) was several-fold higher than when Abbott-157378 was administered alone. Plasma radioactivity levels persisted at >1 μg eq/mL for 7-10 hours after intravenous dosing of the combination and 9-12 hours after oral dosing. The $AUC_{0\text{-}72h}$ and C_{max} levels after intravenous dosing of the combination were 4.5-6.0-fold and 1.2-2.2-fold higher, respectively, than when Abbott-157378 was dosed alone. The increase in $AUC_{0\text{-}72h}$ (13.0-14.3-fold) and C_{max} (6.8-fold) was even more pronounced after oral dosing. The results of this study suggest that the duration of inhibition of Abbott-157378 metabolism in rat is dependent on the plasma levels of Abbott-84538, with approximately 0.05-0.3 μg eq/mL of Abbott-84538 being the critical concentration range for producing the inhibitory effect in rat. A higher portion of the total radioactivity was excreted as the primary and secondary, metabolites when Abbott-84538 and Abbott-157378 were co-administered compared to when Abbott-157378 was administered alone.

Analytical Method

69. **Abbott-157378 Drug Metabolism Report No. 10 -A[redacted]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); non-GLP; Vol 39; page 191-233**

[redacted]

Summary of Non-Clinical Pharmacology

Loponavir (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. Retonavir is a very potent inhibitor of Abbott-15738 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.

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%).

Summary of Safety Pharmacology

Modest effects on CNS receptor or ion channel functions of ABT-378 were found in cells from rats and mice at therapeutic to super therapeutic doses/plasma concentrations. Co-administration of ABT-378 and ritonavir increases barbital or ethanol induced sleep times in mice, as well as decreases electroconvulsive shock threshold in mice at 10/5 and 30/15 mg/kg. Intravenous administration of the 30/15 mg/kg

produced negative chronotropic effects in conscious male rats. Oral administration of 100/50 mg/kg produced no cardiac effects in conscious male rats. However, it was found that intravenous administration at 20/10 or 20/1.5 mg/kg produced marked and sustained decreases in blood pressure, heart rate, and left ventricular contractility, as well as prolonged PR interval, but not QT interval in pentobarbital-anesthetized male dogs.

Summary of Non-Clinical Toxicology

Nonclinical toxicology studies performed with ABT-378/ritonavir included acute oral and i.v. dose studies in mice and rats; two–week repeated-dose oral studies in rats and dogs; two–week repeated-dose oral studies in neonate rats; four–week repeated-dose oral studies in juvenile rats; three-month repeated-dose oral studies in rats (with one-month recovery period) and dogs; three-month oral MTD study in mice; three-month oral toxicity studies with impurities or degradants in dogs; six-moth oral toxicity studies in rats and dogs, and one nine-month oral toxicity studies in dogs. Three different formulations (liquid, semi-solid and soft elastic capsule) were used in the preclinical studies. Potential test article-related toxicities were associated with liver toxicity (liver enzymes, hepatocellular changes, ↑serum triglyceride, ↑cholesterol) and hematological changes in mice, rats and dogs, renal changes in mice (proximal tubular deposition of materials with cellular degeneration), thyroid changes (↑TSH and T4↓) in rats, and gastrointestinal toxicity and testicular changes in dogs. ABT-378/ritonavir- related toxicities reported in this NDA include:

Single Dose Acute Toxicity

ABT-378/ritonavir (2:1 ratio) has a low order of acute toxicity in rodents by the oral route but is more toxic when administered as an intravenous injection. Transient clinical signs (decreased activity, ataxia, dyspnea, increased salivation and/or squinting) were seen in rats and mice at 20/10 - 1250/625 mg/kg for mice and 78/39 - 1250/625 mg/kg in acute toxicity studies. No deaths occurred in rats or mice at 1250/625 mg/kg, and no deaths or histopathologic changes were seen at 2500 mg/kg ABT-378 (AUC value of 45 μg•hr/ml). Summary of single dose acute toxicity studies and repeated dose toxicity studies in mice, rats or dogs are given in Appendices 4 and 5.

Repeat Dose Chronic Toxicity

Liver toxicity: In a 3-month oral mice study, increases in cholesterol and liver weights were seen at 60/30 mg/kg/day (AUC: 121/12μg•hr/ml). Increases in hepatic enzymes (ALT, AST, GGT), triglyceride, liver weights, as well as histopathological changes (cytoplastmic vacuolation, necrosis, subacute inflammation and hepatocytomegaly) were seen at higher doses. Two- to six-month repeat oral rat studies demonstrated increases in cholesterol, liver weights and hepatic enzymes (ALT, AST, GGT), as well as histopathological lesions which were not resolved in rats during the one-month of recovery (multinucleated hepatocytes, single cell necrosis, histocytosis, hepatocytomegaly, increases lysosomal inclusions and smooth endoplasmic reticulum in hepatocytes) (two-weeks oral rats: ≥ 30/15 mg/kg/day, AUC: 44/3 μg•hr/ml; 3-month oral rats: ≥ 50/25 mg/kg/day, AUC: 76/8μg•h/ml; six-month oral rats: ≥ 50/25 mg/kg/day, AUC: 81/10 μg•hr/ml; four-week oral juvenile rats: ≥ 35/15 mg/kg/day, AUC: 52/3 μg•hr/ml). **The no-hepatotoxic-effect dosage level for the six-month oral rat was considered to be 10/5 mg/kg/day (AUC: 8/1 μg•hr/ml).** Neonatal rats appear to be less sensitive to the hepatic toxicity produced by ABT-378/ritonavir combination when compared with adult rats. Note that significant higher drug exposure was observed in neonate than the adult given a similar dose. In neonates at 40/20 mg/kg/day for two weeks (AUC 140/13μg•hr/ml) an increase in liver weights was observed. However, no microscopic findings were seen in the liver. Long-term repeat dose dog studies demonstrated increases in cholesterol, liver weights and hepatic enzymes (ALT, AST, ALP), as well as hepatocellular lesions and hepatocytomegaly in dogs at ≥ 25/8 mg/kg/day (three-month oral dogs: ≥ 35/15 mg/kg/day, AUC: ≥ 95/23 μg•hr/ml; six-month oral dogs: ≥ 25/8 mg/kg/day, AUC: ≥ 54/6 μg•hr/ml). In a nine-month repeat dose dog study, ALP elevations and increased relative liver weights, but without any hepatic microscopic changes were seen in dogs at ≥ 25/12.5 mg/kg/day (AUC: ≥ 55/17 μg•hr/ml). Thus,

species differences in hepatic toxicities were seen between rats and dogs. The rat appears to be a sensitive species for the evaluation of ABT-378/ritonavir-induced liver toxicity. Based on the recommended therapeutic dose for ABT-378/ritonavir combination (400/100 mg BID; 13.3/3.3 mg/kg/day; AUCs: 160/9 µg•hr/ml), no safety margin regarding the hepatotoxicity can be demonstrated in repeated toxicity studies in mice, rats and dogs (Appendix 5).

Serum cholesterol and triglyceride: Drug related-elevations in serum cholesterol levels were seen in mice and rats, and an increase in triglyceride levels was limited to mice. No effects on cholesterol or triglycerides occurred in dogs receiving the drug combination for up to nine months. Increases in cholesterol and triglycerides occurred in mice at 100/50 mg/kg/day (AUCs 292/29µg•h/ml) for two weeks or at ≥ 60/30 mg/kg/day (AUCs ≥121/12 µg•hr/ml) for three months. Increased cholesterol levels were evident in juvenile rats at ≥ 30/15 mg/kg/day (AUCs ≥ 62/3 µg•h/ml) for four weeks and in adult rats at ≥ 50/25 µg•h/ml (AUCs of 65/7 - 73/8 µg•h/ml) for three or six months.

Thyroid toxicity: Repeat dose toxicity studies demonstrated the thyroid toxicity of ABT-378/ritonavir in rats. Dose-dependent decreases in serum T4 levels and elevated serum TSH were seen in adult rats (two-weeks oral rats: ≥10/5 mg/kg/day, AUC: 21/1.2 µg•hr/ml; 3- and 6-month oral rats: ≥ 50/25 mg/kg/day, AUC: ≥74/8µg•hr/ml) and juvenile rats (four-weeks: ≥100/50 mg/kg/day, AUC: 172/10µg•hr/ml). Drug -related thyroid hypertrophy was seen in rats (two-week oral: ≥100/50 mg/kg/day (male), AUC: ≥121/10 µg•h/ml, ≥35/15 mg/kg/day (female), AUC: ≥ 44/3.3 µg•h/ml; 3- and 6-month oral: ≥50/25 mg/kg/day, AUC: ≥74/8µg•h/ml). Neonatal rats appear to be less sensitive to the thyroid toxicity produced by ABT-378/ritonavir combination when compared with adult rats. No thyroid change was seen in neonate rats at 40/20 mg/kg/day (AUC: 140/13µg•hr/ml). All changes were reversible following a one-month recovery period. No drug-related effects on thyroid gland were seen in mice (3-month repeat oral dose MTD study) and dogs (up to nine months repeat oral studies).

Hematological toxicity: Decreases in erythrocytic variables (erythrocyte count, hematocrit, hemoglobin) and anisocytosis and poikilocytosis were seen in adult rats treated with ABT-378/ritonavir combination at ≥ 50/25 mg/kg/day (for three to six-month oral rats: AUC ≥ 65/14 µg•hr/ml). Erythrocyte morphological changes in rats persisted through the one-month recovery period. Similar erythrocytic changes also occurred in one six-month oral female dog at 45/15 - 60/20mg/kg/day (mean AUC values of approximately 205/53µg•h/ml). Erythrocytic changes were not seen in the mice at 200/100 mg/kg/day (three-month MTD mice: AUCs 458/62 µg•hr/ml) or in dogs at ≥ 50/25 mg/kg/day (nine-month oral dogs: AUCs 78/39 µg•hr/ml). Because a direct Coomb's test was negative, the acanthocytosis was probably a secondary effect of the liver alteration caused by ABT-378/ritonavir. Elevations in clotting times were noted in rats in the 3- or 6-month repeated dose studies.

Spleen. Changes in spleen (histiocytosis and increased spleen weight) were limited to rats at 50/25 mg/kg/day or higher (AUCs ≥ 73/8 µg•hr/ml) for six months.

Renal toxicity: No kidney changes were observed in rat or dog studies during the six- (rats) or nine months (dogs) treatment period. Changes in kidney (microvesicular cytoplasmic vacuolation) occurred only in mice that received a combination dosage of 200/100 mg/kg/day (AUCs 458/62 µg•hr/ml) for three months (MTD study).

Gastrointestinal (G.I. toxicity) and related ECG changes: In dogs that received ABT-378/ritonavir combination, emesis, diarrhea and loose stools were seen generally within 1-2 hours after dosing at all dosages tested in the repeated dose studies. In the three-month toxicity study, moderate to severe GI distress occurred in dogs at 70/35 - 100/50 mg/kg/day (AUCs 189/65µg•hr/ml). Hypokalemia, hyponatremia, hypochloridemia and variable blood acid-base imbalances were seen in the affected dogs. In general, anorexia, weight loss, acid-base, electrolyte and fluid-balance alterations that included alkalosis, hypochloridemia and hypokalemia were noted prior to death. ECG changes (prominent U wave, fusion of T and U waves, triggered ventricular extrasystoles and first degree of atrioventricular block) were seen in six male and one female dogs at 70/35-100/50 mg/kg/day. The occurrence of large U waves as well as T waves flattening are typical ECG abnormalities related to hypokalemia. Note that hypokalemia (< 3.5 mEq/L) was not observed during the treatment period for all the affected dogs. ABT-378/ritonavir combination in the SEC formulation at dosages up to 60/20 mg/kg/day (AUCs 264/50

µg•hr/ml) did not cause any electrocardiogram (ECG) or electrolyte changes and no early deaths occurred in dogs (in the presence of early and aggressive dietary supplementation). There were no ECG changes seen in dogs that received the drug combination for nine months.

Testis. Testicular degeneration (loss of germ cells, germ cell degeneration and tubular vacuolization) was seen in dogs that received the drug combination at dosages of 10/3 to 60/20 mg/kg/day (six-month oral dogs: AUC values of 20/2 - 206/53µg•hr/ml). However, no testicular changes were seen in dogs at 50/25 mg/kg/day (AUC values of 78/39 µg•hr/ml) for nine months. Based on the lack of a dose response and the absence of similar findings in dogs exposed to higher plasma exposures for a longer treatment period, the toxicological significance of the changes seen in dogs in the six-month study is unknown.

Reproductive Toxicicology Summary

ABT-378/ritonavir combination was tested for potential reproductive hazards in separate studies covering all phases of the reproductive process. The individual segment I, II, and III studies are summarized in Appendix 6. In the segment I rat study, no drug-related effects on male or female rat fertility & embryonic development were seen at dosages up to 100/50 mg/kg/day (AUCs 114/8 µg•hr/ml). The AUC levels in rats at 100/50 mg/kg/day is approximately 71% of that achieved with the recommended therapeutic dose of 400/100 mg bid (AUCs 160/9 µg•hr/ml). In the segment II study in rats, the drug combination was given during the period of organogenesis. Fetal toxicity in rats was observed at a maternally toxic dosage (100/50 mg/kg/day, AUC 116/16 µg•hr/ml) and was characterized by reduced fetal viability, reduced fetal weight, delayed skeletal ossification and an increased incidence in skeletal variations (14^{th} ribs and 27 presacral vertebrae). In a segment II study in rabbits, maternal toxicity including reductions in food consumption, decreased body weight gain and emaciation was seen at 80/40 mg/kg/day. The AUCs (90/9µg•hr/ml) are approximately 56% of that achieved with the recommended therapeutic dose. No developmental toxicity, including teratogenicity was seen in this study. In a segment III study, the drug combination was given during late gestation through weaning. A reduction in pup survival during lactation was noted at 40/20 and 80/40 mg/kg/day (AUC was not measured in this study but was estimated to be 93/9 µg•hr/ml). No drug-related malformations were observed.

Genetic Toxicology Summary

ABT-378 alone or in combination with ritonavir was tested in three *in vitro* and one *in vivo* genotoxicity assays. The genetic toxicity studies are summaries as follows. All assays had negative findings for genetic toxicity. No mutagenic or clastogenic effects were seen in the listed test. Additionally, *in vitro* mutagenicity tests (Ames and cytogenetic assay) were conducted with several different lots of ABT-378 and ritonavir containing varying amounts of related substances. No mutagenicity was detected in any of these studies (Appendix 7).

Carcinogenicity

Two carcinogenicity studies are in progress by the sponsor, which include a two-year oral carcinogenicity study of Abbott-157378/ritonavir combination in mice (Study No. TD98-160) and a two-year oral carcinogenicity study of Abbott-157378/Abbott-84538 (ritonavir) combination in rats (Study No. TA98-024). The reports of these studies are scheduled to be issued to the division in November 2001. The studies will be completed as a Phase 4 commitment. Two-year carcinogenicty studies have been carried out on ritonavir.

Summary of Special Studies

Abbott-157378/ritonavir with several different lots of ABT-378 and ritonavir containing varying amounts of related substances or impurities in different liquid formulations and SEC formulations were tested in three 3-month toxicity studies in dogs, for the effect of these impurities on the toxicological profile of the test articles. Concentrations of the impurities or related substances in the test articles and the highest daily dosages of the related substances or impurities administered in these dog studies were listed in

Appendices 2 and 3. No evidence was found that the presence of these impurities or related substances increased the level of toxicity of the test articles in these studies. Additionally, all of the identified impurities or related substances do not alter the toxicity profile of the parent compounds. Target organs have generally been the same in these three special studies. All of these related substances or impurities in the test articles occur only at low concentrations resulting in a dosage of ≤ 0.08 mg/kg/day in humans (Appendices 2 and 3). An ABT-378/ritonavir SEC formulation (lot 61003-16) that contained total propylene glycol-esters of up to 2.3% (23 mg/g) have been tested in a nine-month dog study and an ongoing two-year carcinogenicity rat study. No toxicity associated with the increased levels of propylene glycol-esters or propylene glycol-oleate has been noted in this formulation, in which the glycerol adduct level was 5-fold higher than that in the formulations (0.41%) used in the previous studies. However, two related substances or impurities [redacted] in the SEC formulations of ABT-378/ritonavir tested in the dog studies were considered to be higher than the potential human dose (based on 400/100 mg, BID). The toxicological significance of these two substances is still unknown.

Pharmacokinetics Summary

The absorption, distribution, metabolism and excretion of [^{14}C]Abbott-157378 (ABT-378; lopinavir) were investigated in rats and dogs. These studies included a single oral or i.v. 10 mg/kg with or without 5 mg/kg ritonavir (2/1 ratio). Pharmacokinetic and toxicokinetic parameters were determined in mice, rats, dogs, and monkeys. Studies included single i.v. dose rat and dog studies or rising oral doses of Abbott-157378 administered with or without ritonavir, multiple oral dose rat and dog studies with administration of Abbott-157378 and ritonavir (2/1 ratio), and repeat oral dose toxicokinetic studies (up to 9 months). *In vitro* studies have been conducted with rat, dog, monkey and human plasma and also in liver microsomes. *In vitro* models of rat and human liver metabolism have also been utilized to help understand the beneficial pharmacokinetic interaction that ritonavir exerts on the disposition of ABT-378 in humans. The concentrations of drug used in *in vitro* plasma protein binding determinations were chosen to encompass the broad range of ABT-378 plasma concentrations anticipated in clinical and toxicology studies. Concentrations of ABT-378 and ritonavir in plasma were determined with [redacted] using ultraviolet detection. ABT-378 bearing a carbon-14 radiolabel in the dimethylphenoxyacetyl moiety of the molecule was used in all of the metabolism, distribution and protein binding studies. Metabolic patterns in plasma, bile, feces, urine and *in vitro* microsomal incubations were determined by [redacted] radiometric assays.

Absorption and Pharmacokinetics

ABT-378 is not a substrate for P-glycoprotein mediated transport. However, ABT-538 exhibits a large degree of polarized transport across Caco-2 cells. ABT-378 and ABT-538 can inhibit the active transport of vinblastine. ABT-378 and ABT-538 increase the absorptive transport of, and decreases the secretive transport of vinblastine. ABT-378 administered alone was poorly bioavailable in rats, dogs and monkeys. Single oral doses of 5-10 mg/kg did not result in detectable plasma concentrations in dogs and monkeys (Table 52). Single oral doses of 5-10 mg/kg ABT-378 resulted in peak plasma concentrations (C_{max}) <1 µg/mL and AUC values of 1.9 and 0.67 µg•h/mL in rats (10 mg/kg: 0-8 h). Coadministration with ritonavir resulted in an increase in both C_{max} and AUC in rats, dogs, and monkeys. In multiple dose studies, the exposure (AUC) of rats and dogs to ABT-378 was much less than that in humans at comparable doses and ratios. Rats, mice and dogs exhibit higher apparent oral clearances (CL/F) of ABT-378 compared with humans at single doses of ABT-378/ritonsvir (Table 53). The C_{max} and AUC of ABT-378 in mice, rats, dogs and humans generally increased with increasing dose of the ABT-378/ritonavir combination. A consistent sex difference in plasma drug levels was apparent in rats, but not in mice or dogs. In rats, plasma levels were generally higher in females than in males. These parameters increased in an approximately proportional manner in mouse and a less than dose-proportional manner in rats and dogs.

Table 52. Pharmacokinetic Properties of ABT-378 following a Single i.v. Dose in Rat and Dog or a Single Oral Dose in Rats, Dogs, Monkeys and Humans (Single Dose or Multiple Dose)

Species	n	Dose (mg/kg/day) ABT-378/ritonavir	ABT-378 Cmax (μg/mL)	ABT-378 Tmax (h)	ABT-378 AUC (μg•h/mL)	F (%)	ABT-378 CL/F (L/h/kg)
Rat (Single,i.v)	3	5/0	–	–	3.8	–	1.3
Rat	4	5/10	3.1	–	19.9	–	0.3
(Single, p.o)	4	10/0	1.0	0.8	1.9	25.2	5.3
	4	10/5	2.1	4.5	12.3	–	0.8
	6	20	0.9	0.5	2.5	18.7	8.0
	6	100	1.9	1.4	15.3	20.1	6.5
	6	500	4.1	3.8	46.3	12.3	10.8
	6	2500	3.9	4.8	44.2	2.30	56.6
(Multiple, p.o)	10	**10/5 (9 days)**	**2.0**	**5.8**	**20.7**	–	**0.49**
Dog (Single, i.v)	3	5/0	–	–	3.9	–	1.3
Dog	6	5/0	–	–	–	–	–
(Single, p.o)	3	5/2.5*	2.5	1.3	11.3	–	0.4
	3	5/5*	5.6	2.0	36.4	–	0.1
(Multiple,p.o)		5/2.5 (13 days)	4.3	2.0	20.8	–	0.2
Monkey	3	10/0	–	–	–	–	–
(Single, p.o)	3	10/10	3.1	3.3	14.7	–	0.7
Human**							
Adult (2x, p.o)	3	11.4/0	0.7	3.4	2.5	–	4.6
(BID, p.o)		13.3/3.3 (16 days)	11.0	–	160/9	–	0.07
Child		24/6 (300/75 mg/m^2)	12.5	–	116	–	0.20

*Experimental semi-solid capsule formulation. ** Soft elastic capsule formulation under non-fast condition. Equivalent to a single 400 mg dose of ABT-378 given twice daily, or a multiple 400/100 mg/kg doses of ABT-378 and ritonavir (BID) for 16 days normalized to body weight assuming a 60 kg body weight.

Table 53. Estimated Oral Clearance of ABT-378 for Mouse, Rat, Dog and Human after Multiple Dosing When Co-administered With Ritonavir

Species	ABT-378 Dose (mg/kg/day)[a]	AUC $_{0\text{-}24}$ (μg•h/mL)	ABT-378 (CL/F)[b] (L/h/kg)
Mouse, Male (Female)[c,d]	20	50.8 (35.0)	0.39 (0.57)
	60	108.3 (133.6)	0.55 (0.45)
	200	520.5 (395.5)	0.38 (0.51)
Rat, Male (Female) c,d			
	10	7.35 (19.6)	1.36 (0.51)
	50	46.6 (82.5)	1.07 (0.61)
	150	127.2 (195.4)	1.18 (0.77)
Dog[d,e]	10	59.2	0.17
	30	95.8	0.31
	100/70[f]	163.9/239.4	0.61/0.29
Human			
Adult	13.3[g]	188[h]	0.06
Pediatric	300/75 mg/m^2	116[i]	0.20

[a] ABT-378 was dosed in a 2:1 ratio with ritonavir in mouse, rat and dog but 4:1 in human; [b] Apparent oral clearance CL/F = Dose/AUC; [c] Values in parentheses are for female mice and rats; [d] AUC values at each dose from the 3-month toxicology study; [e] Average of male and female data; [f] Dose reduced from 100/50 mg/kg/day ABT-378/ritonavir to 70/35 mg/kg/day ABT-378/ritonavir on Day 30; [g] Equivalent to a single 400 mg dose of ABT-378 given twice daily normalized to body weight (mg/kg) assuming a 60 kg body weight. [h] The estimate of steady-state ABT-378 pharmacokinetics for the 400/100 mg/kg ABT378/ritonavir q 12h anticipated clinical regimen. [i] The pharmacokinetics of ABT-378/ritonavir 300/75 mg/m^2 BID (24/6 mg/kg) have been studied in a total 27 pediatric patient, ranging in age from 6 months to 12 years.

Protein Binding

In vitro, the mean plasma protein binding percentages of ABT-378 ranged from 98.9-97.6% in mouse, 99.8-95.9% in rat, 99.5-96.7% in dog, 98.3-95.4% in monkey and 99.7-97.4% in human plasma at concentrations from 0.1 to 100 μg/mL. The extent of protein binding decreased with increasing drug concentration in all five species. Ritonavir decreased the plasma protein binding of ABT-378 in human plasma when its concentrations are in excess of ABT-378. At concentrations below 2μg/mL, ABT-378 was more extensively bound to physiological concentrations of human α1-acid glycoprotein (AAG; >99%) than to human serum albumin (HSA; 96%). Binding to AAG and HSA appears to diminish at drug concentrations > 4 μg/mL. Ibuprofen, saquinavir, nelfinavir or amprenavir did not displace ABT-378 from its protein binding sites in human plasma. The combination of ABT-378 and ritonavir did not displace a selection of drugs known to be bound to AAG and/or HSA (e.g. warfarin, digoxin and imipramine).

Tissue Distribution

The ABT-378 plasma clearance in rats and dogs at 5 mg/kg ABT-378 (i.v.) was 1.3 L/h/kg. Mean apparent elimination half-lives was 0.6-1.1 h. In rats after oral administration of a 10 mg/kg dose of [^{14}C]ABT-378, the Cmax for total plasma radioactivity was 0.18-0.26 μg equivalents/mL at 4-6 hours. The mean total radioactivity AUC value for female rats (1.46 μg eq/mL) was similar to that of male rats (1.25 μg eq/mL). Coadministration of [^{14}C]ABT-378 with 5 mg/kg ritonavir by the oral route produced a substantial increase in both the Cmax (7-fold) and AUC (13-fold) of total radioactivity compared to dosing ABT-378 alone. The percentage of radiolabeled dose absorbed was 57% for rats and 48% for dogs. Unchanged radioactive ABT-378 was the major component in both rat and dog plasma collected 0-4 hours after intravenous and oral (dog only) dosing, accounting for greater than 80% and 95% of plasma AUC in rats and dogs, respectively. ABT-378 was widely distributed in the tissues of rats. At 4 hours oral administration of a 10/5 mg/kg [^{14}C]-ABT-378/ritonavir, peak plasma concentration were found in the liver, adrenals and thyroid, affording respective tissue to plasma (T/P) ratios of 22.5, 2.07 and 1.90, respectively. ABT-378 also distributes into lymphatic tissue despite high plasma protein binding. By 48 hours, the liver was the only organ containing appreciable radioactivity (0.26μg eq/mL). The highest mean plasma radioactivity level (1.76 μg eq/mL) in maternal plasma was observed in pregnant rats (gestation day 18) at 6 h after dosing, with no detectable levels at 72 h after dosing. The T/P values in the placenta were 0.30 and 0.94 after 1 and 6 h after dosing. The T/P values in the uterus were 0.24, and 0.80 after 1 and 6 h after dosing. However, the T/P values for amniotic fluid were lower, 0.013 and 0.049 after 1 and 6 h postdosing. Accordingly, the fetal radioactivity levels were <0.14 μg eq/g. Detectable levels of radioactivity were observed fetal tissues only at 6 h which represented the peak plasma radioactivity level.

Metabolism

ABT-378 is metabolized exclusively by oxidative pathways in rat and dog. The 4-oxo derivative of ABT-378 M-1 and the epimeric pair of 4-hydroxylated ABT-378 metabolites M-3/4 were found in the rat plasma. The dimethylphenoxy-hydroxylation product of ABT-378 (M-5) and M3/4 were detected in dog plasma. The *in vitro* antiviral activity showed that M-1 and M-3/4 have potency comparable to that of the parent drug. M-1 and M-3 through M-12 were identified in rat and dog excreta while M-13, M-14 and M-15 have been identified in dog excreta. M-16 is a dog specific metabolite (Figures 1 and 2). The major metabolites seen in bile from rats and dogs after intravenous dosing of ABT-378/ritonavir were M-3/M-4, accounting for 30.8 and 38.3% of the total biliary radioactivity, respectively. The dihydroxylated derivatives of ABT-378 (M-11, M-12, M-13, M-14 and M-15) accounted for 8.7 and 18.4% of the total biliary radioactivity in rat and dog bile, respectively. The major metabolites observed in bile after intraduodenal dosing were also M-3/M-4, accounting for 39.5 and 14.3% of the total biliary radioactivity in rats and dogs, respectively. M-1, M-5 and M-9/10 were also observed, accounting for approximately

10% of biliary radioactivity in rats and dogs. The metabolites M-6/7/8 and M-16 were found in dog bile, and together accounted for approximately 6% of the biliary radioactivity. Unchanged [^{14}C]ABT-378 accounted for 2.1 or 0.7% (rats) and 7.3 or 1.3% (dogs) of biliary radioactivity after intravenous or oral dosing, respectively. Polar metabolites accounted for 50.3 or 38.2% in rat and 21.7 or 44.1% in dog of the total biliary radioactivity after intravenous or intraduodenal dosing, respectively. Radioactivity in rat and dog feces consisted largely of unchanged parent drug (19.8-52.8% of total dose) after oral administration of [^{14}C]ABT-378/ ritonavir. Unchanged [^{14}C]ABT-378 was also a major component of rat feces (28.1% of dose) after intravenous dosing with ritonavir. Unchanged [^{14}C]ABT-378 was not a major component in dog feces (<5% of dose) after intravenous dosing. Rat and dog urine contained an insignificant amount of unchanged ABT-378 and polar metabolites.

Excretion

Feces. Rats and dogs at 5-10 mg/kg intravenous or oral [^{14}C]ABT-378/ritonavir displayed high fecal excretion (>80% of dose) of the radiolabeled dose within 3-8 days.

Bile, Urine and Milk. 66.8% of the dose was excreted in the bile within 24 hours after dosing, accompanied by minimal dose recovery in urine (<2%). Intraduodenal administration of a 10 mg/kg dose resulted in the recovery of 24.7% of the dose in the 0-24 hour bile of rat. Recovery of radioactivity in dog bile (0-6 hours) was 19.6 and 8.0% of the administered dose after intravenous and intraduodenal dosing, respectively. [^{14}C]Abbott-157378 associated radioactivity is excreted into the milk in rats. The milk to plasma ratio of radioactivity ranged from 0.084 to 1.53 through 24 hours after dosing. The milk to plasma radioactivity ratios during 3-8 hours after dosing were 0.29 to 0.63.

Enzyme Induction and Inhibition

An *ex vivo* examination of liver microsomal enzyme induction after multiple dosing with ABT-378 and ritonavir in combination has not been conducted in mice, rats, dogs and monkeys. Induction of the CYP3A subfamily of microsomal monooxygenases *in vitro* was observed as an increase in immunoreactive CYP3A protein in human hepatocyte cultures incubated with ritonavir but not in cultures incubated with ABT-378. Incubation of ABT-378 with rat and dog hepatic microsomes resulted in metabolite profiles that were quantitatively similar to the corresponding metabolite profiles of the drug in rat and dog bile. Human liver microsomes and Human hepatocytes converted [^{14}C]ABT-378 largely to M-1, M-3 and M-4. Additionally, the metabolism of ABT-378 in rat liver microsomes was sensitive to inhibition by ritonavir (K_i: 0.013 μM or 9 ng/mL). The kinetics of the metabolism of [^{14}C]ABT-378 were determined with microsomes from four human livers, with K_m values of 6.81μM and mean V_{max} values of 9.38 nmoles/min/mg protein. CYP3A4 and CYP3A5 are the exclusive contributors to the human liver microsomal metabolism of ABT-378. ABT-378 was found to be a weak inhibitor of ritonavir metabolism, with a K_i of 130 μM (82 μg/mL).

CONCLUSIONS

There are no pharmacology issues that would preclude the approval of this NDA.

Labeling Review (NDA):

Carcinogenesis and Mutagenesis

Long-term carcinogenicity studies of KALETRA in animal systems have not been completed.

However, neither lopinavir nor ritonavir was found to be mutagenic or clastogenic in a battery of *in vitro* and *in vivo* assays including the Ames bacterial reverse mutation assay using S. *typhimurium* and E. *coil*, the mouse lymphoma assay, the mouse micronucleus test and chromosomal aberration assays in human lymphocytes.

Carcinogenicity studies in mice and rats have been carried out on ritonavir. In mice, at levels of 50, 100 or 200 mg/kg/day, there was a dose dependent increase in the incidence of combined adenomas and

carcinomas in the liver of both males and females. Based on AUC measurements, the exposure at the high dose was approximately 0.3 and 0.6 fold for males and females, that of the exposure in the clinic at the approved high dose. In rats, dosed at levels of 7, 15 or 30 mg/kg/day, there were no carcinogenic effects. In this study, the exposure at the high dose was approximately 6 per cent that of the exposure in the clinic. Based on the low exposures in the animal studies, the significance of the carcinogenic effects is not known.

Pregnancy, Fertility, and Reproduction

Pregnancy Category C: Lopinavir in combination with ritonavir at a 2:1 ratio produced no effects on fertility in male and female rats at levels of 10/5, 30/15, or 100/50 mg/kg/day. Based on AUC measurements, the exposures in rats at the high doses were approximately 0.7 fold for lopinavir and 0.9 fold for ritonavir of the exposures in the clinic at the recommended therapeutic dose. No treatment-related malformations were observed when Lopinavir in combination with ritonavir was administered to pregnant rats or rabbits. Embryonic and fetal developmental toxicities (early resorption, decreased fetal viability, decreased fetal body weight, increased incidence of skeletal variations and skeletal ossification delays) occurred in rats at a maternally toxic dosage (100/50 mg/kg/day). Based on AUC measurements, the drug exposures in rats at 100/50 mg/kg/day were approximately 0.7 fold for lopinavir and 1.8 fold for ritonavir for males and females, that of the exposures in the clinic at the recommended therapeutic dose. In a peri- and postnatal study in rats, a postnatal developmental toxicity (a decrease in survival in pups between birth and postnatal day 21) occurred at 40/20 mg/kg/day and greater.

No embryonic and fetal developmental toxicities were observed in rabbits at a maternally toxic dosage (80/40 mg/kg/day). Based on AUC measurements, the drug exposures in rabbits at 80/40 mg/kg/day were approximately 0.6 fold for lopinavir and 1.0 fold for ritonavir, that of the exposures in the clinic at the recommended therapeutic dose. There are no adequate and well-controlled studies in pregnant women. KALETRA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

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

Nursing Mothers: The Centers for Disease Control and Prevention recommend that HIV-infected mothers not breastfeed their infants to avoid risking postnatal transmission of HIV. Studies in rats have demonstrated that lopinavir is secreted in milk. It is not known whether lopinavir is secreted in human milk. Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers should be instructed **not to breast-feed if they are receiving KALETRA.**

RECOMMENDATIONS

NDA issues-Phase IV Commitments:

1. Please complete and submit report for two-year carcinogenicity studies with ABT-378/ritonavir combination in rats and mice.

APPEARS THIS WAY
ON ORIGINAL

Hao Zhang, M.D.
Pharmacologist

Concurrences:

HFD-530/WDempsey
HFD-530/JFarrelly
HFD-530/HZhang

Disk:
HFD-530\JFarrelly

cc:

HFD-530/NDA-21-226
HFD-530/SLynche
HFD-530/HZhang
HFD-530/KStruble
HFD-530/K-YLo
HFD-530/JO'Rear

Figures, Appendices and Attachments

Figures

Figure 1. Chemical Structures of ABT-378 and Metabolites
Figure 2. Proposed metabolic Pathway for [^{14}C]ABT-378 in the Rat, Dog and Human *In Vivo*

Appendices

1. Histopathology performed on all tissues in mice, rats and dogs with administration of Abbott-157378 and ritonavir
2. ABT-378 Impurities, Proposed Specification Limits and Levels of Toxicity Studies
3. Ritonavir Impurities/Degradants, Proposed Specification Limits and Levels in Toxicity Studies
3b. Summary of Levels of Impurities in SGE Formulations of ABT-378/Ritonavir in Toxicity Studies
4. Summary of Single Dose Acute Toxicity Studies
5. Summary of Repeated Toxicity Studies in Rats, Dogs and Monkeys
6. Summaries of Reproductive toxicity Studies (segment I, II and III)
7. Summary of Genotoxicity Studies

Attachments

1. Pharmacologist's Review: Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Mice (Study No. TD96-220, R&D/96/458, 1996)
2. Pharmacologist's Review: Acute Oral Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-218, R&D/96/456, 1996).
3. Pharmacologist's Review: Acute Intravenous Toxicity Evaluation of Abbott-157378 and Abbott-84538 Combination in Rats (Study No. TA96-219, R&D/96/457, 1996)
4. Pharmacologist's Review: Single-Dose Oral Toxicity Study of Abbott-157378 in Rats (Study No. TA96-315, R&D/96/669)
5. Pharmacologist's Review: Two-year dietary carcinogenicity study of Abbott-84538 in rats (R&D/98/136)

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Appendix 1. Histopathology performed on all tissues in mice, rats and dogs with administration of Abbott-157378 and ritonavir

Tissue	0 *(mg/kg)*	Treatment *(mg/kg)*
Eye	X	X
Brain	X	X
Pituitary gland	X	X
Sternebrae	X	X
Bone marrow	X	X
Lungs	X	X
Heart	X	X
Aorta	X	X
Thyroid	X	X
Parathyroid	X	X
Trachea,	X	X
Esophagus	X	X
Thymus	X	X
Salivary gland	X	X
Liver	X	X
Adrenal glands	X	X
Spleen	X	X
Pancreas	X	X
Lymph node(s)	X	X
Mesenteric	X	X
Submandibular	X	X
Sciatic nerve	X	X
Testes	X	X
Epididymides	X	X
Seminal vesicles	X	X
Prostate gland	X	X
Ovaries	X	X
Uterus	X	X
Vagina	X	X
Stomach	X	X
Duodenum	X	X
Jejunum	X	X
Ileum	X	X
Colon	X	X
Cecum	X	X
Urinary bladder	X	X
Spinal cord	X	X
Thoracic	X	X
Lumbar	X	X
Skeletal muscle	X	X
Skin with Mammary gland	X	X

Histopathologic examination of gallbladder was performed in dogs.

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Appendix 4. Summary of Single Dose Acute Toxicity Studies

Species	Route	Sex	ALD (mg/kg)[a]	NOEL (mg/kg)[b]	HED (mg/kg)[b]
Mouse	p.o	Combined	>1250/625	<20/10	<1.7/0.8
Rat	p.o	Combined	>1250/625	39/20	6.4/3.4
Rat	p.o[c]	Combined	>2500	100	16.7
Mouse	i.v.	Male	>62.5/31.3	<1.0/0.5	0.08/0.4
	i.v.	Female	>62.5/31.3	2.0/1.0	0.16/0.08
Rat	i.v.	Male	31.3/15.6	3.9/2.0	0.65/0.32
	i.v.	Female	31.3/15.6	1.0/0.5	0.17/0.08

a. ALD: approximately lethal dose (ABT-378/ritonavir); b. NOEL: no-observed effect level (ABT-378/ritonavir combination); c. ABT-378 alone; HED: human equivalent dose (NOEL)

Appendix 5. Summary of Repeated Toxicity Studies in Mice, Rats and Dogs

Species	Group Size	Dose mg/kg/day (Duration)	Target Organ	NOAEL[a] mg/kg/day	HED[b] mg/kg/day
Mice	10M, 10F	3-month; 20/10-200/100	Liver (↑ALP, AST, ALT), ↓RBC,Hct, Hb	60/30	5/2.5
Rat					
Adult	10M, 10F	2-week; 10/5 to 100/50	Liver (↑ALP, AST, ALT), thyroid (↓T4, TSH↑), spleen	30/15	5/2.5
	15M, 15F	3-month; 10/5-150/75	Liver, thyroid, ↓RBC	50/25	8.3 /4.1
	20M, 20F	6-month; 10/5-150/75	Liver, thyroid spleen, RBC	50/25	8.3/4.1
Neonate	10M, 10F	2-week; 10/5-40/20	↑Liver weight	40/20	6.7/3.3
Juvenile	10M, 10F	4-week 10/5-100/50	Liver, thyroid	30/15	5/2.5
Dog	3M, 3F	2-week 5/2.5-50/25	None	50/25	28/14
	4-6M, 4-6F	3-month; 10/5-100/50[d]	Liver (hepatocellular changes, ↑liver wt., ALP, AST and ALT)	35/15	19/9.5
	4M, 4F	6-month 5/2.5-60/20[e]	Liver (↑liver wt., ALP, AST and ALT), testis (↓germ cells, degeneration)	25/8	14/4
	4M, 4F	9-month; 10/5-100/50	Liver (↑liver wt., ALP)[c]	25/12.5	14/7
Human					
Adult	--	13.3/3.3[f]	--	--	--
Child	--	24/6[g]	--	--	--

The repeated dose toxicity of ABT-378/ritonavir has been assessed in mice, rats and dogs in studies ranging in duration from two weeks to nine months of oral administration. Studies in neonatal and juvenile rats have also been conducted. [a]NOAEL: non observed adverse effect level; [b]HED: human equivalent dose. [c] No histopathologic changes were seen in dogs at 50/25 mg/kg/day for nine month. [d]The high dosage was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30 due to toxicity; [e] The high dosage was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 30 due to toxicity. [f] Equivalent to 400/100mg ABT-378/ritonavir BID, which was normalized to body weight (mg/kg) assuming a 60 kg body weight. [g]ABT-378/ritonavir 300/75 mg/m^2 BID

Appendix 6. Summaries of Reproductive toxicity Studies (segment I, II and III)

Species	Study Type	Group Size	Oral Dosage* (mg/kg/day)	Endpoint	NOAEL (mg/kg/day)	HED (mg/kg/day)
Rat	Seg I	24M, 24F	10/5 - 100/50	Fertility & embryonic development	100/50	16.7/8.3
	Seg II	24F	20/10-100/50	Embryonic and fetal development	100/50	16.7/8.3
	Seg III	25F	20/10-80/40	Pre- and postnatal development	40/20	6.7/3.3
Rabbit	Seg II	19-20F	30/15-80/40	Embryonic and fetal development	80/40	26.7/13.3

* ABT-378/ritonavir

Appendix 7. Summary of Genetic Toxicology Studies

Test System	Dosage	Impurity (%)	Result	Review Study No.
Bacteria reverse mutation assay –Ames	100-10,000 µg/plate (+/-S9)	Normal*	Negative	No 27 (R&D96/439)
Bacteria reverse mutation assay –Ames	100-5,000 µg/plate (+/-S9)	6.5	Negative	No 28 (R&D98/303)
Bacteria reverse mutation assay –Ames	100-10,000 µg/plate (+/-S9)	9.1	Negative	No 29 (R&D98/589)
Bacteria reverse mutation assay –Ames	30/15–5000/2500 µg/plate (+/-S9)	9.1	Negative	No 30 (R&D99/447)
Bacteria reverse mutation assay –Ames	1-10,000 µg ABT-538/plate (+/-S9)		Negative	No 31 (R&D96/375)
Mammalian cell human lymphocytes (in vitro - chromosome aberration)	1-10 µg/ml (-S9) 3-50 µg/ml (+S9)	Normal*	Negative	No 32 (R&D96/440)
Mammalian cell human lymphocytes (in vitro - chromosome aberration)	3-30 µg/ml (-S9) 20-50 µg/ml (+S9)	6.3	Negative	No 33 (R&D98/304)
Mammalian cell human lymphocytes (in vitro - chromosome aberration)	10-30 µg/ml (-S9) 10-50 µg/ml (+S9)	9.1	Negative	No 34 (R&D98/645)
Mammalian cell human lymphocytes (in vitro - chromosome aberration)	2/1-50/25 µg/ml (-S9) 5/2.5-30/15 µg/ml (+S9)	9.1	Negative	No 35 (R&D99/448)
Mammalian cell human lymphocytes (in vitro - chromosome aberration)	10-100 µg ABT-538/ml (-S9) 10-100 µg ABT-538/ml (+S9)	4.5	Negative	No 36 (R&D96/746)
Mouse micronucleus assay (in vivo)	625, 1250, 2500 mg/kg/day (ABT-378 alone); 78/39, 156/78 , 313/156 (ABT-378/ritonavir)	Normal*	Negative	No 37 (R&D96/250)
L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay (forward mutation)	1-20 µg/ml (-S9) 25-120 µg/ml (+S9)	Normal	Negative	No 38 (R&D96/773)

*Levels of impurities in Abbott-157378/ritonavir: <2.8% (Lot: 40979-TL, 16276-AL)