

Summary of histopathology findings

Organ/Tissue	Principle Drug Related Finding	TPV/RTV	TPV	RTV
Multiple tissues	Excessive hemorrhage	X	X	
	Lymphoid depletion	X	X	X
Liver	Hypertrophy, hepatocellular, centrilobular	X	X	X
	Karyomegaly	X		X
	Cholangiohepatitis, subacute			X
	Increased mitotic index			X
	Hyperplasia, Kupffer cell			X
Lung	Microgranuloma, histiocytic			X
Lymph node, mesenteric	Hyperplasia, reticuloendothelial cell	X		X
Skin	Fat depletion, subcutis	X	X	X
Stomach	Necrosis, mucosal, glandular	X	X	X
Testis	Degeneration, seminiferous tubule, bilateral	X		
Thymus	Lymphocytolysis	X	X	X
Thyroid gland	Hypertrophy, follicular epithelial	X	X	X

Toxicokinetics:

Plasma Concentrations of TPV and RTV on Drug Week 26 at 8 hours after TPV dose and 9 hours after RTV dose:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV (μ M)	RTV (ng/ml)
120/32	F	133 (Week 14)	1882 (higher than expected - protocol deviation)
	M	198	585
600/160	F	406	418
	M	303	141
1200/320	F	488	462
	M	344	0 (below 10)
1200/0	F	361	0
	M	290	0
0/160	F	0	1689
	F	0	1555

Other:

Histopathology inventory

Note: Tissues were examined histopathologically for animals that died or were sacrificed moribund and for all terminal sacrifice Control, 1200/320 mg/kg/day TPV/RTV, TPV only and

RTV only group animals. A “+” denotes tissues from the 120/32 and 600/160 mg/kg/day TPV/RTV groups that were also examined.

Study U04-3111 Species Rat	Dose (mg/kg/day)			
	Control	TPV/RTV 1200/320	TPV 1200	RTV 160
Adrenals	X	X	X	X
Aorta	X	X	X	X
Bone Marrow, femur +	X	X	X	X
Bone Marrow, sternum				
Bone (femur with stifle joint) +	X	X	X	X
Bone, sternum	X	X	X	X
Brain +	X*	X*	X*	X*
Cecum +	X	X	X	X
Cervix	X	X	X	X
Colon +	X	X	X	X
Duodenum +	X	X	X	X
Epididymis +	X	X	X	X
Esophagus	X	X	X	X
Eye +	X	X	X	X
Fallopian tube				
Gall bladder				
Gross lesions	X	X	X	X
Harderian gland +	X	X	X	X
Heart	X	X	X	X
Ileum +	X	X	X	X
Injection site				
Jejunum +	X	X	X	X
Kidneys +	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver +	X*	X*	X*	X*
Lungs +	X	X	X	X
Lymph nodes, bronchial	X	X	X	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric +	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves +	X	X	X	X
Ovaries +	X*	X*	X*	X*
Pancreas +	X	X	X	X
Parathyroid +	X	X	X	X
Peripheral nerve				

Pharynx				
Pituitary	X	X	X	X
Prostate	X	X	X	X
Rectum +	X	X	X	X
Salivary gland +	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X	X	X	X
Skeletal muscle	X	X	X	X
Skin +	X	X	X	X
Spinal cord +	X	X	X	X
Spleen +	X	X	X	X
Sternum	X	X	X	X
Stomach +	X	X	X	X
Testes +	X*	X*	X*	X*
Thymus +	X	X	X	X
Thyroid +	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X
Zymbal gland				

X, histopathology performed
*, organ weight obtained

Study title: 26-Week oral (gavage) interaction/toxicity study in the Beagle dog on tipranavir and ritonavir.

(See IND 51,979 (474) *Summary of Immunotoxicity Findings for Tipranavir in Appendix for discussion of findings related to immunotoxicity in this study.*)

Key study findings: Target organs when TPV and RTV were co-administered were the liver and urinary bladder. There was no significant increase in toxicity when the two compounds were co-administered. Additional target organs noted when TPV and RTV were administered alone included the gallbladder, bone marrow (sternum, rib) and spleen. There was no NOAEL for the co-administration of TPV and RTV due to the presence of hepatocellular hypertrophy in the liver of one male dog in the low dose TPV/RTV group.

Study no.: U04-3011

Volume # and page #: Module 4, M002, vol. 1.24, page 1.

Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877

Date of study initiation: November 27, 2001

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot no. 113010, 100% purity; RTV, Lot no. 66729 TL and TSA-02-001, 100% purity.

Methods

Doses: Initial doses were 0, 15/4, 37.5/10 and 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV. These doses continued to the end of Drug Week 12 due to a high incidence of emesis. Doses were escalated in Drug Week 13 from 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV to 150/40, 150 and 40, respectively.

Species/strain: Beagle dogs [1

Number/sex/group or time point (main study): 3

Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 2 ml/kg for tipranavir and 2ml/kg for ritonavir; animals received RTV first followed by TPV. Due to the bitter taste of TPV a cherry syrup wash was added in Drug Weeks 7 and 8.

Satellite groups used for toxicokinetics or recovery: None.

Age: 7 – 9 months

Weight: 6 – 11 kg

Sampling times:

Unique study design or methodology (if any):

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.)

Mortality: Room checks for morbidity and mortality were performed once daily during the Pretest Phase and twice daily during the Drug Phase.

Clinical signs: Clinical observations were recorded at least once daily during the Pretest Phase. Clinical observations were made 1 and 4 hours after dosing. Physical examinations, including measurement of reflexes (patella, pupillary and pain reflex), heart rate, rectal temperature and respiratory rate were performed in Pretest Weeks -4 and -2 and Drug Weeks 3, 7, 11, 19 and 24 at 2 – 4 hours after dosing.

Body weights: Body weights were measured once weekly during Pretest and Drug Phases.

Food consumption: Food consumption was measured daily in the Pretest and Drug Phases.

Ophthalmoscopy: Both eyes of all dogs were examined in Pretest Week -4 and Drug Weeks 7, 13 and 26.

EKG: Blood pressure and electrocardiograms were measured in Pretest Weeks -4 and -2 and in Drug Weeks 3, 7, 11, 19 and 25, 2 – 4 hours after dosing.

Hematology: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, blood samples were collected from the jugular vein for performance of a standard hematology battery of tests.

Clinical chemistry: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, blood samples were collected from the jugular vein for performance of a standard clinical chemistry battery.

Urinalysis: In Pretest Weeks -4 and -1 and in Drug Weeks 4, 8, 12, 18 and 26, animals were placed in metabolism units overnight with access to water but without food, for collection of urine.

Gross pathology: A complete necropsy was performed on early death and terminal sacrifice animals.

Organ weights (specify organs weighed if not in histopath table):

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (), no ()

Results

Mortality: No drug-related deaths occurred during the study. Two dogs died from dosing accidents.

Clinical signs: Clinical signs considered related to TPV and RTV administration were increased salivation, emesis and soft stool/diarrhea. The incidence of these signs was dose-related. No drug-related effects were observed on physical examinations during the course of the study.

Body weights: No drug-related effects were observed on body weights.

Food consumption: No drug-related effects were observed on food consumption.

Ophthalmoscopy: No drug-related changes in ophthalmology were observed in the study.

EKG: No drug-related changes in electrocardiograms or blood pressure were observed in the study.

Hematology: There were no drug-related changes in hematology parameters.

Clinical chemistry: Significant drug-related changes in clinical chemistry when data from both sexes were combined included elevations of 93% to 134% in mean alkaline phosphatase in the 37.5/10 mg/kg/day TPV/RTV, 75-150/20-40 mg/kg/day TPV/RTV and 20-4 mg/kg/day RTV. One 20-40 mg/kg/day RTV male was noted to have elevations of 84% and 200% AST and ALT, respectively, as compared to its Pretest values. A slight decrease (13%) in mean albumin level was noted in 75-150/20-40 mg/kg/day TPV/RTV animals in Drug Week 26 only.

Urinalysis: There were no drug-related changes in urinalysis.

Gross pathology: Drug-related macroscopic enlargement and/or prominent lobular architecture in the liver were observed in males and females from the high dose TPV/RTV group and in

males from the TPV group and the RTV group. These changes correlated with diffuse centrilobular hypertrophy microscopically.

Organ weights (specify organs weighed if not in histopath table): There were no changes in organ weights except for liver weights. The percentage differences for the group mean absolute liver weights compared to control group means were as follows: 1) +20% (M) and +14% (F) in the low dose TPV/RTV group; 2) +6% (M) and +3% (F) in the middle dose group; 3) +19% (M) and +10% (F) in the high TV/RTV dose group; 4) +14% (M) and +7% (F) in the TPV group and 5) +31% (M) and +25% (F) in the RTV group.

Histopathology: Adequate Battery: yes (x), no ()
Peer review: yes (), no ()

Drug-related microscopic changes were observed in the liver, bone marrow (sternum, rib), lymph nodes, gallbladder, thymus, spleen and urinary bladder.

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Histopathology Findings

Group:	Control		Low-Dose TPV/R TV		Mid-Dose TPV/R TV		High-Dose TPV/R TV		High TPV		High RTV	
	0		15/4 TPV/R TV		37.5/10 TPV/R TV		75/20 TPV/R TV		75/0 TPV		0/20 TPV	
Dose (mg/kg/day): Test Article: Formulation: TPV Aqueous pH 10.5/RTV Propylene glycol												
Gender:	M	F	M	F	M	F	M	F	M	F	M	F
No. in Group	3	3	3	3	3	3	3	3	3	3	3	3
Bone Marrow, Rib Granulocytic hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++ +	1/3 ++	0/3	0/3

Bone Marrow, Sternum Granulocytic hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	1/3 ++	0/3	0/3	
Lymph Node Lymphoid hyperplasia	0/3	0/3	0/3	1/3 + 1/3 ++	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++ +	
Mesenteric Lymph Node Lymphocytic depletion, cortical	0/3	0/3	1/3 ++	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3	0/3	0/3	
Spleen Lymphocytic depletion	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3	1/3 ++ 1/3 ++ +	1/3 +	
Thymus Lymphocytic depletion, cortical	1/3 ++ 1/3 ++ ++ +	1/3 ++ 1/3 ++ ++ +	1/3 + 1/3 ++ ++ +	3/3 ++	1/3 ++ 1/3 ++ ++ +	1/3 ++ 1/3 ++ ++ ++	1/3 ++ 1/3 ++ ++ 1/3 ++ +	1/3 ++ 1/3 ++ ++ ++ 1/3 ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++	1/3 ++ 1/3 ++ ++ ++ ++ ++
Liver Centrilobular hypertrophy	0/3	0/3	1/3 +	0/3	1/3 ++	1/3 ++	1/3 ++ 1/3 ++ ++ +	1/3 ++ ++ ++ ++	2/3 ++ ++ ++ ++	2/3 ++ ++ ++ ++	2/3 ++ ++ ++ ++	1/3 ++ ++ ++ ++	1/3 ++ ++ ++ ++	
Urinary Bladder Hypertrophy of transitional epithelium	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3	0/3	
Gall Bladder Cystic glandular hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	2/3 ++	1/3 ++	1/3 ++	1/3 ++	

Number affected/Number examined.

+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Toxicokinetics: Blood was collected for the measurement of plasma levels of TPV and RTV in Drug Weeks 3, 7, 13, 18 and 26. Blood samples were collected prior to dosing and approximately 1, 2, 4, 8, and 24 hours after dosing.

TPV and RTV co-administration caused increases in plasma levels of TPV and decreases in RTV, as compared to the individual drugs administered alone. In most instances, TPV and RTV

plasma levels increased with increasing dose level and there were no gender differences for either compound given alone or co-administered.

C_{max} and AUC of TPV and RTV on Drug Week 26:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV		RTV	
		C _{max} (M)	AUC _{0-24h} (M.h)	C _{max} (ng/ml)	AUC _{0-24h} (ng.h/ml)
15/4	F	34	157	554	1,603
	M	19	72	61	274
37.5/10	F	41	217	438	2,031
	M	37	236	372	2,014
75-150/20-40	F	64	598	416	5,121
	M	66	722	852	7,780
75-150/0	F	16	74	6	18
	M	40	346	0	0
0/20-40	F	0	0	10,970	97,340
	F	0	0	10,574	79,014

Histopathology inventory

Study U04-3011 Species Dog	Dose mg/kg/day					
	0	15/4	37.5/10	75/20- 150/40	75-150	20-40
	Control	TPV/RTV	TPV/RTV	TPV/RTV	TPV	RTV
Adrenals	X	X	X	X	X	X
Aorta	X	X	X	X	X	X
Bone Marrow, rib	X	X	X	X	X	X
Bone Marrow, sternum	X	X	X	X	X	X
Bone, femur	X	X	X	X	X	X
Bone, sternum	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix	X	X	X	X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X

Epididymis	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder	X	X	X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland						
Heart	X	X	X	X	X	X
Ileum	X	X	X	X	X	X
Injection site						
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*
Lachrymal gland						
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X	X
Lymph nodes, bronchial	X	X	X	X	X	X
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X*	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve						
Pharynx						
Pituitary	X	X	X	X	X	X
Prostate	X	X	X	X	X	X
Rectum	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles						
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X	X	X	X	X	X
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*
Thymus	X	X	X	X	X	X
Thyroid	X	X	X	X	X	X
Tongue	X	X	X	X	X	X
Trachea	X	X	X	X	X	X

Urinary bladder	X	X	X	X	X	X
Uterus	X	X	X	X	X	X
Vagina	X	X	X	X	X	X
Zymbal gland						
Tonsils	X	X	X	X	X	X

X, histopathology performed

*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 26-week oral (gavage) interaction/toxicity study in beagle dogs with ritonavir co-administration.

(Reviewed under U04-3011 26-Week oral (gavage) interaction/toxicity study in the beagle dog on tipranavir and ritonavir.)

Study no.: U03-3193

Study title: 26-Week Oral (Capsule) Safety Study in the Beagle Dog on Tipranavir and Ritonavir in SEDDS.

Key study findings: The purpose of this study was to determine the safety of the self-emulsifying drug delivery system (SEDDS) formulation, with TPV/RTV co-administration. Exposure to CrEL in the SEDDS formulation was of particular interest because IV administered CrEL is known to cause anaphylactoid reactions and it seemed possible that orally administered CrEL might pass from the GI tract into systemic circulation with long treatment duration. Consequently, exposures to the SEDDS formulation were chosen to achieve 1 (91 mg/kg/day SEDDS containing 1 mg/g CrEL), 10 (910 mg/kg/day SEDDS) and 30-fold (2720 mg/kg/day SEDDS) exposure to CrEL in humans.

Hematology and clinical chemistry changes were observed in three animals exposed to the highest dose level of SEDDS vehicle, including one Control female and two high dose animals. These changes included elevated white blood cell counts, accompanied by neutrophilia and monocytosis and increased liver alkaline phosphatase. In the two high dose animals, these changes were reversible and without microscopic changes. The Control female was sacrificed moribund in Week 13. In view of the similar changes noted in this female and two other animals receiving the high dose of SEDDS, this death was judged to be related to SEDDS vehicle administration. Target organs of SEDDS vehicle toxicity noted in the early death female were the stomach, intestine and mesenteric lymph nodes. These organs displayed microscopic changes consistent with an infectious etiology but in the absence of a causative agent.

CrEL plasma levels were detectable 2 hours after the first or second dose in several animals administered 2720 mg/kg/day SEDDS and one animal receiving 910 mg/kg/day SEDDS. Plasma CrEL levels ranged from 0.1 to 1 mg/ml. Given that a 10 kg Beagle has a blood volume of 850 ml, the total dose in the blood at the point of measurement would range from 87 to 192 mg. This is considerably lower than the IV doses of CrEL, e.g. approximately 27,000 mg

in Paclitaxil, known to cause anaphylactoid reactions in humans. It should also be noted that there were no signs of a hypersensitivity reaction (e.g. edema, hives, rash) in the animals in the study and no SEDDS-related fecal occult blood was detected.

In conclusion, the NOEL for SEDDS is considered to be 91 mg/kg/day SEDDS due to the appearance of detectable levels in CrEL in the plasma of one animal dosed at 910 mg/kg/day. However, due to the lack of observed toxicities in animals dosed at 910 mg/kg/day, the NOAEL is considered to be 910 mg/kg/day SEDDS. This dose gives a 10-fold safety factor for the amount of CrEL in the proposed human dose of 500/200 mg/kg/day TPV/RTV in the SEDDS vehicle. However, in light of the large amount of CrEL in this oral drug, including the amount of CrEL in the recommended human dosage of TPV/RTV 500/200 mg BID in the label should be considered.

Study no.: U04-3184

Volume #, and page #: Module 4, M002, Vol., 1.40; page 1.

Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, Ridgefield, CT 06877-0368.

Date of study initiation: 2-10-03

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: TPV stock SEDDS formulation, lot no. 6257-15 (purity %); SEDDS vehicle formulation, lot no. 5804-91; RTV lot no. TSA-02-001 (purity %)

Methods

Doses: 0 TPV/RTV in 2720 SEDDS; 15/6 TPV/RTV in 91 SEDDS; 15/6 TPV/RTV in 910 SEDDS; 15/6 TPV/RTV in 2720 SEDDS mg/kg/day (twice daily dosing with half the dose administered followed by half the dose 4 hours later).

Species/strain: Beagle dog

Number/sex/group or time point (main study): 3/sex/group.

Route, formulation, volume, and infusion rate: Oral via gelatin capsules.

Satellite groups used for toxicokinetics or recovery:

Age: 7 – 9 months.

Weight: 6 to 10 kg

Sampling times:

Unique study design or methodology (if any):

The purpose of the study was to investigate the safety of the TPV SEDDS formulation, with the dose levels selected based on exposure to Cremophor EL (CrEL) at the proposed human dose level of 500/200 mg TPV/RTV. The clinical TPV SEDDS capsule of 250 mg TPV contains 20 mg CrEL in one gram. The RTV formulation contains 100 mg RTV and 20 mg CrEL per capsule. Therefore, at 500/200 mg TPV/RTV, humans receive per day four tipranavir capsules and four RTV capsules. Consequently, humans are being exposed to a total of 200 mg CrEL/day or 3.3 mg/kg/day based on a 60 kg human. Exposures to the SEDDS formulation

were chosen to achieve 1, 10 and 30-fold the exposure to CrEL in humans. A low dose of 91 mg/kg/day SEDDS (containing — mg/g CrEL) approximates (— ng/kg/day) the equivalent human exposure (— mg/kg/day) to CrEL at the 500/200 TPV/RTV BID dose level on a body weight basis (mg/kg/day). The middle dose of 910 mg/kg/day SEDDS approximates 10-fold the human exposure; the high dose of 2720 mg/kg/day SEDDS approximates 30-fold the human exposure.

The dose level of TPV/RTV was selected based on results of a 26-week oral toxicity study in dogs such that the lowest dose would result in minimal toxicity and a low incidence of emesis. The dose level was adjusted to — mg/kg/day to reflect the ratio of 500/200 mg TPV/RTV BID or 2.5:1.

Observations and times:

Mortality: Morbidity and mortality checks were performed once daily during the Pretest Phase and twice daily during the Drug Phase.

Clinical signs: Clinical observations performed once daily during the Pretest Phase and at least once before and approximately 30 minutes after each dose administration during the Drug Phase throughout the study. Physical examinations, including measurement of reflexes, heart rate, rectal temperature and respiratory rate were performed in Pretest Weeks -4 and -2 and Drug Weeks 3, 7, 11, 19 and 25 at 1 to 2 hours after the second dose.

Body weights: Body weights were recorded once weekly during the Pretest and Drug Phases of the study.

Food consumption: Food consumption was evaluated daily in the Pretest and Drug Phases of the study.

High dose control female 03-00001 received special food due to retained deciduous loose canines. The canines were removed surgically on Drug Day 23 and the animal was maintained on wet food or a mix of wet food/kibble. On Drug Day 33 subcutaneous fluids were administered because of dehydration. This animal continued to exhibit decreased food consumption and showed high white blood cell count with neutrophilia and monocytosis. The animal was sacrificed on Drug Day 81.

High-dose female 03-00019 displayed a high white blood cell count with neutrophilia as well as neck swelling and decreased food consumption starting in Drug Weeks 3 to 4. These effects were determined to be due to a neck lesion and with appropriate treatment the lesion healed and the adverse events resolved.

Ophthalmoscopy: Ophthalmoscopy was not performed.

EKG: EKGs and blood pressure were measured in Pretest Weeks -4 and -2 and in Drug Weeks 3, 7, 11, 19 and 25 1 to 2 hours after the second dose. Heart rate, PQ, QRS and QT durations were quantitated and evaluated.

Fecal analysis: Occult blood monitoring was performed for all dogs in Pretest Weeks -4 and -2 and in Drug Weeks 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26.

Hematology: Blood samples were collected from the jugular vein in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Blood was analyzed for standard hematology parameters.

Clinical chemistry: Blood samples were collected from the jugular vein in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Blood was analyzed for standard clinical chemistry parameters and for alkaline phosphatase (AP) isoenzymes.

Urinalysis: Urine samples were collected in Pretest Weeks -4 and -2 and in Drug Weeks 4, 8, 12, 18 and 26. Animals were placed in metabolism units overnight with access to water, but without food, for collection of urine. Urine was analyzed for standard urinalysis parameters.

Gross pathology: A complete necropsy was performed on all animals at the end of the study and on early death Control female 03-00001.

Organ weights (specify organs weighed if not in histopath table): See table at the end of this study.

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

Results

Mortality: One Control female was sacrificed moribund in Drug Week 13. This death was considered related to administration of high dose SEDDS. This animal displayed clinical signs of decreased motor activity, thinness/emaciation, decreased food consumption, decreased body weight and clinical pathology changes including elevated white blood cell counts with neutrophilia and monocytosis, increased alkaline phosphatase and secondary effects considered related to the anorexia observed.

Clinical signs: Clinical signs considered related to test article and/or vehicle administration were soft stools and diarrhea. These signs increased in frequency with the dose of SEDDS and are considered related to the SEDDS vehicle.

Emesis was not a predominant sign as it had been in earlier studies using an aqueous solution of TPV, where the taste of the solutions was judged to be the cause of excessive emesis. Taste was not a factor in this study since TPV was administered in a capsule.

No overt evidence of hypersensitivity (e.g. edema, hives or erythema) to the SEDDS formulation was noted.

Body weights: Body weights were decreased in two animals, including an early death control female (Week 13) and a High dose female. The effects on these animals were considered related to high dose SEDDS.

For Control female 03-00001, an overall body weight loss of 2 kg between Drug Days 1 to 78 was observed.

High dose female 03-00019 lost a total of 0.9 kg between Drug Day 1 and 36 but recovered by Drug Week 13.

Food consumption: Food consumption was decreased in two animals, including an early death control female (Week 13) and a High dose female. The effects on these animals were considered related to high dose SEDDS.

Control female 03-00001 exhibited decreased food consumption starting on Drug Day 10. The adjustments to her diet are described under methods. Decreased food consumption continued until this animal was sacrificed moribund.

High dose female -3-00019 exhibited decreased food consumption transiently until recovery in Drug Week 13.

Ophthalmoscopy: Ophthalmoscopy was not performed.

EKG: No drug- or vehicle-related effects were observed.

Fecal analysis: Results of occult blood testing in fecal samples did not reveal any definitive pattern that can be related to drug or vehicle. Fecal samples from a number of animals in all groups displayed positive results for occult blood during the Pretest phase. However, the same animals did not necessarily test positive during the Drug Phase. The sponsor states that part of this may be explained by sampling effects.

Hematology: No hematology changes occurred that were considered related to TPV/RTV administration. There were no coagulation changes that were judged drug or vehicle related.

In three animals, one Control and two High dose animals, all of which received 2720 mg/kg/day, white blood cell counts were elevated at various time points, accompanied by neutrophilia and monocytosis (see Table below). These changes were judged by the sponsor to be related to the SEDDS vehicle.

Control female 03-00001 displayed increased white blood cell counts 4-fold and 7-fold above Pretest levels in Drug Weeks 8 and 12, respectively. The increase in white cell counts was due to an increase in absolute neutrophil and monocyte counts, which in Drug Week 12 were 10-fold to 11-fold higher than Pretest levels, respectively. Red blood cell parameters (RBC, hemoglobin and hematocrit) were decreased between 32 to 35% in Drug Week 12.

High dose male 03-00022 displayed an elevated white blood cell count in Drug Week 18 that was 3-fold above Pretest Week -1 value. An increase in absolute neutrophil and monocyte counts of 4.6-fold and approximately 3-fold above Pretest Week -1 levels was observed. White blood cell parameters had returned to normal in Drug Week 26.

High dose female 03-00019 displayed an elevated white blood cell count in Drug Week 4 that was 3.5-fold above its Pretest Week -1 value, due to approximately 6-fold increases in absolute

neutrophil and monocyte counts. The white blood cell count returned to normal by Drug Week 8 measurement. Slight decreases of 15 to 17% in red blood cell parameters were observed during the study but the values were within normal limits.

Clinical chemistry: Changes in clinical chemistry parameters included increased alkaline phosphatase (AP), shown to be of hepatic origin through isoenzyme analysis. Total AP levels administered TPV/RTV remained elevated over time, although not in all individual animals, but those of Control animals tended to decrease over the course of the study. This was due to TPV/RTV-related increases in hepatic AP isoenzymes concurrent with a normal expected decrease in bone AP isoenzymes as dogs aged. Hepatic origin AP isoenzymes levels ranged from 6 to 34 U/L in all animals during Pretest Phase and in Control animals during Drug Phase (except for the early death Control female, whose levels were 236 and 332 U/L in Drug Weeks 8 and 12, respectively), while hepatic AP isoenzymes levels ranged from 1 to 150 U/L in TPV/RTV-treated animals.

Many clinical chemistry changes were noted in three dogs, Control female 03-00001, High dose male 03-00022 and High dose female 03-00019 (see Table below).

Hematology and clinical chemistry findings in individual animals:

Animal/Sex	03-00001/F	03-00022/M	03-00019/F
Dose TPV/RTV (mg/kg/day)	0 (Control)	15/6	15/6
SEDDS (mg/kg/day)	2720	2720	2720
Sampling Time Drug Phase versus (Pretest Week)	Week 12 ^a (Pretest Week -1)	Week 18 (Pretest Week -1)	Week 4 (Pretest Week -1)
WBC count (10 ³ /μl)	69.84 (9.95)	27.44 (8.77)	30.61 (8.77)
Neutrophils (10 ³ /μl)	58.56 (5.48)	23.31 (5.10)	24.50 (4.38)
Monocytes (10 ³ /μl)	5.48 (0.48)	1.55 (0.49)	1.86 (0.30)
Alkaline phosphatase (U/L)	389 (116)	140 (111)	188 (91)
Hepatic AP isoenzymes (U/L)	332 (16)	94 (12)	150 (20)
BUN (mg/dL)	5 (13)	9 (15)	10 (15)
ALT (U/L)	15 (33)	-	17 (39)
Glucose (mg/dL)	26 (98)	-	51 (95)
Total protein (g/dL)	5.5 (5.4)	-	6.4 (5.3)
Albumin (g/dL)	1.5 (3.2)	-	2.8 (3.1)
Globulin (g/dL)	4.0 (2.2)	-	3.6 (2.2)
Calcium (mg/dL)	9.3 (11.6)	-	-
Phosphorus (mg/dL)	5.4 (7.4)	-	4.6 (6.4)
Cholesterol (mg/dL)	219 (144)	-	251 (117)
Total bilirubin (mg/dL)	0.9 (0.1)	-	-

Urinalysis: No drug- or vehicle-related changes were observed.

Gross pathology: Drug-related macroscopic changes were not observed in the study. However, vehicle-related changes were evident in Control animal 03-00001 that was sacrificed moribund. Mesenteric lymph node enlargement and discoloration with local adhesion to the cecum and pancreas were noted. This correlated with the histopathological finding of subacute lymphadenitis. Bronchial lymph node enlargement was also noted and correlated with subacute lymphadenitis.

Organ weights (specify organs weighed if not in histopath table): There were no significant treatment-related weight changes in organ weight parameters.

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

Drug-related microscopic observations were not observed. Vehicle-related changes were observed.

Subacute to marked pyogranulomatous inflammation in multiple organs (brain, cecum, colon, duodenum, heart, liver, bronchial lymph nodes, mandibular lymph nodes, mesenteric lymph nodes, spleen, stomach, thyroid glands, thymus and urinary bladder) was observed in Control female 03-00001. The inflammation was centered in mesenteric lymph nodes and the stomach with secondary lesions in other organs and consisted of infiltrates of neutrophils, macrophages and multinucleate giant cells mixed with edema fluid, hemorrhage and cellular debris. Special stains did not demonstrate organisms within the lesions. Amyloidosis was present in the liver, bronchial lymph node, mandibular lymph node and spleen. The bone marrow demonstrated granulocyte hyperplasia secondary to the inflammatory processes present in other organs.

A finding of uncertain nature was present within the lung. The change occurred in animals from middle dose (0/3 males and 1/3 females) and high dose (2.3 males and 0.3 females) groups and consisted of infiltrates of macrophages and granulocytes into alveolar spaces and septa. This change is most likely iatrogenic in nature.

Another change worthy of mention was minimal lymphoid depletion in Peyer's patches of the ileum. This change was evident in animal from the low dose (1/3 males and 0/3 females), middle dose (1/3 males and 0/3 females), high dose (1/3 males and 2/3 females) groups but not in Control animals. This change was not considered drug-related as it was not evident in other lymphoid tissues.

Toxicokinetics: Blood was collected for the measurement of plasma levels of TPV, RTV and CrEL via the jugular vein prior to dosing and 1, 2, 4, 6, 8 and 24 hours after the first daily dose in Drug Weeks 3, 7, 13, 18 and 26 from all animals on study.

Cmax levels and AUC exposures of TPV and RTV were similar across all time points, all groups and both sexes, as they all received the same dose level of both compounds but in different volumes of SEDDS vehicle. Levels of both test articles were highly variable. Neither compound was detected in the plasma of Control animals.

Levels of CrEL plasma levels were below detectable limits (LOD = --- mg/ml) in the low dose group receiving 91 mg/kg/day SEDDS. One male of the middle dose group receiving 910 mg/kg/day CrEL displayed a CrEL level above the limit of detection (--- mg/ml) at one time point. CrEL levels were variably above the limit of detection in males of the high dose group and females of the Control group, both receiving 2720 mg/kg/day SEDDS. CrEL levels detected ranged from [---] mg/ml and were noted at 2 hours after the first or second capsule administration.

Toxicokinetic parameters for tipranavir, ritonavir and cremophor EL.

Dose TPV/RTV mg/kg/day	0/0 (Control)		15/6		15/6		15/6	
Dose SEDDS mg/kg/day	2720		91		910		2720	
Sex: No. of animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
TPV Cmax (μM)	0	0	18.7	15.6	22.9	16.4	18.9	14.5
TPV AUC ₀₋₂₄ ($\mu\text{M}\cdot\text{h}$)	0	0	99.1	107	161	118	143	96.0
RTV Cmax (ng/ml)	0	0	160	92.5	440	141	167	84.3
RTV AUC ₀₋₂₄ (ng.h/ml)	0	0	654	710	2715	829	1080	520
Cremophor EL (mg/ml) ^a	[---]		[---]		[---]		[---]	

^a Expressed as number of animals displaying CrEL levels above the limit of detection (LOD = --- mg/ml) – total number of time points for all animals; note that all time points > LOD occurred 2 hours after first or second dose.

Other:

Histopathology inventory

Study: U04-3184		Dose Group mg/kg/day			
Species:	TPV/RTV (mg/kg/day)	0	15/6	15/6	15/6
Dog	SEDDS (mg/kg/day)	2720	91	910	2720
Adrenals		X	X	X	X

Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Rib & Sternum				
Bone	X	X	X	X
Rib & Sternum				
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	X	X	X	X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X	X	X	X
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, Bronchial	X	X	X	X
Lymph nodes, Mandibular	X	X	X	X
Lymph nodes, Mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves	X	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Parathyroid	X	X		X*
Peripheral nerve				
Pharynx				
Pituitary	X	X	X	X
Prostate	X	X	X	X
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles				
Skeletal muscle	X	X	X	X

Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X	X	X	X
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X	X	X	X
Thyroid	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				
Additional Tissues				
Tonsils	X	X	X	X

X, histopathology performed

*, organ weight obtained

Study title: Toxicokinetics of tipranavir in a 26-week safety study in beagle dogs following oral (capsule) dosing of tipranavir SEDDS formulation with ritonavir co-administration.
(Reviewed under U04-3184 26-Week oral (capsule) safety study in the beagle dog on tipranavir and ritonavir in SEDDS.)

Study no.: U04-3295

Study title: 39-Week Oral Toxicity Study in the Dog Followed by a 9-Week Recovery Period

Key study findings: No drug-related deaths occurred. Clinical signs were related to local gastrointestinal effects of the dosing solution and increased in frequency with dose. All signs stopped when dosing stopped. Reversible decreases in body weight gain were noted in mid- and high-dose groups. Mild, reversible decreases in red blood cell numbers and hemoglobin levels were observed in the high-dose group. Reversible, dose-related increases in serum alkaline phosphatase and decreases in albumin and total protein were observed at the mid- and high-doses. A reversible, dose-related increase in liver weight was observed in the mid- and high-dose groups. Histopathology revealed reversible hepatocyte hypertrophy in the liver and increased hematopoiesis in the spleen and incompletely reversible cystic hyperplasia of the gall bladder epithelium at the mid and high doses. Reversible, minimal bile duct hyperplasia in the liver of high-dose females was also noted. The NOAEL for both sexes was 20 mg/kg/day which gives an AUC corresponding to 0.03 to 0.04 of the expected human AUC (500/200 mg TPV/RTV BID). The high dose used in this dog study produces an AUC approximately equivalent to the human AUC.

Study no.: U00-3270 (Pharmacia & Upjohn Study Report a0017797)

Volume #, and page #: Module 4, M002, Vol. 1.25, page 1.

Conducting laboratory and location: Drug Development Toxicology, Worldwide Toxicology, Pharmacia & Upjohn, Kalamazoo, MI

Date of study initiation: May 7, 1997

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: TPV (PNU-140690E), (A1)5134-AS-163 [] purity) and (A1)5134-AS-1650 ([] purity).

Methods

Doses: 0, 20, 75 or 320 mg/kg/day (0, 10, 37.5 or 160 mg/kg twice daily)

Species/strain: Beagle dog

Number/sex/group or time point (main study): 7/sex/group

Route, formulation, volume, and infusion rate: Oral via gastric intubation, purified water with pH adjusted to approximately 10.5 with 10% sodium hydroxide.

Satellite groups used for toxicokinetics or recovery: Blood samples were collected from the last 3 dogs/sex/group and 3 of the original 7 animals/group constituted the recovery group.

Age: 9 – 10 months.

Weight: Males – 8.2 to 11.6 kg; Females – 6.0 to 8.4 kg

Sampling times: Blood and urine samples were collected on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338. Toxicokinetics samples were collected predose (AM) and 2, 8, 10 and 14 hours after the AM dose on Days 1, 182 and 273 and predose (AM) and 10 hours after the AM dose on Days 28 and 84.

Unique study design or methodology (if any):

Observations and times:

Mortality: Clinical observations were performed 3 times prior to dose initiation and at least 2 times daily, 1 hour after each dose. Recovery dogs were observed once daily during the recovery period.

Clinical signs: Clinical observations performed 3 times prior to dose initiation and at least 2 times daily, 1 hour after each dose. Recovery dogs were observed once daily during the recovery period.

Body weights: Body weights were recorded four times prior to dose initiation and once weekly throughout treatment and recovery periods and prior to each scheduled necropsy following an overnight fast.

Food consumption: Food consumption was evaluated daily starting on Day -26 and continued throughout treatment and recovery periods except for Days when the dogs were fasted overnight for blood collection the following day. Each dog received 500 grams [] Diet [] 1 hour before the morning dosing. Uneaten food was weighed the next morning. Canned food was used as dietary supplements at the discretion of the Study Director. Data on amounts and days are retained in raw data.

Ophthalmoscopy: All dogs were given an ophthalmoscopic examination on Days -8, 269 and 332.

EKG: ECGs were collected from all dogs during the pretest period on Days -18 and -4, during treatment at 2.5 hours after dosing on Days 88, 179 and 270 and from 3/sex/group during recovery on Day 333.

Hematology: Blood samples were collected from the jugular vein on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Blood was analyzed for standard hematology parameters.

Clinical chemistry: Blood samples were collected from the jugular vein on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Blood was analyzed for standard clinical chemistry parameters.

Urinalysis: Urine samples were collected on Days -27, -6, 30, 58, 86, 177, 268, 303 and 338 from all surviving dogs. Urine was analyzed for standard urinalysis parameters.

Gross pathology: A complete necropsy was performed on all animals euthanized on Days 274, 275 and 339 and in dog number 54 following her death on Day 245.

Organ weights (specify organs weighed if not in histopath table): See table at the end of this study.

Histopathology: Adequate Battery: yes (x), no ()
Peer review: yes (x), no ()

Results

Mortality: Dog number 54 possibly aspirated some of the dosing solution during the morning dosing on Day 245. She was found dead 1 hour after the afternoon dosing period. No other unscheduled deaths occurred in the study.

Clinical signs: Clinical signs were observed at all drug levels and included emesis, soft feces and diarrhea and salivation before and after dose administration. These clinical signs occurred with greater frequency in the treated groups than in the control group and generally frequency increased as the dose increased.

Body weights: There were no statistically significant changes in body weight data when individual dose groups were compared to controls. However, the mid- and high-dose males and high-dose females lost a small amount of weight as shown in the table below.

Food consumption: High dose males consumed more food per day (398 g) than the other groups (302 – 334 g). Thus, their weight loss and slower weight gain occurred in spite of adequate food consumption.

Ophthalmoscopy: No drug-related changes were observed.

EKG: No drug-related effects were observed.

Hematology: See the table below. All effects reversed during the recovery period.

Clinical chemistry: See the table below. All effects reversed during the recovery period.

Urinalysis: No drug-related effects were observed.

Gross pathology: No treatment-related effects were observed at interim and final necropsies. The unscheduled necropsy of dog number 54 revealed gross lesions associated with aspiration of the dosing solution and included dark red coloration of multiple lung lobes, red mottling in other areas of the lung and red foci in the thymus.

Organ weights (specify organs weighed if not in histopath table): Liver weights showed a statistically significant dose-related increase in males and females in the mid- and high-dose groups. These increases were apparently related to hepatocyte hypertrophy seen microscopically. This change reversed during the recovery period. No other organ weights showed a treatment effect.

Histopathology: Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

See the table below for histopathological findings. The sponsor states that the data on testicular degeneration and/or atrophy were re-evaluated by an expert panel. The findings of the panel are reported in study U04-3531. The panel concluded that the findings in the dog were within normal limits of variation.

Table: Percent change (+/-) compared to controls.

Parameter (Week 39)	Dose (mg/kg/day)			
	75		320	
	Male	Female	Male	Female
Body weight	-9.2%	+3.6 %	-9.5%	-8.3%
Red blood cells	-2.0 %	-11.2 %	-4.2 %	-15.7 %
Hemoglobin	-0.6 %	-6.8 %	-4.4 %	-9.0 %
Mean cell volume	+0.7	+5.9 %	+0.5	+10.8 %
Alkaline phosphatase	+285 %	+167 %	+ 875 %	+791 %
Albumin	-12.6 %	-6.7 %	- 19.2 %	-24.2 %
Total protein	-8%	-2%	-10%	-14%
Albumin/globulin ratio	-8.6 %	-11.4 %	-19.0 %	-22.8 %
Calcium	-1.8%	-3.2 %	-4.5 %	-3.8 %
Cholesterol	+9%	+15%	-13%	-40%
Liver weight (% body weight)	+38.4 %	+41.6 %	+75.2 %	+57.5 %

Hematopoiesis spleen (increased)	0/4	1/4 mild	2/4 minimal 1/4 mild	3/4 mild 1/4 moderate
Hepatocellular hypertrophy	2/4 minimal 2/4 mild	1/4 minimal 2/4 mild	1/4 minimal 3/4 moderate	1/4 mild 3/4 moderate
Bile duct hyperplasia	0	0	0	3/4 minimal
Cystic hyperplasia of gallbladder epithelium	2/4 mild 1/4 moderate NR	3/4 minimal 1/4 mild NR	2/4 mild 2/4 moderate NR	2/4 mild 2/4 moderate
Mild vacuolar degeneration of seminiferous tubules	1/4		2/4 NR	
Atrophy of seminiferous tubules	1/4 mild		1/4 moderate NR	

NR = Not completely reversible after the 9-week recovery period.

Toxicokinetics: Cmax and AUC increased with dose with values for mid-dose males being greater than predicted by linear dose relationship. No gender differences were noted and there was no evidence of increased clearance or decreased absorption over time.

Dose mg/kg/day	0 (Control)		20		75		320	
	Males	Female	Male	Female	Male	Female	Male	Female
Cmax (µM)	0	0	6.5	7.9	69	38	127	101
AUC ₀₋₂₄ (µM.h)	0	0	50	55	490	290	1130	1180
Fold versus Human AUC*			0.03	0.04	0.32	0.19	0.73	0.77

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 µM.h.

Histopathology inventory

Study: U00-3270 Species: Dog	Dose Group mg/kg/day			
	0	20	75	320
Adrenals	X*	X*	X*	X*
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)				

Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	X	X	X	X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland	X	X	X	X
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, cervical				
Lymph nodes, Mandibular	X	X	X	X
Lymph nodes, Mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves				
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Parathyroid	X*	X*	X*	X*
Peripheral nerve				
Pharynx				
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles				
Skeletal muscle				
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Sternum	X	X	X	X
Stomach	X	X	X	X

Testes	X*	X*	X*	X*
Thymus	X	X	X	X
Thyroid	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				
Additional Tissues				
Diaphragm	X	X	X	X
Psoas Muscle	X	X	X	X
Proximal Tibia	X	X	X	X
Tattoo	X	X	X	X

X, histopathology performed

*, organ weight obtained

Study title: PNU-140690: 2-Week oral toxicity study in the male cynomolgus monkey.

Key study findings: TPV was well-tolerated at all doses (20, 80 and 320 mg/kg/day) in the cynomolgus monkey. There were no deaths and clinical signs were limited to diarrhea, soft stool and emesis. Fibrinogen was increased in all dose groups by Day 14. On Day 15, C_{max} values for TPV increased in proportion to dose while the dose-related increase of AUC was greater than dose proportional. A metabolite peak, previously seen only in human plasma after TPV treatment, was identified in the monkey. At steady-state, the ratios of parent drug to metabolite, based on AUC values, were 0.8, 1.9 and 4.9 for the low, middle and high doses. The NOAEL level was 320 mg/kg/day.

Study no.: U00-3191 (Pharmacia & Upjohn Study Report a0006007)

Volume #, and page #: Module 4, M002, vol. 1.27, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI.

Date of study initiation: September 8, 1997

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: TPV, Lot no. (A1)5134-AS-1586, [] purity.

Methods

Doses: 0, 20, 80 or 320 mg/kg/day, administered in two equally divided doses 8 hours apart.

Species/strain: Monkey/cynomolgus

Number/sex/group or time point (main study): 3 males/group

Route, formulation, volume, and infusion rate: Oral (nasal intubation), aqueous solution pH 10.5.

Satellite groups used for toxicokinetics or recovery: None

Age: Mature

Weight: 4.5 to 7.4 kg

Sampling times:

Unique study design or methodology (if any):

Observations and times:

Mortality: Mortality was checked at least once daily on Days 1 through 15.

Clinical signs: Clinical signs were checked at least once daily on Days 1 through 15.

Body weights: Body weights were measured once before treatment and weekly during the treatment period.

Food consumption: Not performed.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Blood samples for a standard battery of hematology tests were taken on Days -1, 7 and 14.

Clinical chemistry: Blood samples for a standard battery of hematology tests were taken on Days -1, 7 and 14.

Urinalysis: Not performed.

Gross pathology: Not performed.

Organ weights (specify organs weighed if not in histopath table): Not performed.

Histopathology: Adequate Battery: yes (), no () Not performed.

Peer review: yes (), no ()

Results

Mortality: There were no deaths.

Clinical signs: Clinical signs related to TPV administration were emesis, diarrhea and soft stool. Emesis was due to a gag reflex from oral intubation. Emesis did not occur when nasal intubation was used for dose administration. The frequency of clinical signs was related to dose with soft stool noted only in the high dose group.

Body weights: There were slight decreases in body weights over the course of the study. There were not of a magnitude to be considered significant.

Food consumption: Not performed.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: There were slight decreases in group mean hematocrit, hemoglobin and red blood cell count at the 80 and 320 mg/kg/day dose levels. Only hematocrit and hemoglobin were slightly decreased in the low dose group. Group mean mean cell hemoglobin concentration was slightly decreased by Day 14 in all dose groups. Group mean mean cell volume was slightly increased in the high dose group on Days 7 and 14. None of these changes were of a magnitude to be considered toxicologically significant.

Increases in group mean fibrinogen and group mean platelet counts were seen in all groups by Day 14. The increases in platelet counts were not of a magnitude to be considered toxicologically significant. However, fibrinogen values were increased 41% to 73% over baseline values by Day 14. These increases may be associated with a TPV effect on the liver.

Minimal changes in the high dose group were seen in group mean white blood cell count, basophil count, large unstained cell count, monocyte count and in mean segmented neutrophils. These changes were due to one animal and the individual values were within the range of control values.

Clinical chemistry: There were slight decreases in group mean aspartate aminotransferase, blood urea nitrogen, total protein and cholesterol and increases in triglycerides compared to control means at the high dose level only. There were slight increases in group mean total bilirubin at the middle and high doses and slight decreases in group mean glucose in all dose groups. These changes may have been secondary to a TPV effect on the liver but none were of a magnitude to be considered toxicologically significant.

Urinalysis: No performed.

Gross pathology: Not performed.

Organ weights (specify organs weighed if not in histopath table): Not performed.

Histopathology: Adequate Battery: yes (), no () Not performed.

Peer review: yes (), no ()

Toxicokinetics: On Day 15, C_{max} (μM)/AUC(μM.h) values for TPV were 0.62/2.18, 2.21/18.70 and 9.00/94.00 for the low, middle and high dose groups, respectively. There was a 2- to 4-fold decrease of mean C_{max} and AUC values in the middle and high dose groups after repeated dosing for 15 days, suggesting decreased absorption or increased clearance. This effect was not observed in the low dose group. Increases in C_{max} and AUC increased with dose. The C_{max} dose was proportional to dose but the AUC increase was greater than proportional to dose. There was no evidence of saturation of absorption at these doses.

A human metabolite, previously only observed in human plasma after eight days of dosing, was formed in vivo in monkeys. Its appearance in monkey plasma was established by retention time only. On Day 15, C_{max} (μM)/AUC(μM.h) values for the metabolite were 0.26/2.72, 0.58/10.10 and 0.99/19.30 for the low, middle and high dose groups, respectively. There was a decrease of mean C_{max} and AUC values after repeated dosing similar to that seen for the parent compound. C_{max} and AUC values increased with dose but the increase was not dose proportional for the high dose. At steady-state on Day 15, the ratios of parent drug to metabolite based on AUC were 0.8, 1.9 and 4.9 for the low, middle and high doses.

2.6.6.4 Genetic toxicology

Study title: U-140690E: Evaluation of U-140690E in the preincubation mutagenesis assay in bacteria (Ames assay).

Key findings: There were no increases in revertant frequencies to the levels required for a positive finding in any strains at any dose. TPV was judged to be nonmutagenic under the conditions of this test.

Study no.: U00-3090 (Pharmacia & Upjohn Technical Report 7228-96-101)

Volume #, and page #: M002, vol. 1.27, page 1

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI

Date of study initiation: June 21, 1996

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-1720, τ τ purity

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA-97A, TA-98, TA-100, TA-1535 and *Escherichia coli* strain WP2 uvrA

Doses used in definitive study: 114.3, 228.5, 457.0, 914.1, 1828.1 μg/plate

Basis of dose selection: Precipitation was observed in the preliminary range-finding experiment with TA-100 and WP2 uvrA at 2500 and 5000 μg/plate with metabolic activation and at 1250 to 5000 μg/plate without metabolic activation. No toxicity was observed in either strain at any dose.

Negative controls: DMSO

Positive controls: With metabolic activation: 2-Aminoanthracene (all strains); without metabolic activation: 4-Nitro-o-phenylenediamine (strain TA-97A), 2-Nitrofluorene (strains TA-98 and TA-100), Sodium Azide (strain TA-1535) and ENNG (strain WP2 uvrA).

Incubation and sampling times: The preincubation method was used. Tester strains were grown overnight in Oxoid broth No. 2. TPV was diluted in DMSO at a concentration of 20 mg/ml and serial dilutions were made from this stock solution. Bacteria ($1 - 3 \times 10^8$ in 100 μ l) were combined with 100 μ l of either the test agent, DMSO or a positive control and either 0.5 ml of the metabolic (phenobarbital and β -naphthoflavone induced rat liver S9) activation mixture or 0.5 ml of phosphate buffer (pH 7.4) and preincubated at 37^o C for 20 minutes. After the preincubation period, the contents of each tube were combined with 2 ml of top agar (45^o C) containing a small amount of histidine and tryptophan to give a total volume of 2.7 ml. The molten mixture was then poured onto a petri plate containing histidine/tryptophan deficient base agar and was incubated at 37^o C for 2 days. After incubation, the plates were examined for evidence of precipitation and for toxicity as shown by a diminished or absent background lawn or reduced colony numbers. Colonies were counted using an automatic colony counter. A confirmation experiment was performed using the same doses. Due to contamination of TA-1535 in the first experiment, a third experiment was performed using only this strain.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all criteria for validity.

Three replicate cultures were used per dose. Appropriate negative and positive controls were used. Positive and negative controls results fell within acceptable ranges. Criteria for a positive response in the assay were as follows: 1) a dose-related increase in frequency of revertants ; 2) a reproducible elevation of mutant colonies per plate at a single dose which amounts to a tripling of the control background frequency of revertants in TA-98, TA-1535, TA-1537 and WP2 uvrA or a doubling in TA-97A, TA-100 and TA-102. Criteria for a negative response in the assay were as follows: 1) No evidence of systematic reproducible elevation of revertant frequency in any strain or activation group; 2) No reproducible doubling (TA-97A, TA-100, TA-102) or tripling (TA-98, TA-1535, TA-1537, WP2 uvrA) of the solvent control background frequency of revertants at any dose level.

Study outcome: There were no increases in revertant frequencies to the levels required for a positive finding in any strains at any dose. TPV was judged to be nonmutagenic under the conditions of this test.

Study title: U-14069E: Evaluation of U-140690E in the in vitro unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes.

Key findings: Under the conditions of this in vitro rat hepatocyte DNA repair assay, the results with TPV, evaluated at doses ranging from 0.027 to 2.72 µg/ml, were negative.

Study no.: U00-3091 (Pharmacia & Upjohn Technical Report 7228-96-092)

Volume #, and page #: Module 4, M002 vol. 1.27, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of study initiation: May, 1996.

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)0403-AS-049, 100% purity.

Methods

Strains/species/cell line: Rat primary hepatocytes.

Doses used in definitive study: Doses are expressed as TPV free acid equivalents. Doses in the first experiment were 0.027, 0.091, 0.270, 0.906, 2.72, 9.06, 27.20 and 90.60 µg/ml. Doses of 9.06, 27.20 and 90.60 µg/ml were toxic and the slides were unscorable. Precipitation occurred at 90.60 µg/ml. Doses in the second experiment were 0.1, 0.3, 0.6, 1.0, 2.0 and 3.0 µg/ml.

Basis of dose selection: A large number of doses were used in the first experiment to establish solubility and toxicity. Doses in the second, confirmatory, experiment were based on the results of the first experiment.

Negative controls: The negative control was the vehicle, DMSO.

Positive controls: The positive control was 2-acetylaminofluorene (2-AAF).

Incubation and sampling times: Hepatocytes were dissociated from rat liver by collagenase perfusion, placed in monolayer culture and incubated in the presence of TPV or controls and [³H]TdR for 18 to 20 hours. The cultures were fixed, mounted on microscope slides and DNA repair (unscheduled DNA synthesis, UDS) was evaluated by measuring the incorporation of [³H]TdR by autoradiography. For each cell, the silver grains over the nuclear area and two nuclear-sized cytoplasmic areas were counted by a Beckman LS 5000TD colony counter.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all criteria for validity. The positive and negative controls gave appropriate results.

Duplicate cultures were used and 30 cells were analyzed per culture (slide). Excessive cytotoxicity was determined by microscopic examination of the slides and indicated by loss of cells, abnormal cell morphology or pyknotic nuclei.

The criteria for a positive outcome were that UDS net grain count of any test dose is ≤ 5 net grains per nucleus in both the preliminary and replicate experiments and the percentage of cells in repair is ≥ 10 .

The criteria for a negative outcome were that UDS net grain count of all test doses is ≤ 0 and that testing was performed to the limits of solubility or cytotoxicity or at 3 mg/ml.

Study outcome: All net grains/nucleus at nontoxic doses in the first experiment and all net grains/nucleus in the second experiment were less than zero and, therefore, met the criteria for a negative response.

Study title: PNU-140690E: Evaluation of PNU-140690E in the AS52/XPRT mammalian cell mutation assay with and without rat liver S9 metabolic activation.

Key findings: TPV did not induce gene mutation at the XPRT locus in AS52 cells at doses of 0.05 to 12.5 $\mu\text{g/ml}$ without metabolic activation and 0.5 to 50 $\mu\text{g/ml}$ with metabolic activation. Therefore, TPV is considered to be negative in the AS52/XPRT mammalian cell mutation assay under the conditions of the assay.

Study no.: U00-3169 (Pharmacia & Upjohn Technical Report 7228-97-052)

Volume #, and page #: Module 4, M002 vol. 1.28, page 1.

Conducting laboratory and location:

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Date of study initiation: February 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-2363

Methods

Strains/species/cell line: AS52 Chinese hamster ovary cells.

Doses used in definitive study: Without activation: 0.05, 0.5, 5.0, 10, 20 and 40 µg/ml. With activation: 0.5, 5.0, 25, 50, 100 and 200 µg/ml.

The highest two concentrations of 20 and 40 µg/ml without activation and 100 and 200 µg/ml with activation could not be cloned for mutant selection due to toxicity. Based on the results of the parallel toxicity test of the definitive mutation assay, the confirmatory mutation assay was performed at the following concentrations: Without activation: 0.5, 5.0, 10, 12.5, 15 and 20 µg/ml. With activation: 1.0, 5.0, 10, 20, 40 and 80 µg/ml.

Basis of dose selection: A range-finding experiment was performed with TPV at doses of 0.05 to 2000 µg/ml with and without Aroclor-induced rat liver S9 activation. Severe toxicity was observed at 50 to 2000 µg/ml without activation and at 500 to 2000 µg/ml with activation. The definitive assay doses were based on this range-finding experiment and, as explained above, the confirmatory assay doses were based on the results of the definitive assay.

Negative controls: Untreated cultures and vehicle (DMSO) treated cultures.

Positive controls: Ethyl methanesulfonate without metabolic activation and dimethylnitrosamine with activation. DMSO was also the vehicle for positive controls.

Incubation and sampling times: Duplicate cultures were treated with TPV or controls in serum-free culture medium for five hours, washed and cultured in fresh complete medium for an additional 18 to 24 hours. The cells were then subcultured for XPRT mutant phenotypic expression and for a parallel cytotoxicity test. After an expression period of 8 to 9 days, mutant cells were selected in culture medium containing 6-thioguanine (TG) and mutant colonies were scored after 7 days of growth.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all criteria for validity.

Duplicate cultures were used and 1×10^6 cells were analyzed per time per dose. The metabolic activation system (Aroclor-induced rat liver S9) was appropriate. The number of mutant colonies and cloning efficiency (cytotoxicity) were determined by counting by eye after fixing and staining the colonies.

The cytotoxicity curve was steep and in the definitive assay dropped from 96% to 5% cloning efficiency (with activation) and from 83% to 60% (without activation) cloning efficiency with the latter being the highest cytotoxicity achieved in that arm of the study. However, there was no increase in mutant frequency at the high dose (with activation) with 5% cytotoxicity and adequate cytotoxicity was achieved in the confirmatory assay.

Criteria for a positive response: The test article shows a positive dose-response trend and at least one test dose show either a statistically significant increase in mutant frequency or a two-fold increase in the number of mutants, which represents a net increase of at least 30 mutants per 1×10^6 surviving cells. In the absence of a positive dose-response trend, at least two consecutive test article doses show a statistically significant increase in mutant frequency.

Criteria for a negative response. The test article is considered to have caused a negative response if it shows neither a dose-dependent response nor a two-fold, statistically significant increase ≥ 30 mutants per 1×10^6 surviving cells relative to the pooled, concurrent negative controls.

Study outcome: TPV did not induce gene mutation at the XPRT locus in AS52 cells with or without metabolic activation. Therefore, TPV is considered to be negative in the AS52/XPRT mammalian cell mutation assay under the conditions of the assay.

Study title: PNU-140690E: Evaluation of PNU-140690E in the in vitro chromosome aberration assay in human peripheral lymphocytes.

Key findings: There was no significant increase in the percentage of cells with chromosome aberrations in any test group when compared to the concurrent solvent control. No statistically significant increase in polyploidy (including endoreduplication) was observed in any test group when compared to the solvent control. TPV was not clastogenic under the conditions of this assay.

Study no.: U00-3172 (Pharmacia & Upjohn Study Report 7228-97-037)

Volume #, and page #: Module 4, M002 vol. 1.28, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI.

Date of study initiation: February 1997.

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-2363, τ 3 purity.

Methods

Strains/species/cell line: Primary human lymphocytes.

Doses used in definitive study: Doses are expressed as free acid equivalents. The doses used were 0 (solvent DMSO), 3.27, 6.54, and 13.1 $\mu\text{g/ml}$ for 24 hour treatment without metabolic activation (Aroclor-induced rat liver S9) and 0, 2.18, 4.36 and 8.73 $\mu\text{g/ml}$ for three hour treatment (with and without metabolic activation) and for 48 hour treatment (without metabolic activation).

Because of the lack of cytotoxicity observed in the first experiment, a second chromosome aberration experiment was conducted. The doses used in this experiment were 0, 10.91, 21.82 and 43.6 µg/ml for three hour treatment without metabolic activation and three, 24 and 48 hour treatments with metabolic activation.

Basis of dose selection: A range-finding cytotoxicity test was conducted with TPV at doses of 0.22 to 17.5 µg/ml based on the toxicity of TPV in previous studies. Cytotoxicity was measured by mitotic inhibition. The doses in the first experiment were based on the findings in this range-finding study. Due to a lack of cytotoxicity in the first experiment, the doses for the second experiment were altered as explained above.

Negative controls: The negative controls were medium and vehicle (DMSO).

Positive controls: The positive control for experiments without metabolic activation was mitomycin C and for experiments with metabolic activation, cyclophosphamide. The vehicle for positive controls was culture medium.

Incubation and sampling times: Lymphocytes were obtained from two donors (one female and one male). Duplicate cultures were treated in complete culture medium containing 10% serum for 24 or 48 hours without metabolic activation before the cells were harvested. With metabolic activation, duplicate cultures were treated in serum-free medium for three hours and cultured for an additional 21 hours in fresh complete medium before the cells were harvested. Cells were swelled in a hypotonic solution and fixed. The fixed cell suspension was dropped onto microslides and stained with Giemsa. For each dose, three slides per duplicate culture were made. All slides were scored blind except the positive controls, for which 50 metaphase cells per duplicate culture were scored prior to scoring the coded slides. A total of 200 metaphase cells per time point/dose were scored for chromosome aberrations. A total of 400 cells per time point/dose were scored for polyploidy including endoreduplication.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all validity criteria. Positive and negative controls performed adequately. Adequate cytotoxicity was achieved in the second experiment.

The criterion for a positive result was that the test article induces a dose-dependent and statistically significant increase in the percentage of cells with chromosome aberrations when compared to the concurrent solvent control at one or more dose levels or the test article induces a statistically significant increase in the percentage of cells with chromosome aberrations when compared to the concurrent solvent control at two or more dose levels when there is no positive dose response.

The criterion for a negative result was that the test article did not induce a statistically significant increase in the percentage of cells with chromosome aberrations when compared to the concurrent solvent control at any dose level.

Study outcome: There was no significant increase in the percentage of cells with chromosome aberrations in any test group when compared to the concurrent solvent control. No statistically significant increase in polyploidy (including endoreduplication) was observed in any test group when compared to the solvent control.

Study title: PNU-140690E: Evaluation of PNU-140690E in the in vivo micronucleus test in mouse bone marrow.

Key findings: TPV was negative in the in vivo micronucleus test in mouse bone marrow and, therefore, did not show clastogenic potential.

Study no.: U00-3196 (Pharmacia & Upjohn Technical Report 7228-97-051)

Volume #, and page #: Module 4 M002 vol.1.28, page 1.

Conducting laboratory and location: 1

Date of study initiation: January 21, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-2363, } purity.

Methods

Strains/species/cell line: CD-1 Mice

Doses used in definitive study: 520, 1300 and 2600 mg/kg.

Basis of dose selection: Treatment groups of three mice per sex were treated with 0, 10, 50, 100, 500, 1000 or 2000 mg/kg. The mice were observed for three days for treatment-related deaths, clinical signs and body weights. Since no clinical symptoms were observed, a dose of 2620 mg/kg was added a week later. At 2620 mg/kg, salivation, soft stool, wet/stained anogenital area, inactivity, and labored breathing were observed in most animals on the day of dosing. No clinical symptoms were observed after the day of dosing.

Negative controls: The negative control was vehicle (distilled water adjusted to pH 10.5) administered by oral gavage.

Positive controls: The positive control was triethylenemelamine administered by IP injection.

Incubation and sampling times: Animals were dosed by oral gavage with two equal administrations of test article separated by approximately 6 hours to give a total of 0, 520, 1300 or 2600 mg/kg TPV. Animals were sacrificed 24, 48 or 72 hours after the first dose to collect bone marrow. Only one sampling time (24 hours) was used for the positive control animals. Five male and five female animals per dose/time point were treated with the test article and vehicle control. Bone marrow cells were collected from femurs and stained with Wright-Giemsa and 2000 polychromatic erythrocytes (PCE) per animal were scored for micronucleated polychromatic erythrocytes (MPCE).

On the day of dosing, observations at 2600 mg/kg included the same signs seen in the range-finding experiment. Very few clinical signs were observed after the day of dosing.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all criteria for validity. Clinical signs at 2600 mg/kg provided evidence of exposure to TPV.

The criteria for a positive response were a positive dose-response trend and a statistically significant increase in the number of MPCE at one or more dose levels over that of the concurrent vehicle control. If there is no positive dose-response trend, at least two consecutive test doses must produce a statistically significant increase in the number of MPCE.

The criterion for a negative response is that none of the test doses show a statistically significant increase in the number of MPCE when compared to the vehicle control.

Study outcome: TPV, at doses of 520, 1300 and 2600 mg/kg, is concluded to be negative in the micronucleus test under the conditions of the test. TPV did not show clastogenic potential.

Study title: A 13 week oral (gavage)micronucleus assay in rats administered tipranavir SEDDS formulation (Study No. 03R066)

Key findings: Neither TPV nor degradation products formed in the SEDDS formulation induced chromosome damage in rats following thirteen weeks dosing at levels that produced detectable drug levels at all doses.

Study no.: U04-3013

Volume #, and page #: Module 4 M002 vol. 1.38, page 1

Conducting laboratory and location: Boehringer Ingelheim Pharmaceuticals, Inc., Toxicology & Safety Assessment, 900 Ridgebury Rd., Ridgefield, CT 06877-0368

Date of study initiation: May, 2003

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV SEDDS (unstressed), Lot No. 6380-1; TPV SEDDS with degradation products (stressed), Lot No. NB4987/136.

Methods

Strains/species/cell line: ~ CD(SD) BR Sprague-Dawley rats

Doses used in definitive study: 0, 125 or 400 mg/kg/day.

Basis of dose selection: Doses were selected on the basis of previous repeat-dose toxicology studies in the rat.

Negative controls: The negative control was stressed SEDDS vehicle.

Positive controls: The positive control was 10 mg/kg/day cyclophosphamide at 10 mg/kg orally for three days.

Incubation and sampling times: Male and female rats were dosed for thirteen weeks via oral gavage. The test article was TPV SEDDS formulation that had been prepared at a total concentration of 250 mg TPV/gm. A sample was stressed to provide a formulation that included degradation products. The formulation was administered at dose levels of 125 or 400 mg/kg/day TPV. Details of the treatment of the animals can be found in the review of study number U04-3154.

Approximately 24 hours after the last dose, the rats were sacrificed and bone marrow smears were prepared for micronucleus evaluation. Bone marrow smears from animals treated with stressed SEDDS or stressed TPV SEDDS formulation were stained with acridine orange and evaluated under fluorescent microscopy for alteration in the ratio of polychromatic to normochromatic erythrocytes (PCE:NCE ratio) and the frequency of micronucleated polychromatic erythrocytes (MN-PCE).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study met all criteria for validity. Five animals/sex/group were used for bone marrow analyses. Up to 2000 PCE were scored for the presence of micronuclei for each animal. The ratio of PCE to NCE in up to 200 erythrocytes was determined as an indicator of alteration of erythropoiesis. Positive and negative controls performed adequately. Toxicokinetic data provided evidence of exposure as there were detectable drug levels at all doses.

Study outcome: There was no indication of a drug-induced effect on the ratio of PCE to NCE or on micronucleated-PCE. Neither TPV nor degradation products formed in the SEDDS formulation induced chromosome damage in rats following thirteen weeks dosing at levels that produced detectable drug levels at all doses.

2.6.6.5 Carcinogenicity

Rat and mouse carcinogenicity studies are ongoing and will be completed as a post-marketing commitment.

[See Appendix Review of Carcinogenicity Study Design/Dose Selection Proposals and Executive CAC Memo, IND 51,979 Submission 48 (SX) May 22, 1999 (rat) and IND 51,979 Submission 217 (SX), March 24, 2003 (mouse)]

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: PNU-140690E: Male and female fertility and early embryonic development study (oral) in Sprague-Dawley rats.

Key study findings: TPV was given orally daily at doses of 0, 40, 400 or 1000 mg/kg/day in two equal doses eight hours apart to male and female rats. Treatment was from 4 weeks prior to cohabitation until killed (males) or from 2 weeks prior to cohabitation through gestation day 12 (females). TPV plasma concentration in females in the 1000 mg/kg/day group on treatment day 2 ranged from \square μM , with a mean of 258 μM , which is approximately two-fold the human C_{max} of 103 μM at the clinical dose of 500/200 TPV/RTV BID. Exposure (AUC) was not determined in this study. However, an exposure in pregnant rats corresponding to two-fold the human exposure at 500/200 TPV/RTV BID can be extrapolated based on the finding of an AUC of 1670 $\mu\text{M}\cdot\text{h}$ (C_{max} 157 μM) in pregnant rats in Study Number U00-3254. TPV administration to females at 1000 mg/kg/day resulted in treatment related death, body weight loss and decreased body weight gain, decreased food consumption, clinical signs and hepatomegaly. Treatment-related findings for females given 400 mg/kg/day were limited to clinical signs and hepatomegaly. Treatment-related effects in males at 400 and 1000 mg/kg/day included slightly decreased body weight and body weight gain, postdose salivation and hepatomegaly. TPV effects on coagulation may have made rats more susceptible to hemorrhage following accidental injury. TPV did not affect spermatogenesis, estrous cycle, copulation, conception, fertility, implantation or early embryonic development at the doses administered.

The NOAEL for systemic toxicity was 40 mg/kg/day for males and 400 mg/kg/day for females. The NOAEL for reproductive effects was 1000 mg/kg/day.

Study no.: U00-3170 (Pharmacia & Upjohn Study Report a0066139)

Volume #, and page #: Module 4, M002, vol. 1.31, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI.

Date of study initiation: May 7, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot # (A1)5134-AS-1636, 99.9% purity.

Methods

Doses: 0, 40, 400 and 1000 mg/kg/day (0, 20, 200 and 500 mg/kg/dose administered BID 8 hours apart) in aqueous solution, pH 10.5.

Species/strain: Rat — CD(SD)BR Sprague-Dawley.

Number/sex/group: 24/sex/group

Route, formulation, volume, and infusion rate: Oral by gastric intubation.

Satellite groups used for toxicokinetics:

Study design: Males were administered TPV (or vehicle) twice daily for 51 consecutive days beginning 4 weeks (29 days) prior to cohabitation and continuing until killed. Males were cohabited (1:1) with females which were dosed for 28 to 39 consecutive days beginning 2 weeks prior to cohabitation and continuing through gestation day 12. Females were killed on gestation day 13 for uterine examinations. Data collected on gestation day 13 included the numbers of corpora lutea and live and dead embryos.

Parameters and endpoints evaluated: Clinical signs, body weight, food consumption, estrus monitoring, reproductive data, necropsy, histology (reproductive organs from males that did not impregnate a female, organs from animals that died on study and gross lesions), toxicokinetics, uterine examinations and evidence of copulation (= gestation Day 0).

Results

Mortality: One male in the 400 mg/kg/day group and 4 males in the 1000 mg/kg/day group died. The cause of death for all 5 males was injuries associated with the dosing process and/or accidental trauma. Microscopic evidence of hemorrhage was observed in these rats. Dose-related prolongation of PT and APTT times has been noted in other rat studies of TPV effects. Prolongation of clotting times in rats is consistent with increased susceptibility to accident-related hemorrhagic episodes.

Seven females in the 100 mg/kg/day group died. Three deaths were due to dosing injuries as confirmed by necropsy findings. Four deaths were found dead before the morning dose on female treatment day 2. The cause of death for these four females was unclear but attributed to TPV treatment based on histopathology findings.

Clinical signs: In males, clinical signs were from four males in the 1000 mg/kg/day group that died and included swollen muzzle, nasal or oral bleeding and labored breathing. Most of the males in the 400 mg/kg/day group and all of the males in the high dose had postdose salivation.

In females, postdose salivation was also observed in most of the animals in the middle and high dose groups. Resistance to dosing was also noted in these two groups and was probably caused by lack of palatability in the dosing solutions.

Body weight: In males, statistically significant decreases in body weights were noted in the 400 and 1000 mg/kg/day groups. After 50 days of dosing, the percent decrease in group mean weights compared to control group mean weights was 3%, 6% and 11% for low, middle and high dose groups, respectively.

Female mean body weights and body weight changes in the 40 and 400 mg/kg/day groups were comparable to the control group prior to dose initiation and throughout the pre-mating treatment period. Female mean body weights in the high dose group were slightly, but significantly, decreased on treatment days 8 and 11 of the pre-mating period. A mean body weight loss occurred during the initial pre-mating treatment period (days 1 – 4) and this resulted in a statistically significant decrease in body weight gain during the entire pre-mating treatment period (days 1 – 15) in the high dose group. Female mean body weights and mean body weight changes during the gestation period were comparable to control means.

Food consumption: In males, group mean food consumption compared to control group mean was reduced 2%, 3% and 4% in the low, middle and high dose groups.

In females, group mean food consumption in the pre-mating period after two weeks of dosing compared to control group mean was increased (2%) in the low dose group but decreased 2% and 8% in the middle and high dose groups. At day 12 of the gestation period, group mean food consumption compared to control group mean was increased (2%) in the low dose group and decreased (3%) in the middle and high dose groups. Mean gestation food consumption in the treated groups was comparable to the control group.

Toxicokinetics: Plasma samples from 10 females in the 1000 mg/kg/day group were obtained 10 hours after the morning dose on female treatment day 2. This sampling was prompted by the unexpected deaths of four females in this group. TPV mean plasma concentration on treatment day 2 in females was 258 μM . This is approximately 50% of the dose day 1 C_{max} after the morning dose in a four-week oral dose toxicity study in rats. This decrease from day 1 suggests that induction of metabolism had already occurred by dose day 2.

Necropsy: In males, hepatomegaly was observed in all treated groups with an increase in incidence in the middle and high dose groups (20/24) as opposed to the low dose group (3/24) and control group (0/24). Other observations were related to dosing or cage injuries.

Microscopic observations of centrilobular hepatocellular necrosis were noted in three males in the high dose group and were thought to be due to hypoxic conditions associated with acute blood loss. Microscopic examination of reproductive organs of males that failed to impregnate did not determine the cause.

Hepatomegaly was also observed in females with a greater incidence in the 400 (20/24) and 1000 (19/26) mg/kg/day groups as compared to the low dose (6/24) and control (0/24) groups. An enlarged cecum was also seen in three females in the high dose group but this was not considered drug-related due to the low incidence. Other observations were related to injuries or were not drug-related. Histopathological examinations of females found dead did not resolve the cause of death.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Estrous cycle monitoring:

Estrous cycle monitoring showed that there were no relevant differences in the mean number of estrous cycles, the mean estrous cycle length or the number of females with anestrus when the treated groups were compared to the control group.

Copulation and fertility:

TPV treatment did not affect the ability of male and female rats to copulate. The mean precoital interval in the treated groups was comparable to the control group. The male and female fertility and conception indices were not affected in this study.

Reproductive data at gestation day 13 uterine examination:

The following reproductive parameters were comparable between the control group and all treated groups: mean number of implantations, mean number of dead embryos, mean preimplantation loss percent, mean postimplantation loss percent, number of females with preimplantation loss and the number of females with postimplantation loss. The mean number of corpora lutea and the mean number of live embryos in the 1000 mg/kg/day group were statistically significantly decreased as compared to the control groups. The decreased mean number of live embryos was due to the decreased number of corpora lutea and there was no corresponding increase in the mean number of dead embryos. The decreased mean number of corpora lutea (15.3 in the treated group versus 17.2 in the control group) was not considered treatment-related because the decrease was slight and the mean value was within historical control ranges of the means for the Sprague-Dawley rat.

Embryofetal development

Study title: PNU-14069E: A dose range-finding study (oral) in pregnant rats.

Study no.: U00-3190 (Pharmacia & Upjohn Study Report a0014475)

Discussed under U00-3254 PNU-140690E: Embryo-fetal development study (oral) in Sprague-Dawley rats.

Study title: PNU-140690E: Embryo-fetal development study (oral) in Sprague-Dawley rats.

Key study findings: TPV was given orally twice daily at doses of 0, 40, 400 or 1000 mg/kg/day to bred rats on gestation days 6 through 17. Increased postdose salivation, increased liver weight, decreased maternal body weight and food consumption and decreased fetal body weight and sternebrae ossification were present in the 400 and 1000 mg/kg/day groups. There was no evidence of drug-related embryo lethality or teratogenicity at any dose. The NOAEL for both maternal and developmental toxicity was 40 mg/kg/day. Mean overall C_{max} at steady state (dose day 12, gestation day 17) for the 40 mg/kg/day group was 30.4 and mean AUC was 340 µM which is 0.2-fold of the expected human exposure at the proposed dose of 500/200 TPV/RTV BID.

Study no.: U00-3254 (Pharmacia & Upjohn Study Report a0035138)

Volume #, and page #: Module 4, M002, vol. 1.32, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI.

Date of study initiation: February 27, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-2363, purity.

Methods

Doses: 0, 40, 400, or 1000 mg/kg/day (dosing occurred twice daily, with half the daily dose administered 8 hours apart).

Species/strain: Rat — .CD(SD)BR Sprague-Dawley

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: Oral (gavage), purified water pH adjusted to 10.5, 20 mg/kg/day.

Satellite groups used for toxicokinetics: 5 females/group

Study design: In the TPV oral dose range-finding study (U00-3190) in pregnant rats at doses of 0, 40, 100, 400, 700 and 1000 mg/kg/day, embryo/fetotoxic effects were not seen at any dose. Maternal effects were minimal, consisting of a dose-related increase in relative liver weight in all TPV-treated groups, clinical observations of postdose salivation in all rats at 400 mg/kg/day and above and decreased body weight gain from gestation days 6 through 9 in the

1000 mg/kg/day group. Toxicities observed in a two-week preliminary TPV oral dose study in rats (U00-3085) and in a four-week TPV oral dose study in rats (U00-3087) were also taken into consideration. Based on the results of these previous studies, dose levels for this study were chosen so that minimal maternal toxicity would be expected at the high dose (1000 mg/kg/day) and a NOAEL would be expected at the low dose (40 mg/kg/day). A middle dose of 400 mg/kg/day was approximately mid-way between the low and high doses.

TPV in purified water (pH 10.5) was given orally twice daily to groups of 24 time-mated female rats at 0, 40, 400 or 1000 mg/kg/day in Segment A of the study. In Segment B of the study, plasma levels of TPV were evaluated in each of the TPV-treated satellite groups. All rats were dosed twice daily beginning on gestation day 6 (day 0 was the day of insemination) and continuing through gestation day 17. Segment B rats were sacrificed on gestation day 18 and a necropsy was performed to determine pregnancy status. Segment A rats were sacrificed and necropsied (cesarean sections performed) on gestation day 20.

Parameters and endpoints evaluated:

Rats were observed for general appearance on gestation day 0 and daily during the treatment and posttreatment periods; maternal body weights and food consumption were recorded. Serial blood samples were taken for toxicokinetic evaluation from each rat in Segment B on the first day (gestation day 6) of dosing and the last day (gestation day 17) of dosing. For each Segment A rat, the abdominal and thoracic viscera were examined, the intact uterus was weighed and the numbers of corpora lutea, live and dead fetuses and early and late resorptions were recorded. Each fetus was sexed, weighed, grossly (externally) examined and sacrificed by lethal injection. Half of the fetuses per litter were examined viscerally and the remaining fetuses were processed and examined for skeletal anomalies.

Results

Mortality (dams): No premature deaths or signs of morbidity occurred during the study.

Clinical signs (dams): The only drug-related clinical sign was postdose salivation in the 400 and 1000 mg/kg/day groups.

Body weight (dams): Body weights in the treated groups decreased. At the end of the dosing period, the percent differences of mean body weights of treated groups versus the control group were -1% (40 mg/kg/day), -5% (400 mg/kg/day) and -10% (1000 mg/kg/day).

Food consumption (dams): Mean group food consumption was statistically significantly decreased in the 400 (-14%) and 1000 (-22%) mg/kg/day groups when compared to control group mean.

Toxicokinetics:

Dose (mg/kg/day)	40	400	1000
Sex: No. of animals	F: 5	F: 5	F: 5
Gestation Day 6			
C _{max} ₀₋₂₄ (µM)	90	418	474
AUC ₀₋₂₄ (µM.h)	1000	6700	8300
Fold versus Human AUC*	1.85	4.35	5.38
Gestation Day 17			
C _{max} ₀₋₂₄ (µM)	30.4	118	157
AUC ₀₋₂₄ (µM.h)	340	1310	1670
Fold versus Human AUC*	0.22	0.85	1.08

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 µM.h.

Terminal and necropsopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

Mean absolute liver weight was statistically significantly increased in the 400 (+ 32%) and 1000 (+ 31%) mg/kg/day groups when compared to mean liver weight for the control group.

Mean pregnant uterus weight in the 1000 mg/kg/day group was statistically significantly less (- 7%) than the control group. This is a reflection of decreased fetal body weight.

No drug-related effects were demonstrated for mean numbers of corpora lutea, implantations, live or dead fetuses, late, early or total resorptions, preimplantation loss or postimplantation loss.

Offspring (malformations, variations, etc.):

Statistically significant differences were seen in mean fetal body weights. The mean for the 40 mg/kg/day group was increased (+ 6%) while the means for the 400 and 1000 mg/kg/day groups were decreased (- 4% and - 11%, respectively) when compared to the mean for the control group. The decreases in the middle and high dose groups were considered drug-related.

Fetal examinations revealed an increase (51 fetuses in 18 litters versus 27 fetuses in 11 litters in the control group) in the incidence of unossified sternebrae in the 1000 mg/kg/day group. Although not statistically significant, the incidence of unossified # 5 and/or #6 sternebrae was also increased in the 400 mg/kg/day group. This effect may be secondary to the decreased fetal body weight in the middle and high dose groups.

Study title: PNU-14069E: A dose range-finding study (oral) in New Zealand White rabbits.
Study no.: U00-3273 (Pharmacia & Upjohn Study Report a0066438)
 Discussed under U00-3140 PNU-140690E: Embryo-fetal development study (oral) in New Zealand White rabbits.

Study title: PNU-140690E: An embryo-fetal study (oral) in New Zealand White rabbits.

Key study findings: TPV was given orally twice daily at doses of 0, 75, 150 or 375 mg/kg/day to timed-mated female New Zealand White rabbits. Treatment was for 15 consecutive days during the major period of organogenesis (gestation days 6 through 20). Group mean C_{max} and AUC were dose-related but not linear on gestation day 6. On gestation day 15, group mean C_{max} and AUC were dose-related for the low and middle doses but were unexpectedly low for the high dose. This may be due to extensive hepatic microsomal enzyme induction which has been documented in several species. Maternal toxicity (drug-related death of one female, abortions, body weight and food consumption decreases and increased clinical signs) and developmental toxicity (slightly decreased fetal body weights, fetuses with wavy ribs and bent femurs and increased incidence of fetuses with gross malformations) occurred at 375 mg/kg/day. Maternal toxicity (abortions) occurred at 150 mg/kg/day. No developmental toxicity was observed at 150 mg/kg/day. Interpretation of the fetal findings at 375 mg/kg/day was complicated by the occurrence of maternal toxicity. A single litter was responsible for 75% of the fetuses with a gross malformation, 80% of the fetuses with a visceral malformation and 50% of the fetuses with a skeletal malformation (including bent femur) and this may have been a litter effect. In addition, similar gross malformations were not observed in fetuses at 375 and 750 mg/kg/day TPV in the dose range-finding study in rabbits. It is unlikely that TPV was teratogenic at 375 mg/kg/day considering the lack of characteristics typical of known developmental toxicants and the maternal toxicity observed at this dose level. The NOAEL for maternal toxicity was 75 mg/kg/day while the NOAEL for developmental toxicity was 150 mg/kg/day. The AUCs associated with these NOAEL doses correspond to 0.04-fold and 0.08-fold, respectively, the human exposure at the proposed clinical dose of 500/200 TPV/RTV BID.

Study no.: U00-3140 (Pharmacia & Upjohn Study Report a0072118)

Volume #, and page #: Module 4, M002, vol. 1.32, page 1

Conducting laboratory and location: Pharmacia & Upjohn, Worldwide Toxicology, Kalamazoo, MI

Date of study initiation: March 27, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A)5075-AS-2363, 100% purity.

Methods

Doses: 75, 150 and 375 mg/kg/day, half doses administered twice daily 8 hours apart.

Species/strain: Rabbit/New Zealand White

Number/sex/group: 20 females/group

Route, formulation, volume, and infusion rate: Oral (gastric intubation), aqueous solution in 0.25N sodium hydroxide, pH 10.5, 10 ml/kg/day.

Satellite groups used for toxicokinetics: 5 females/group

Study design: In the oral dose range finding study (U00-3273) in pregnant New Zealand White rabbits at doses of 0, 75, 150, 375 and 750 mg/kg/day TPV, mean body weight loss in the 375 and 750 mg/kg/day dose groups was indicative of maternal toxicity. Clinical signs in these groups included failure to eat and soft, scant feces. Mean relative liver weights were slightly increased at doses of 150 mg/kg/day and greater. The abortion of two does in the 750 mg/kg/day group was considered drug-related. Developmental toxicity was demonstrated by increased postimplantation loss at 750 mg/kg/day and decreased mean fetal body weight at 375 and 750 mg/kg/day. Therefore, the doses chosen for the definitive study were 75, 150 and 375 mg/kg/day TPV. The intention was to demonstrate a NOAEL at the low dose and to produce minimal maternal toxicity at the high dose. The middle dose of 150 mg/kg/day was previously investigated and is twice the low dose and approximately half the high dose.

In this study, TPV was given orally twice daily at doses of 0, 75, 150 or 375 mg/kg/day to timed-mated female New Zealand White rabbits. Treatment was for 15 consecutive days during the major period of organogenesis (gestation days 6 through 20). Clinical observations, body weights and food consumption were recorded during the study. Rabbits were killed on gestation day 29 for cesarean section examination. Gross necropsies were performed on each animal at this time and fetuses were examined.

Parameters and endpoints evaluated: Clinical observations, body weights and food consumption were evaluated. Gross necropsies were performed on each dam and the condition of reproductive organs was evaluated. Data collected on gestation day 29 included: liver weights, gravid uterus weights and numbers of corpora lutea, live and dead fetuses and early and late resorptions. Fetuses were weighed, sexed and examined for gross, visceral and skeletal malformations and variations. In addition, blood samples were collected on the first and last days of dosing from satellite animals for toxicokinetic assay of TPV in plasma.

Results

Mortality (dams): In the main study group, there were two unscheduled mortalities. One was considered drug-related, i.e. doe 73 in the 375 mg/kg/day group was sacrificed moribund on gestation day 21. The other mortality (doe 40, 75 mg/kg/day group) died from a dosing injury on gestation day 12. In addition, five does were sacrificed early as a consequence of abortion. Control doe 17 aborted on gestation day 23. One 150 mg/kg/day doe (57) aborted on gestation day 18. Three 375 mg/kg/day does aborted: number 61 on gestation day 28 and does 65 and 79 on gestation day 24. One 150 mg/kg/day doe (51) aborted on the day of scheduled cesarean section (gestation day 29).

There were two (does 81 and 82, 75 mg/kg/day groups) unscheduled mortalities in the toxicokinetic group, both due to dosing injury. One doe (93) in the 375 mg/kg/day group aborted on the day prior to and the of scheduled sacrifice.

Clinical signs (dams): A drug-related increase in the incidence and duration of hair loss was observed in the 375 mg/kg/day group. The duration but not the incidence of scant feces was increased in the 375 mg/kg/day group. The absence of feces was noted in two does and six does in the 150 and 375 mg/kg/day groups, respectively. Observations included red fluid on the cage paper for one day from each of the two does in the 150 mg/kg/day group that aborted and moribundity in doe 73 (sacrificed on gestation day 21) in the 375 mg/kg/day group. Fecal and moribund findings in the 375 mg/kg/day group were attributed to drug treatment.

Body weight (dams): At the end of the dosing period, the group mean for the 375 mg/kg/day group was reduced by 6% when compared to the control group mean body weight. There was no change in the low and middle dose mean group body weights.

Food consumption (dams): At the end of the dosing period, the group means for food consumption were reduced by 10%, 12% and 52% in the low, middle and high dose groups, respectively. The reduction in the high dose group was statistically significant.

Toxicokinetics:

Dose (mg/kg/day)	75	150	375
Sex: No. of animals	F: 5	F: 5	F: 5
Gestation Day 6			
C _{max} ₀₋₂₄ (µM)	1.94	6.9	43
AUC ₀₋₂₄ (µM.h)	9.1	32.9	330
Fold versus Human AUC*	0.006	0.02	0.21
Gestation Day 15			
C _{max} ₀₋₂₄ (µM)	4.9	8.4	5.1
AUC ₀₋₂₄ (µM.h)	66	120	82
Fold versus Human AUC*	0.04	0.08	0.05

*Based on 500/200 mg TPV/RTV BID and an AUC of 1542 µM.h.

Group mean C_{max} and AUC were dose-related but not linear on gestation day 6. On gestation day 15, group mean C_{max} and AUC were dose-related for the low and middle doses but were unexpectedly low for the high dose. This may be due to extensive hepatic microsomal enzyme induction which has been documented in several species.

Terminal and necropsopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

There were no drug-related effects on pregnant uterus weights in the 75 and 150 mg/kg/day groups. A 10% reduction in the mean weight of the pregnant uterus was observed at the 375 mg/kg/day dose level but this reduction was not statistically significant.

Group mean liver weight was statistically significantly increased (+14%) in the 375 mg/kg/day group when compared to control group mean liver weight. There were slight (+1%) increases in group mean liver weights in the low and middle dose groups.

There were no drug-related alterations to any of the following parameters: numbers of corpora lutea, implantations, live fetuses, dead fetuses, early resorptions, postimplantation loss or females with no viable progeny. There were no drug-induced changes in percentages of pre- or postimplantation loss.

A slight (- 8%) reduction in the weighted mean body weight of fetuses from does treated with 375 mg/kg/day was evident when compared with controls.

Two abortions at 150 mg/kg/day and four abortions at 375 mg/kg/day, as well as evidence of fetal resorptions, and moribundity in an additional 375 mg/kg/day group female were attributed to treatment. Therefore, it is concluded that TPV at high doses can cause abortions and fetal resorptions in rabbits.

Gross necropsy findings were not attributed to drug treatment. However, doe number 31 (75 mg/kg/day dose group) had a red thymus which correlated with microscopic evidence of thymic hemorrhage. Doe number 61 (375 mg/kg/day dose group), sacrificed on gestation day 28 due to abortion, had diffuse hepatocellular vacuolation, consistent with lipid infiltration. Doe number 73 (375 mg/kg/day dose group), sacrificed moribund on gestation day 21, had microscopic lesions in the adrenals, spleen kidney and liver. Zona glomerulosa cells of the adrenal glands were hyperplastic and hypertrophied. There were multifocal sites of acute hepatocellular necrosis, diffuse hepatocellular hypertrophy and multifocal sites of deposition of a basophilic staining material. Doe number 73 also had renal proximal tubular vacuolation and lymphocytic splenic atrophy. These findings in doe number 73 are consistent with multiple organ toxicity or failure.

Offspring (malformations, variations, etc.):

There were no statistically significant, drug-related effects on visceral, skeletal or total malformations. There was statistically significant increased dose response for the number of fetuses with gross malformations (4 malformations = 3.4%) in the 375 mg/kg/day group. These included dome shaped head (2 fetuses in 1 litter, doe 78), carpal flexures (3 fetuses in 1 litter, doe 78), arthrogryposis (1 fetus in 1 litter, doe 78) and omphalocele (1 fetus in 1 litter, doe 76). Conversely, in the dose range-finding study (U00-3273) no gross malformations were observed among does treated with 375 and 750 mg/kg/day involving 61 fetuses from 7 litters and 46 fetuses from 6 litters, respectively.

One or more of four visceral malformations were observed among five fetuses within two litters exposed to 375 mg/kg/day. None of these were observed among fetuses of does treated with vehicle or lower TPV doses. The visceral malformations observed were: internal hydrocephaly,

unilateral (left) microphthalmia, bulbous aortic arch and retroesophageal aortic arch. Other malformations involving the aortic arch or subclavian were observed among control fetuses. All findings reported in the study fall within reported historical ranges.

Several skeletal malformations were observed among control and drug-treated fetuses and all appeared random with the exception of bent femur. Bilateral bent femurs were observed in five fetuses from the 375 mg/kg/day group doe number 78 and in two from doe 76. This finding was not statistically significant but bilateral bent femurs are rare events. Interpretation of this finding is unclear but it was also associated with wavy ribs (skeletal variation). Bent long bones when associated with wavy ribs are known to be reversible.

There were no drug-related effects on gross or visceral variations, whether analyzed by fetal or litter incidence. The number of fetuses, although not litters, exhibiting skeletal variations was increased in all treated groups relative to the incidence among control fetuses (86 of 154 fetuses, 56%). Among fetuses exposed to 75 mg/kg/day, 150 mg/kg/day and 375 mg/kg/day, 142 of 178 fetuses (80%), 106 of 156 fetuses (68%) and 92 of 117 fetuses (79%), respectively, were affected. The total numbers of skeletal variations among the drug-treated groups are increased, but not in a dose-related manner. Interpretation of these data is unclear and it may represent a ceiling effect.

Similarly, increases in the numbers of fetuses with skeletal variations contributed to the observed increase in fetuses with one or more variations. Among vehicle-exposed does, 93/154 (60%) fetuses were reported with one or more variations. In the drug-exposed groups, significant differences were found in the 75 mg/kg/day and 375 mg/kg/day groups, i.e. 146/178 (82%) and 97/117 (83%) fetuses with one or more variations, respectively. Again, the increases do not appear to be dose-related and may represent a ceiling effect.

Prenatal and postnatal development

Study title: PNU-140690E: Oral pre and postnatal developmental toxicity study in the rat.

Key study findings: TPV was toxic to dams and suckling pups at 400 and 1000 mg/kg/day with dose-relationship. Maternal toxicity was restricted to adverse effects on body weight and food consumption. Pup toxicity consisted of slight (400 mg/kg/day) or marked (1000 mg/kg/day) progressive growth inhibition throughout lactation, resulting in persistent adverse influence on the growth of the pups up to maturity. However, none of the postweaning functions examined in F1 offspring, including reproductive ability, were compromised up to the 1000 mg/kg/day dose. The 40 mg/kg/day dose was an NOAEL for both the dams and offspring.

The effects of TPV on postnatal development of offspring might be a consequence of toxic milk supply to the sucklings, since drug-related material passes into the milk or decreased milk

production, rather than a reflection of impaired development in utero. The absence of drug-related malformations in offspring also supports this assumption and demonstrates lack of teratogenicity of TPV under the conditions of this study.

Study no.: U00-3204 (Pharmacia & Upjohn Study Report 9850091)

Volume #, and page #: Module 4, M002, Vol. 1.33, page 1

Conducting laboratory and location: Pharmacia & Upjohn, Viale Pasteur, 10, 200014 Nerviano (MI), Italy

Date of study initiation: July, 1997

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: TPV, Lot No. (A1)5134-AS-1462, 99.9% purity

Methods

Doses: 0, 40, 400 and 1000 mg/kg/day, half the dose administered twice daily 8 hours apart.

Species/strain: Rat \bar{m} CD(SD)BR Sprague-Dawley

Number/sex/group: 24 females/group

Route, formulation, volume, and infusion rate: Oral (gavage), aqueous solution pH 10.5, 20 mg/kg/day

Satellite groups used for toxicokinetics: Not performed.

Study design: Female rats were mated with untreated males of the same strain and the day on which spermatozoa were found in the vaginal smear was considered Day 0 of pregnancy. Twenty-four bred female rats for each test group were treated with TPV at doses of 0, 40, 400 or 1000 mg/kg/day during the prenatal and postnatal period, from Day 6 of pregnancy to Day 21 postpartum. All bred females were allowed to litter and rear their pups to weaning. Pups were culled to eight per litter (4/sex) and these pups were nursed by their dams for 21 days after delivery. After weaning, selected F1 pups (at least one male and one female pup per dam) were observed up to adult age and were tested for their reproductive ability at sexual maturity.

Parameters and endpoints evaluated: Dams were observed for survival, general condition, course of pregnancy and parturition, lactating condition and care and aptitude in rearing pups and their body weights and food consumption were recorded. Post-mortem examination was carried out on all animals treated. Any tissue showing gross alterations was stored in formalin for possible histological examination. The number of implantation sites was recorded. Uteri of non-pregnant rats were stained to detect the presence or absence of implantation sites.

Pups were observed for survival, growth and development, including behavior. All F1 females from the reproduction test were subjected to cesarean section at mid-pregnancy and the uterine content was examined and recorded. Male F1 rats were killed on completion of the Day 13 to 15 sacrifice of F1 parent females and examined for abnormalities and gross lesions. For males which failed to inseminate females or to mate, testes, epididymides and seminal vesicles were preserved for possible histological examination.

Results

F₀ in-life: No animals died during the study. No drug-related symptoms were observed during pregnancy and lactation in any treatment groups.

Mean group body weights when compared to controls were reduced at the end of the gestation period in the 1000 mg/kg/day group by 4%. Mean group body weights when compared to controls were increased at the end of the lactation period by 6% in the 400 mg/kg/day group and by 3% in the 1000 mg/kg/day group.

Mean group food consumption when compared to controls at the end of the gestation period was unchanged in the high dose group but was increased 3% and 6 % in the 40 and 400 mg/kg/day groups, respectively. Mean group food consumption when compared to controls at the end of the lactation period was slightly (1%) increased in the 40 mg/kg/day group and decreased slightly (1%) in the 400 mg/kg/day group and significantly (10%) in the 1000 mg/kg/day group.

The mean duration of pregnancy was not affected by TPV treatment and none of the treated rats delivered outside the time range for the strain used. The doses tested showed no adverse effects on labor and delivery. The delivery index and pregnancy index were 100% in all groups, since all rats delivered live pups and had live newborns at completion of delivery.

A slight reduction in total litter size at birth occurred at 1000 mg/kg/day (14.5 on average vs. 15.1 for controls), but the group mean value reflected the small litter size of one dam. If that animal was excluded then the mean value of pups born at 1000 mg/kg/day was close to the control value (14.9 vs. 15.1).

The incidence of dams with stillborn pups was higher in treated groups than in the control group (5, 5 and 4 at 40, 400 and 1000 mg/kg/day, respectively, vs. 1 at 0 mg/kg/day). The corresponding number of stillborn pups was 7, 8 and 6 at 40, 400 and 1000 mg/kg/day, respectively, vs. 1 at 0 mg/kg/day. There was no dose-related increase in incidence of dams with stillborn pups or in numbers of stillborn pups. No other pups died at birth at any dose. The proportion of pups dying or cannibalized soon after birth, as a reflection of possible treatment-related maternal disturbances during parturition, was not increased in treatment groups in comparison with the control group.

No changes in maternal behavior were observed during pregnancy at any dose level. Maternal behavior was not affected during the parturition phase. There were no signs of inaptitude in rearing and nursing litters or tendency to cannibalism at any dose during the lactation period. Dams showed no neglect of their litters at any dose.

F₀ necropsy: No TPV-related changes were observed at any dose during post-mortem examination at the end of the lactation period.

F₁ physical development: At birth, mean values of pups born in treated groups were comparable to those of the control group and there was no increase in neonatal losses in any dose group. During lactation, pup survival was not changed at any dose.

At birth, mean pup body weight was minimally reduced, compared to controls, at the highest dose and was similar to controls at the lowest and intermediate doses. Throughout lactation, mean pup body weight showed dose-related reductions at 400 and 1000 mg/kg/day, but was unaffected at 40 mg/kg/day. At the end of the postweaning period, group mean body weights were reduced in the low, middle and high dose groups by 1%, 7% and 22%, respectively.

No differences between treated pups and controls were noted for the mean age for balanopreputial cleavage in F₁ selected male rats and for vaginal opening in F₁ selected female rats.

Post-mortem examinations of pups revealed no drug-related findings.

F₁ behavioral evaluation: Some physical and functional landmarks of development were retarded in pups in the 400 and 1000 mg/kg/day dose groups. A clear indication of developmental retardation occurred for the air righting reflex. The pups in the 1000 mg/kg/day group developed this landmark later than pups in the control group. This sign indicated a relationship with the lower body weight of pups in treated groups and did not reflect any prenatal damage. Pups in the 400 mg/kg/day group, which weighed less than control pups during the third week of lactation, developed this landmark slightly later also. In addition, at 1000 mg/kg/day, minimal or slight differences compared to controls in attaining other landmarks of development (pinna unfolding, eye opening, surface righting and startle reflex) were observed. Pre-weaning development of pups at 40 mg/kg/day showed no harmful effects of maternal treatment with TPV.

Learning and remembering capacity was not impaired by maternal TPV treatment. There were no changes in behavior of F₁ offspring of either sex in terms of emotional status and exploratory activity in any TPV-treated group.

F₁ reproduction: mating behavior and fertility of F₁ offspring were not impaired at any TPV dose. Values for the copulation index and fertility index were comparable to those of controls in all TPV treated groups.

F₂ findings: Group mean values for litter parameters showed no drug-related changes. There were no increases in embryonic death or decreases in live embryos, compared to controls, in any dose group.

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

Study title: U-140690: Industrial Toxicology evaluation – eye irritation study in male New Zealand White rabbits.

Key study findings: TPV powder at the doses tested was minimally irritating to the unrinsed eye of a rabbit. However, rinsing the exposed eye practically alleviated all irritation. In addition, the compound appeared to cause slight cumulative irritation after multiple instillations. There is little likelihood that eye irritation will occur from gross exposure of the eyes to amounts of the powder which may be encountered during clinical use, manufacturing and industrial and transportational handling.

Study no.: U00-3096 (Pharmacia & Upjohn Technical Report 7228-96-116)

Volume # and page #: Module 4, M002, vol. 1.34, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI

Date of study initiation: July 15, 1996

GLP compliance: No

QA reports: yes () no (x)

Drug, lot #, and % purity: TPV, Lot no. 5075-AS-1669, purity not provided.

Formulation/vehicle: Dry powder.

Methods

Doses: 20 mg/eye/day for five consecutive days or 100 mg/eye for one day.

Study design: Twenty-four hours prior to TPV administration, the eyes of at least two rabbits were examined using the fluorescein dye staining procedure to insure that rabbits used in the test had normal corneas and conjunctivas without pre-existing injury. In the testing procedure, each eye of a rabbit was instilled with the designated dose of the dry powder containing TPV. Thirty seconds after the instillation, the powder in the right eye of each rabbit was thoroughly rinsed out with sterile water while the left eye remained unrinsed. The irritancy was scored according to the Draize eye irritation test. The irritant properties of the test material are classified by the Kay and Colandra interpretation of eye irritation properties.

Results: TPV powder at the doses tested was minimally irritating (scores of 2 out of a total possible score of 110 for one instillation of 20 mg TPV powder to 14/110 for repeat dosing for 5 days of 20 mg TPV and 9/110 at 24 hours after a single dose of 100 mg TPV powder) to the unrinsed eye of a rabbit. However, rinsing the exposed eye practically alleviated all irritation. In addition, the compound appeared to cause cumulative irritation after multiple instillations. There is little likelihood that eye irritation will occur from gross exposure of the eyes to amounts of the powder which may be encountered during clinical use, manufacturing and industrial and transportational handling.

Study title: U-140690: Industrial Toxicology evaluation – dermal irritation study in male New Zealand White rabbits.

Key study findings: Based on the PII value of 0.0 for intact skin exposed to TPV paste, TPV is not categorized as a primary dermal irritant to intact skin. However, it is mildly irritating (PII<2) to abraded skin with open wounds. For humans, it is unlikely that skin irritation will occur from a single contact with the powder. Reasonable care should be taken during clinical use, manufacturing and industrial and transportational handling.

Study no.: U00-3095 (Pharmacia & Upjohn Technical Report 7228-96-117)

Volume # and page #: Module 4, M002, vol. 1.34, page 1.

Conducting laboratory and location: Pharmacia & Upjohn, Inc., Worldwide Toxicology, Kalamazoo, MI.

Date of study initiation: July 15, 1996

GLP compliance: No

QA reports: yes () no (x)

Drug, lot #, and % purity: TPV, Lot no. 5075-AS-1669, purity not provided.

Formulation/vehicle: TPV powder ground into a fine powder with mortar and pestle and moistened with water to form a paste.

Methods

Doses: 100 mg/site/day for 5 days and 500 mg/site/day for 1 day.

Study design: Three to four days prior to TPV application, two patches of hair were clipped from the backs of two male rabbits using electric clippers. Immediately preceding TPV application, the skin site on the left side of each rabbit was abraded with a hypodermic needle to produce scratch marks in a one square inch marked area. The scratches were deep enough to penetrate the stratum corneum but not enough to cause bleeding or injury to the dermis. The skin on the right side was also marked with a one square inch area but was left intact. In the testing procedure, the TPV paste was spread on each skin site. The test site was covered and the site was kept in semi-occlusive contact with the TPV paste for 24 hours. At the end of the contact period, the patches were removed and the application sites were wiped with a paper towel to remove residual compound. The skin sites were observed for irritation at selected time points.

The irritancy was classified in terms of a PII calculated from the erythema and edema scores when the response was maximal. A PII of <2 = mildly irritating; 2 to 5 = moderately irritating and >5 = severely irritating.

Results: There were no signs of irritation in the intact skin while mild irritation was observed along the open scratch marks of the abraded skin.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The nonclinical studies demonstrate the safety of TPV. The toxicities observed in animal studies are reversible, manageable, species specific and/or considered secondary to species-specific hepatic enzyme-including effects of TPV in the rodent. The nonclinical studies support the clinical monitoring of liver and GI function.

Unresolved toxicology issues (if any): Carcinogenicity studies in mice and rats are ongoing and will be completed as a post-marketing commitment.

Recommendations: None.

Suggested labeling:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long term animal carcinogenicity bioassays with tipranavir and tipranavir/ritonavir are currently in progress. However, tipranavir showed no evidence of mutagenicity or clastogenicity in a battery of five *in vitro* and *in vivo* tests including the Ames bacterial reverse mutation assay using *S. typhimurium* and *E. coli*, unscheduled DNA synthesis in rat hepatocytes, induction of gene mutation in Chinese hamster ovary cells, a chromosome aberration assay in human peripheral lymphocytes, and a micronucleus assay in mice.

Tipranavir had no effect on fertility or early embryonic development in rats at dose levels up to 1000 mg/kg/day, equivalent to a C_{max} of 258 μM in females. Based on C_{max} levels in these rats, as well as an exposure (AUC) of 1670 $\mu\text{M}\cdot\text{h}$ in pregnant rats from another study, this exposure was approximately $\left[\quad \right]$ the anticipated exposure in humans at the recommended dose level of 500 mg/ 200 mg tipranavir/ritonavir bid.

PREGNANCY

TERATOGENIC EFFECTS

PREGNANCY CATEGORY C

Investigation of fertility and early embryonic development with tipranavir disodium was performed in rats, teratogenicity studies were performed in rats and rabbits, and pre- and post-natal development were explored in rats.

In pre- and post-development studies in rats, tipranavir showed no adverse effects at 40 mg/kg/day, but caused growth inhibition in pups and maternal toxicity at dose levels of 400 mg/kg/day. No post-weaning functions were affected at any dose level.

There are no adequate and well-controlled studies in pregnant women for the treatment of HIV-1 infection. APTIVUS 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 APTIVUS, an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling (800) 258-4263.

NURSING MOTHERS

The Center for Disease Control and Prevention recommends that HIV-infected mothers not breastfeed their infants to avoid risking postnatal transmission of HIV. Because of both the potential for HIV transmission and any possible adverse effects of tipranavir, mothers should be instructed not to breastfeed if they are receiving APTIVUS.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

(1) IND 51,979 (000)

PHARMACOLOGIST'S REVIEW

IND 51,979 (Original)

Date Submitted: November 13, 1996

Date Assigned: November 21, 1996

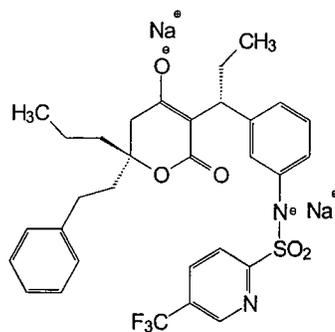
Date Completed: December 10, 1996

HFD-530

SPONSOR: Pharmacia & Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

DRUG: PNU-140690 or U-140690; PNU-140690E or U140690E (the disodium salt of PNU-140690 or U-140690); [R-(R*,R*)]-N-[3-[1-[-[5,6-Dihydro-4-hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H-pyran-3-yl]propyl]phenyl]-5-(trifluoromethyl)-2-pyridinesulfonamide disodium salt.

STRUCTURE:



FORMULATION:
Equivalents (FAE)

100 and 200 mg Free Acid
capsules.

RELATED INDs: None.

INDICATION(S): [

INTRODUCTION: PNU-140690 is a protease inhibitor that binds specifically to the active site of the HIV protease and thereby inhibits protease activity. HIV protease is necessary for the processing of the gag-pol polyprotein and inhibition of the protease yields noninfectious, immature virions. Earlier protease inhibitors, which were peptide derived compounds, had the

undesirable characteristics of low oral bioavailability and rapid excretion. PNU-140690 is a non-peptidic HIV protease inhibitor from the class of 4-hydroxy-5,6-dihydro-2-pyrones. PNU-140690 demonstrated good activity against HIV laboratory viral strains and clinical viral isolates. The objective of the proposed human study described in this submission is to evaluate the safety, tolerance and pharmacokinetics of PNU-140690 after oral administration.

Clinical Protocol: The proposed study is a Phase I, single center, randomized, double-blind, placebo-controlled, escalating single-dose study. Each group will consist of six subjects and within the group subjects will be randomized to drug or placebo. Each subject assigned to receive drug will receive one dose of 100 mg, 300 mg, 500 mg, 700 mg, 900 mg, 1200 mg, 1600 mg or 2000 mg PNU-140690. The primary safety and tolerance variables will be medical events reported. Secondary safety and tolerance variables will be laboratory tests, continuous cardiac monitoring, vital signs, and EKG. Pharmacokinetic variables, AUC, Cmax, Tmax, oral clearance and drug half life, will be determined.

PRECLINICAL PHARMACOLOGY AND TOXICOLOGY REVIEWS:

- 1) **U-109112 and U-140690: Preliminary single-dose oral safety/toxicity and toxicokinetics study in male Sprague-Dawley rats.**
Report No. 7227-96-016; March, 1996.
Test Article Lot No. 28840-HIS-86.

This preliminary study was conducted by Pharmacia & Upjohn, Inc., Kalamazoo, Michigan. The purpose of the study was to determine the acute toxicity and toxicokinetics of U-140690 when administered as a single oral dose to male rats.

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On Original**

Fasted male $\bar{CD}(SD)BR$ Sprague-Dawley rats (3/dose group) received 90, 180 or 360 mg/kg U-140690 in an aqueous solution of 0.1M sodium hydroxide with 0.5% polysorbate 80 orally as a single dose. The rats were observed for 7 days postdose. Daily clinical observations, blood samplings at predose, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 hours post dose for plasma drug concentration determinations, weekly body weights and gross necropsy observations were performed.

U-140690 was well tolerated as a single oral dose at the dose levels administered. All rats survived throughout the 8 day observation period. The only clinical sign observed was soft stool (2 of 3 rats at 180 mg/kg and 1 of 3 rats at 360 mg/kg). All animals gained weight during the study period.

Pharmacokinetic analysis of plasma levels of U-140690 showed that at the 90, 180 and 360 mg/kg doses C_{max} values were 186 ± 68 , 317 ± 67 and 352 ± 4 μM , respectively, and AUC values were 1140 ± 230 , 3200 ± 1600 and 4850 ± 280 $\mu M.h$, respectively. AUC increased in proportion to dose, while C_{max} did not. C_{max} was limited to approximately 350 μM at doses of 180 mg/kg and above.

Comment: The doses of 90, 180 and 360 mg/kg used in this study are equivalent to human doses of approximately 14, 28 and 57 mg/kg or 715, 1430 and 2860 mg/day (based on a weight of 50 kg), respectively. These doses cover the high end of the range of doses (100 mg/day up to 2000 mg/day) to be covered in the proposed human study.

2) U-140690E: One day oral dose preliminary toxicokinetic study in female Sprague-Dawley rats.

Report No. 7270-96-015; October, 1996.

Test Article Lot No. (A)0403-AS-049.

This preliminary study was conducted by Pharmacia & Upjohn, Inc., Kalamazoo, Michigan. The purpose of the study was to determine the maximum tolerated dose and maximum achievable plasma level of U-140690 when administered orally to female rats twice in one day.

Female $\bar{CD}(SD)BR$ Sprague Dawley rats (3/dose group) received 0, 750, 1000 or 1500 mg/kg U-140690E in double distilled water orally by gavage twice in 1 day with 8 hours between doses. The total doses of U-140690E received by the rats were 0, 1500, 2000 or 3000 mg/kg/day. Evaluations included clinical observations, body weights, hematology, serum chemistry and coagulation system tests. Blood samples were taken predose, 2, 4, 6, 8, 10, 12, 14 and 24 hours after the morning dose for determination of U-140690 plasma levels. Gross necropsies were performed on Day 2 and gross lesions were recorded.

One rat from the 1500 mg/kg/day dose group and three from the 3000 mg/kg/day groups died within 24 hours of dosing. Clinical signs included staining around the nose, mouth and anogenital areas and decreased activity in all dose groups. At 2000 and 3000 mg/kg/day soft stool was also noted. In addition, rats in the 3000 mg/kg/day had additional treatment-related

clinical signs including labored breathing, pale in color, prone position and moribund appearance. All rats surviving to the second day lost weight. There were no treatment-related changes in hematology or serum chemistry. Coagulation systems showed a slight prolongation of both the activated partial thromboplastin and the prothrombin times. The gross necropsy observations showed stomachs distended with ingesta in all rats. The small and large intestines of the 3 rats treated at the highest dose were pale and yellow in color. The rat from the 1500 mg/kg/day group found dead had a large intestine that was pale. Animals which survived to terminal necropsy also had pale livers with rounded edges suggesting enlargement.

Toxicokinetic studies showed that maximum plasma concentrations were reached by 2 hours postdose. Drug exposure was similar for the 1500 and 2000 mg/kg/day doses with mean overall C_{max} of 580±40 µM and 500±85 µM, respectively, and AUC of 8370±2260 µM and 8310±1370 µM, respectively. C_{max} for the 3000 mg/kg/day dose was 760±56 µM but AUC could not be calculated because animals did not survive beyond 8 to 10 hours.

The sponsor concluded that a doses of 1500 mg/kg/day or higher in female Sprague-Dawley rats would not be suitable for use in multiple day dosing studies of toxicity.

Comment: The 1500 mg/kg/day rat dose is equivalent to a human dose of 11,900 mg/day and is approximately 6 times higher than the highest human dose proposed for the initial human trial.

3) U-140690E: A series of preliminary one-day oral formulation/safety/toxicity and toxicokinetic study segments in male Beagle dogs.

Report No. 7270-96-013; September, 1996.

Test Article Lot No. (A)0403-AS-049.

This preliminary study was conducted by Pharmacia & Upjohn, Inc., Kalamazoo, Michigan. The purposes of this study were 1) to compare systemic exposure of U-140960E when given in solution by gastric intubation versus U-140960E bulk drug given orally in gelatin capsules and 2) to compare the effect of administration of 300 mg/kg versus 500 mg/kg. The results were to be used to select dose levels and formulation for multi-dose studies in dogs.

Three male Beagle dogs received U-140690E orally as a divided dose with 8 hours between doses on three separate days with a week between dosing days. The three dosing regimens used on the three days were as follows: Part A) 300 mg/kg (150 mg/kg/dose) as an aqueous solution; Part B) 300 mg/kg (150 mg/kg/dose) as bulk drug in capsules; Part C) 500 mg/kg (250 mg/kg/dose) as an aqueous solution. The dogs were evaluated for clinical signs and body weight. Blood samples were taken predose, 1, 2, 4, 6, 8, 9, 10, 12, 16 and 24 hours after the morning dose for determination of blood plasma levels of U-140690E.

U-140690E was well tolerated at the 300 mg/kg/day dose when given as a divided dose in aqueous solution or in capsules. Clinical signs were similar in both 300 mg/kg/day dose groups and consisted of soft stool, emesis and diarrhea. These signs were more intense in the 500 mg/kg/day dose and salivation, inactivity and drinking postdose were also noted in this group.

The toxicokinetic study showed that a concentration maxima was reached approximately 4 hours after dosing. The C_{max} for the 300 mg/kg/day dose in solution was 180±30 µM, while that for the drug in capsule form was 170±70 µM. The purpose. AUCs for the 300 mg/kg/day solution and capsule form doses were 2470±650 µM.h and 1810±640 µM.h, respectively. The AUC(0,24) of the solution formulation was 27% greater than that of the solid salt capsule formulation. The 500 mg/kg/day dose was therefore delivered in solution. This dose yielded a C_{max} of 210±30 µM and an AUC of 2550±550 µM.h, which was very similar to that yielded by the 300 mg/kg/day dose.

Comment: The doses tested in this study, 300 mg/kg/day and 500 mg/kg/day are equivalent to human doses of 8100 mg/day and 13500 mg/day. These doses are approximately 4 and 7 times greater than the highest human dose (2000 mg/day) to be used in the initial trial.

4) U-140690: Two-week preliminary oral safety/toxicity and toxicokinetics study in male and female Sprague-Dawley.

Report No. 1470-96-015; September, 1996.

Test Article Lot No. (A)0399-KEW-077-J380.

This preliminary study was performed by Pharmacia & Upjohn, Inc., Worldwide Toxicology, Tsukuba, Japan. The purpose of the study was to evaluate the safety/toxicity of U-140690 in male and female rats.

Male and female — CD(SD) Sprague-Dawley rats (3/gender/dose) received 0, 64, 126, 250, 500 or 1000 mg/kg/day U-140690 in a 0.3M sodium hydroxide aqueous solution (pH 10) by gastric intubation for Substudy A to determine toxicity of U-140690. Male and female rats (4/gender/dose) received 126, 250, 500 or 1000 mg/kg/day U-140690 for Substudy B to evaluate toxicokinetics of U-140690. For both substudies, the daily doses were divided into two equal doses given approximately 8 hours apart.

The following parameters were evaluated: 1) daily clinical observations; 2) body weights; 3) aspartate aminotransferase and alanine aminotransferase levels; 4) terminal hematology; 5) coagulation and serum chemistry measurements; 6) gross necropsy observations; 7) liver weights; and 8) histopathologic observations. Blood samples were taken for determination of plasma drug levels.

There were no deaths or moribund animals during the study, indicating that U-140690 at doses of 64 to 1000 mg/kg/day administered orally was well tolerated. Salivation was observed in all animals receiving 250 mg/kg/day or more U-140690. Body weight gain was suppressed in males administered 126, 500 and 1000 mg/kg/day.

Effects noted in coagulation indices and serum chemistry liver function parameters were considered to be related to effects of U-140690 on the liver. Changes in coagulation indices were considered toxicologically relevant but not adverse. Changes in electrolytes were also noted but were thought to be secondary due to stress or effects on the endocrine system.

Electrolyte changes and serum chemistry liver function effects were not large enough to be biologically relevant.

Liver weights increased in all dose groups and dose-related periacinar hepatocellular hypertrophy was seen in females at 126 mg/kg/day or greater and in males at 250 mg/kg/day or greater. Minimal to mild follicular cell hypertrophy of the thyroids was also noted at these doses.

CONCLUSION: There are no preclinical safety issues to be conveyed to the sponsors at this time. It is safe to proceed with this study.

C. Anita H. Bigger, Ph.D.
Pharmacologist

Concurrences:

HFD-530/GChikami
HFD-530/JFarrelly
HFD-530/ABigger

Disk:

HFD-530/JFarrelly

cc:

HFD-530 Original IND
HFD-530 Division File
HFD-340
HFD-530/Supervisory Medical Officer
HFD-530/Medical Officer
HFD-530/Microbiology
HFD-530/Chemistry
HFD-530/ABigger
HFD-530/DStaten

(2) IND 51,979 (048, SX)

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals**

Application (IND/NDA) number: 51,979
Division: HFD-530
CAS#:

Drug name: PNU-140690; Tipranavir
 Pharmacological Classification: HIV protease inhibitor
 Sponsor/Applicant: Pharmacia & Upjohn
 Sponsor/Applicant contact name: Leslie Franks, Regulatory Manager
 Sponsor/Applicant telephone and fax number: Phone 616-833-2136; FAX 616-833-8237
 Date submitted (stamp date): April 7, 1999
 45-day date (from submission stamp date): May 22, 1999
 P/T Reviewer(s): Anita Bigger (Reviewer), James Farrelly (Team Leader)
 Date Review Completed: May 5, 1999
 Date of CAC review: May 11, 1999
 CAC members: Joseph DeGeorge, Joseph Contrera and Paul Andrews (Kenneth Hastings)

Summary of Proposal for Review:

Species/strain: Rat, Sprague-Dawley — CD(SD)IGS BR]
 Number/sex/dose: 65/sex/group (main study) and 15/sex/group (toxicokinetics)
 Route: oral gavage (twice a day dosing at least 8 hours apart)

	<u>male</u>	<u>female</u>
Doses proposed: mg/kg/day	30, 100, 300	30, 100, 300
Basis of dose selection:		
MTD	X	X
AUC		
ratio		
saturation		
MFD		
PD		
other		
Kinetics submitted:	<u>rodent</u>	<u>human</u>
pharmacokinetics	X	X
metabolism	X	X
protein binding	X	X

Notable design features: Based on the results of a 26-week study, the proposed high dose of 300 mg/kg/day will result in a 12% to 14% and an estimated 8% to 10% decrease in body weight gain relative to controls in male and female rats, respectively. Additional toxicity endpoints include target organ toxicity in the erythron, thyroid and liver of rats given PNU-140690 at doses of 125 or 400 mg/kg/day. The sponsor proposes to use diet restriction in the carcinogenicity study. However, the 26-week study upon which dose selection is based was conducted with no diet restriction.

Summary of Recommendations to CAC

The protocol design and dose selection are appropriate except for the use of diet restriction. The reviewer concluded that, if diet restriction is used, the sponsor should conduct a second dose range finding study with diet restriction. An alternative would be to conduct the carcinogenicity study with the proposed doses but without diet restriction.

	<u>male</u>	<u>female</u>
Doses recommended by reviewer:	30, 100, 300	30, 100, 300

Basis for recommendation (details): The high dose of 300 mg/kg/day is based on a decrease in body weight gain exceeding the ICH recommendation of no more than 10% decrease and target organ toxicity. Based on the 26-week study, the low dose of 30 mg/kg/day is expected to result in minimal toxicity and gives systemic exposure for rats which is approximately equivalent to the lower range of expected human systemic exposure at therapeutic doses. The mid dose of 100 mg/kg/day is a logarithmic interval between the low and high doses.

Questions for the CAC:

Does the CAC agree that either the sponsor should use an unrestricted diet for the carcinogenicity study or conduct a second dose range finding study with diet restriction?

Executive CAC May 11, 1999

Committee: Joseph DeGeorge, Ph.D., HFD-24, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Paul Andrews, Ph.D., HFD-150, Alternate Member
James Farrelly, Ph.D., HFD-530, Team Leader
Anita Bigger, Ph.D., HFD-530, Presenting Reviewer

Author of Draft: Anita Bigger

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND 51, 979

Drug Name: PNU-140690; Tipranavir

Sponsor: Pharmacia & Upjohn

Rat Carcinogenicity Study and Dose Selection: 30, 100 and 300 mg/kg/day

Background and Discussion

The sponsor proposes to evaluate the carcinogenic potential of PNU-140690 after oral administration to rats twice daily (at least 8 hours apart) of 0, 15, 50 or 150 mg/kg for at least 104 weeks. Toxicokinetic data will be obtained from satellite rats similarly dosed.

Dose selection was based on the results of a 26-week toxicity study in which rats received oral administration twice daily of 0, 10, 20, 62.5 or 200 mg/kg. The high dose of 300 mg/kg/day proposed for the carcinogenicity study is based on an estimated decrease in body weight gain of 12% to 14% in males and 8% to 10% in females, as well as on target organ toxicity. The decrease in body weight gain projected is consistent with the ICH guideline for definition of an MTD. However, the drug-induced target organ toxicities, particularly hematologic changes, did not seem sufficient to support an MTD. Justification of the high dose is also based on a pharmacodynamic endpoint arising from the observation in the 26-week study of prolonged coagulation parameters but at the high dose in the 26-week study the changes in these parameters were decreasing with time. The low dose of 30 mg/kg/day is expected to result in minimal toxicity and give systemic exposure for rats which is approximately equivalent to the lower range of expected human systemic exposure at therapeutic doses. The mid dose of 100 mg/kg/day is a logarithmic interval between the low and high doses.

The sponsor proposes to use dietary restriction in the carcinogenicity study but did not explain the basis for the extent of dietary restriction chosen (16.8 grams for females and 21 grams for males). However, of greater concern is the fact that the 26-week study, upon which dose selection is based, was conducted with no dietary restriction. Therefore, the 26-week study may not serve as an appropriate dose-range finding study for the proposed carcinogenicity study.

Executive CAC Recommendations and Conclusions:

The Committee did not feel that toxicokinetic data are needed beyond the 6-month data already supplied.

The Committee agreed that an MTD had been reached and that the decrease in body weight gain established the MTD. The Committee also noted for the record that the extent of drug-induced change in the hematologic data was not sufficient to support an MTD.

The Committee concurred with the study only if done without dietary restriction. In order to concur with a study with dietary restriction, the dose-range finding study would have to be conducted with dietary restriction. Alternatively, the sponsor could proceed with the study as described and justify the MTD at the end of the study.

15

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

\Division File, HFD-530
\JFarrelly, HFD-530
\ABigger, HFD-530
\JToerner, HFD-530
\ASeifried, HFD-024

(3) IND 51,979 (062)

PHARMACOLOGIST'S REVIEW

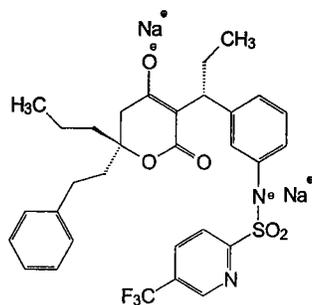
IND 51,979 (062)

Date Submitted: January 21, 2000
Date Assigned: February 2, 2000
Date Completed: April 19, 2001
HFD-530

SPONSOR: Pharmacia & Upjohn Company
7000 Portage Road
Kalamazoo, Michigan 49001

DRUG: PNU-140690; Tipranavir; [R-(R*,R*)]-N-[3-[1-[5,6- Dihydro-4-
hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H- pyran-3-yl]propyl]phenyl]-5-
(trifluoromethyl)-2- pyridinesulfonamide disodium salt.

STRUCTURE:



FORMULATION: 300 mg capsules.

RELATED INDS: None.

INDICATION(S): L

1.

INTRODUCTION: Tipranavir (TPV) is a protease inhibitor that binds specifically to the active site of the HIV protease and thereby inhibits protease activity. HIV protease is necessary for the processing of the gag-pol polyprotein and inhibition of the protease yields noninfectious, immature virions. Earlier protease inhibitors, which were peptide derived compounds, had the undesirable characteristics of low oral bioavailability and rapid excretion. TPV is a non-peptidic HIV protease inhibitor from the class of 4-hydroxy-5,6-dihydro-2-pyrones. TPV demonstrated good activity against HIV laboratory viral strains and clinical viral isolates.

The present submission contains the report on a 2-week oral safety and toxicity study in rats.

REVIEW:

Study Report No.: a0063181; 1/11/2000.

PNU-140690: 2-Week oral safety/toxicity study in Sprague-Dawley rats.

Study No.: 1999-0048; 3/22/1999 - 12/14/1999.

Test Article Lot No.: GLP10586 (Lot C, bulk lot 5435-TLY-9085) and GLP10589 (Lot B, bulk lot 5363-MTM-9801)[purity (%TPV) not specified].

Submission Volume 1, page 108.

This study was conducted for Pharmacia & Upjohn, Kalamazoo, Michigan, by L in compliance with Good Laboratory Practices. The objective of this study was to evaluate the safety and toxicity of two different bulk lots of TPV containing different unqualified impurities based on relative retention times and different amounts of the same impurity as judged by percent area under the peak.

Approximately eight week old male and female — CD VAF/Plus Sprague-Dawley rats L [5/sex/group for toxicity segment (Groups 1-7); 3/sex/group for systemic exposure segment (Groups 8-13)] weighing 214 to 216 grams and 170

to 236 grams, respectively, received 0, 40, 125 or 400 mg TPV/kg/day with twice daily oral dosing via gastric intubation for two weeks. Groups 2-4 and 8-10 received Lot C, while Groups 5-7 and 11-13 received Lot B. The vehicle was 0.25N sodium hydroxide with the pH adjusted to 10.5.

Observations for clinical signs were performed twice daily throughout the study. Body weights were measured three times pretest and weekly during dosing. Blood samples were collected at necropsy and standard hematology and clinical chemistry assays were conducted. Serial blood samples were collected on Day 14 at 2, 8, 10, 14 and 24 hours after the AM dose and analysed for determination of pharmacokinetic parameters.

All rats in the toxicity segment were subjected to complete gross necropsy. The following table shows the tissues chosen for histopathology and organs chosen for weight determination.

Table 1: Histopathology Inventory

Study Dose	0	40	125	400
Organ				
Adrenals	X,*	*	*	X,*
Aorta	X			X
Bone Marrow smear	X			X
Bone(femur)	X			X
Brain	X,*	*	*	X,*
Cecum	X			X
Cervix	X			X
Colon	X			X
Duodenum	X			X
Epididymis	X			X
Esophagus	X			X
Eye	X			X
Fallopian tube				
Gall bladder				
Gross lesions				

Harderian gland				
Heart	X,*	*	*	X,*
Ileum	X			X
Injection site				
Jejunum	X,			X
Kidneys	X,*	X,*	X,*	X,*
Lachrymal gland				
Larynx				
Liver	X,*	X,*	X,*	X,*
Lungs	X			X
Lymph nodes, cervical				
Lymph nodes, mandibular	X			X
Lymph nodes, mesenteric	X			X
Mammary Gland	X			X
Nasal cavity				
Optic nerves	X			X
Ovaries	X			X
Pancreas	X			X
Parathyroid	X,*	*	*	X,*
Peripheral nerve				
Pharynx				
Pituitary	X,*	*	*	X,*
Prostate	X			X
Rectum				
Salivary gland	X			X
Sciatic nerve	X			X
Seminal vesicles				

Skeletal muscle	X			X
Skin	X			X
Spinal cord	X			X
Spleen	X,*	*	*	X,*
Sternum	X			X
Stomach	X			X
Testes	X			X
Thymus	X,*	*	*	X,*
Thyroid	X,*	X,*	X,*	X,*
Tongue				
Trachea	X			X
Urinary bladder	X			X
Uterus	X			X
Vagina	X			X
Zymbal gland				

X, histopathology performed; *, organ weight obtained

RESULTS: There were no deaths during the course of the study. Treatment-related increases in the incidence of brown body surface staining were seen in all high-dose animals and occasionally in mid-dose animals. Increased salivation was noted occasionally in high-dose animals. No treatment-related effects on body weights were seen. High-dose males showed a slight decrease of 4%, while females showed a slight increase of 2%.

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The sponsor noted mild elevations in platelet counts in high-dose males and females and in mid-dose females and stated that these changes were possible effects of TPV. The sponsor noted occasional significant changes in other hematology parameters but did not consider these treatment-related. The percent change in means for these parameters are shown in Table 2 below.

The sponsor found treatment-related increases in TSH in animals in the high-dose groups. Total protein and globulin were slightly higher in females in the high-dose group. A similar trend was noted in males but reached statistical significance in Lot B high-dose animals. The sponsor noted that alanine aminotransferase values were higher and triglyceride values lower in males in the high-dose groups. Other values (Table 2) were also significantly different from controls but the sponsor did not consider these treatment-related.

Table 2: Percent Change in Means for Hematology and Clinical Chemistry Parameters in High-Dose Groups versus Control Groups.

HEMATOLOGY	Lot C		Lot B	
	Males	Females	Males	Females
Prothrombin Time	+28	+5	+29	+8
APTT	+17	+18	+39	+8
Reticulocyte Count	0	+27	+13	+25
Platelet Count	+10	+24	+12	+12
White Blood Cell Count	-34	+11	-5	+8
CLINICAL CHEMISTRY				
Total Protein	+7	+10	+9	+10
Globulin	+7	+17	+11	+17
Alanine Aminotransferase	+60	+14	+23	+3
Aspartate Aminotransferase	-15	-23	-15	-13
Alkaline Phosphatase	-14	-25	-25	-16
Creatine Kinase	-33	-56	-33	-40
Triglycerides	-26	-9	-30	-3
TSH	+196	+306	+77	+275

Treatment-related increases in liver and thyroid/parathyroid weights were observed. In the high-dose groups, increases in absolute liver weights were 34 to 39% in males and 72 to 80% in females. Increases in absolute thyroid/parathyroid weights were 14 to 19% in males and 20 to 53% in females. Increases were dose-related in both sexes for liver and in males for thyroid/parathyroid. Increases in adrenal weights were seen in males (18 to 22%) in the high-dose groups and in females in the mid- (27 to 34%) and high-dose (21 to 27%) groups. These adrenal findings were not confirmed microscopically.

There were no treatment-related gross findings. Treatment-related microscopic effects were found in the liver and thyroid glands. Centrilobular hepatocellular hypertrophy was observed in high-dose animals. Centrilobular hepatocytes had increased amounts of acidophilic cytoplasm. Thyroid follicular cell hypertrophy was seen in all high-dose groups, in mid-dose groups except Lot C females and in Lot B low-dose males. Altered thyroids were characterized by follicles with absent or pale staining colloid and lining epithelium that was more columnar and basophilic than controls.

The results of plasma analyses are shown in Table 3 below.

Table 3: Systemic Exposure on Day 14.

Dose Level (mg/kg/day)	AUC ₀₋₂₄ μM.h	C _{max0-24} μM
Lot C		
40	M:172±49 F:214±118	M:24±8 F:29±11
125	M:270±47 F:505±226	M:32±7 F:71±27
400	M:430±210 F:1310±540	M:49±21 F:146±59
Lot B		
40	M:49±13 F:216±27	M:6±2 F:43±14
125	M:390±59 F:370±270	M:51±13 F:70±52
400	M:570±260 F:1120±380	M:97±44 F:119±21

SUMMARY/CONCLUSIONS: It should be noted that the sponsor plans to use a different formulation (TRIS/SEDDS-2 Vehicle) in future studies. This study will be used for comparison with the bridging study for this new formulation.

Treatment-related toxicity was clearly demonstrated in both sexes administered TPV (Lots C and B) for two weeks at 125 and 400 mg/kg/day. At 40 mg/kg/day, thyroid follicular cell hypertrophy was found in Lot B males. Therefore, no NOEL was established in this study.

The sponsor noted the following treatment-related or possibly treatment-related toxicities observed in the study: 1) brown-body surface staining; 2) increased salivation; 3) increased TSH levels, thyroid/parathyroid weights and thyroid follicular cell hypertrophy; 4) increased liver weights and centrilobular hepatocellular hypertrophy; 5) decreased body weights in males; 6) increased platelet counts, total protein and globulin in both sexes and alanine aminotransferase activity in Lot C males; 7) decreased triglyceride values in males; 8) increased adrenal weights. In addition to these effects, a review of the data revealed other effects seen in one or two groups (males versus female or Lot C versus Lot B) but not considered treatment-related by the sponsor: 1) increased prothrombin time and APTT (more pronounced in males); 2) increased reticulocyte count (more pronounced in females)/ 3) decreased white blood cell count (males Lot C); 4) decreased aspartate aminotransferase levels (more pronounced in females Lot C); 5) decreased alkaline phosphatase and creatinine kinase levels.

There were no overall meaningful differences in toxicity observed following treatment with Lot C or Lot B.

C. Anita H. Bigger, Ph.D.
Pharmacologist

Concurrences:

HFD-530/JFarrelly
HFD-530/ABigger 4-19-01

Disk:

HFD-530/JFarrelly

cc:

HFD-530 Original IND
HFD-530 Division File
HFD-340
HFD-530/JToerner
HFD-530/Microbiology
HFD-530/Chemistry
HFD-530/ABigger

HFD-530/LStephens

(4) IND 51,979 (067)

PHARMACOLOGIST'S REVIEW

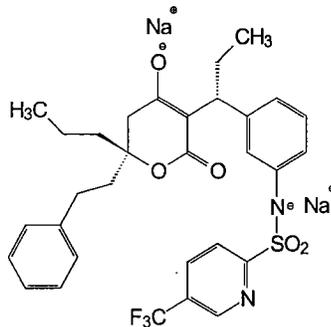
IND 51,979 (067)

Date Submitted: April 19, 2000
 Date Assigned: April 27, 2000
 Date Completed: March 22, 2001
 HFD-530

SPONSOR: Pharmacia & Upjohn Company
 7000 Portage Road
 Kalamazoo, Michigan 49001

DRUG: PNU-140690; Tipranavir; [R-(R*,R*)]-N-[3-[1-[-[5,6- Dihydro-4-
 hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H- pyran-3-yl]propyl]phenyl]-5-
 (trifluoromethyl)-2- pyridinesulfonamide disodium salt.

STRUCTURE:



FORMULATION: TRIS/SEDDS-2 Vehicle (TRIS/Capmul MCM vehicle)

Component	Percent
-----------	---------

Capmul Medium Chain Monoglyceride (MCM)	
Tromethamine (TRIS)	
Total	

RELATED INDS: None.

INDICATION(S): C

I

INTRODUCTION: Tipranavir (TPV) is a protease inhibitor that binds specifically to the active site of the HIV protease and thereby inhibits protease activity. HIV protease is necessary for the processing of the gag-pol polyprotein and inhibition of the protease yields noninfectious, immature virions. Earlier protease inhibitors, which were peptide derived compounds, had the undesirable characteristics of low oral bioavailability and rapid excretion. TPV is a non-peptidic HIV protease inhibitor from the class of 4-hydroxy-5,6-dihydro-2-pyrones. TPV demonstrated good activity against HIV laboratory viral strains and clinical viral isolates.

This submission contains the final research reports on two studies, one on male and female fertility and early embryonic development in Sprague-Dawley rats and one on oral toxicity in the rat after administration for two weeks of TPV using a TRIS/SEDSS-s vehicle.

REVIEW:

- 1) **Study Report No.: a0066139; 2/23/2000.**
PNU-140690E: Male and female fertility and early embryonic development study (oral) in Sprague-Dawley rats.
Study No.: 96-492; 1997 - 2000.
Test Article Lot No.: (A1)5134-AS-1636 (C I purity).
Submission Volume 1, page 22.

- 2) **Study Report No.: a0045169; 3/7/2000.**
PNU-140690: 2-Week oral toxicity study in the rat using a TRIS/SEDSS-2 vehicle (Capmul MCM as lipid component).

Study No.: 1998-0206; 11/16/1998 - 3/7/2000.
Test Article Lot No.: (A1)5399-MTM-9802 (100% purity).
Submission Volume 1, page 238.

Appears This Way
On Original

This study was conducted by Pharmacia & Upjohn, Kalamazoo, Michigan, in compliance with Good Laboratory Practices.

Previous preclinical toxicity studies of TPV in aqueous solution were conducted in rats and dogs. The bioavailability of TPV in other formulations was tested in dogs. A self-emulsifying drug delivery system (SEDDS) containing tromethamine (TRIS) was found to have the highest bioavailability in dogs. It was also shown to be bioavailable in rats.

Therefore, a two-week oral toxicity study in male and female rats was conducted. The objectives of the study were as follows: 1) to compare the safety of TPV in a formulation with minimal TRIS (unstressed) with that of TPV in a formulation in which the TRIS component contained TRIS (stressed) and 2) to compare the findings with those from previous studies in which TPV had been administered in aqueous solution at dose levels expected to yield similar plasma levels to the doses used in the present study.

Nine to twelve week old male and female CD(SD)BR Sprague-Dawley rats (5/sex/group for toxicity segment; 3/sex/group for systemic exposure segment) weighing 313 to 369 grams and 228 to 290 grams, respectively, received 0, 63, 125 or 400 mg/kg/day with twice daily oral dosing via gastric intubation for two weeks. Each dose level was administered in two vehicles: 1) unstressed TRIS/SEDDS-2 and 2) stressed TRIS/SEDDS-2. Observations for clinical signs were performed twice pretest and at least once daily during dosing. Body weights were measured twice pretest and weekly during dosing. Blood samples were collected at necropsy and standard hematology and clinical chemistry assays were conducted. Serial blood samples were collected on Days 1 and 14 at 2, 4, 8, 10, 14 and 24 hours after the AM dose and analysed for determination of pharmacokinetic parameters.

All rats in the toxicity segment were subjected to complete gross necropsy. The following table shows the tissues chosen for histopathology and organs chosen for weight determination.

Table 1: Histopathology Inventory

Study Dose	0	63	125	400
Organ				
Adrenals	X,*	*	*	X,*
Aorta	X			X
Bone Marrow smear				
Bone (femur)				

Reviewer:

NDA No.

Brain	X,*	*	*	X,*
Cecum	X			X
Cervix	X			X
Colon	X			X
Duodenum	X			X
Epididymis	X			X
Esophagus	X			X
Eye	X			X
Fallopian tube				
Gall bladder				
Gross lesions	X			X
Harderian gland	NE			NE
Heart	X,*	*	*	X,*
Ileum	X			X
Injection site				
Jejunum	X,*	*	*	X,*
Kidneys	X			X
Lachrymal gland				
Larynx				
Liver	X,*	X,*	X,*	X,*
Lungs	X			X
Lymph nodes, cervical				
Lymph nodes, mandibular	X			X
Lymph nodes, mesenteric	X			X
Mammary Gland	X			X
Nasal cavity				
Optic nerves				
Ovaries	X,*	*	*	X,*

Reviewer:

NDA No.

Pancreas	X			X
Parathyroid	X,*	*	*	X,*
Peripheral nerve				
Pharynx				
Pituitary	X,*	*	*	X,*
Prostate	X,*	*	*	X,*
Rectum	NE			NE
Salivary gland	X			X
Sciatic nerve	X			X
Seminal vesicles	NE			NE
Skeletal muscle	X			X
Skin	X			X
Spinal cord	X			X
Spleen	X,*	*	*	X,*
Sternum	X			X
Stomach	X			X
Testes	X,*	*	*	X,*
Thymus	X			X
Thyroid	X,*	X,*	X,*	X,*
Tongue	NE			NE
Trachea	X			X
Urinary bladder	X			X
Uterus	X,*	*	*	X,*
Vagina	X			X
Zymbal gland				
Knee Joint	X			X
Diaphragm	X			X

X, histopathology performed, *, organ weight obtained; NE, collected but not examined.

RESULTS: There were no deaths and the only clinical sign was salivation. This occurred in all groups, including controls, but was noted for more days in the treated

groups and increased with dose. Body weight gain was slightly suppressed (2% to 10%) in treated males and slightly increased (7% to 12%) in treated females. These changes were similar in both vehicle groups. There were statistically significant treatment-related increases in prothrombin time for males in the high-dose groups (6% in unstressed vehicle group, 12% in stressed vehicle group) and in activated partial thromboplastin time for males in the mid- (13% in unstressed vehicle group, 17% in stressed vehicle group) and high-dose (35% in unstressed vehicle group, 42% in stressed vehicle group) groups. These changes were considered toxicologically relevant by the sponsor and thought to be due to effects on the liver, such as alteration of synthesis of coagulation factors, rather than a direct effect on the coagulation system.

There were treatment-related, toxicologically relevant changes in thyroid function tests. There were statistically significant decreases in males in triiodothyronine at the mid- (10% in unstressed vehicle group, 18% in stressed vehicle group) and high-doses (21% in unstressed vehicle group, 28% in stressed vehicle group) and in thyroxine values at the mid- (7% in unstressed vehicle group, 17% in stressed vehicle group) and high-doses (28% in unstressed vehicle group, 40% in stressed vehicle group). There were statistically significant increases in thyroid-stimulating hormone in females at all doses (60% to 452% in unstressed vehicle group, 171% to 464% in stressed vehicle group) and in males at the high-dose (54% in unstressed vehicle group, 298% in stressed vehicle group). These changes were consistent with increased mean thyroid weights and the observation of follicular cell hypertrophy in the thyroids of TPV-treated animals and thought to be secondary to the effect of TPV on the liver.

Other treatment-related changes which the sponsor described as slight and/or not toxicologically relevant are shown in the table below:

Table 2: Percent Change in Means for Hematology and Clinical Chemistry Parameters in High-Dose Groups versus Control Groups.

	Unstressed Vehicle		Stressed Vehicle	
	Males	Females	Males	Females
HEMATOLOGY				
RBC Count	-8	-10	-1	-13
Hemoglobin	-7	-9	-2	-12
Hematocrit	-2	-3	+1	-4
Mean Cell Hemoglobin Concentration	-2	-2	-3	-1
Reticulocyte Count	+13	+18	-5	+41
Platelet Count	+23	+29	+11	+28
White Blood Cell Count	-7	-17	-11	-19
CLINICAL CHEMISTRY				

Total Protein	+2	+12	+10	+5
Albumin	+2	+14	+10	+1
Globulin	+1	+10	+9	+9
Calcium	+2	+2	+1	+1
Aspartate Aminotransferase	-20	-10	-29	-19
Alkaline Phosphatase	+4	-18	-37	-42
Triglycerides	-81	-49	<-73	-60
Glucose	+7	-5	-3	-19
Blood Urea Nitrogen	+8	+20	+1	+15
Cholesterol	-6	+12	-24	0

There were no treatment-related gross necropsy findings. The sponsor stated that there were mild to moderate increases in absolute and relative liver and thyroid gland weights in all treated groups as shown below in Table 3. Other organs exhibiting moderate increases in weight are also shown in this table.

Table 3. Percent Difference in Mean Liver, Thyroid, Adrenal and Uterus Weights of the High-Dose Treated Groups versus Control Groups.

Unstressed Vehicle		Stressed Vehicle	
Male	Female	Male	Female
Absolute Liver Weight			
+35	+99	+34	+94
Relative Liver Weight*			
+37	+93	+41	+83
Absolute Thyroid Weight			
+29	+68	+11	+69
Relative Thyroid Weight*			
+29	+62	+19	+58
Absolute Adrenal Weight			
+6	+31	+25	+28
Relative Adrenal Weight*			
+6	+25	+32	+20

Absolute Uterus Weight			
	0		+24
Relative Uterus Weight*			
	-4		+17

*, Percent body weight.

Histopathologic changes involved the liver and thyroid glands and were similar in groups treated with either the unstressed or stressed formulation. Dose-related minimal to moderate hepatocellular hypertrophy of the liver was observed in males and females but was more prominent in females. Dose-related minimal to moderate follicular cell hypertrophy was observed in males and females but was more prominent in females.

Pharmacokinetic parameters are shown in Table 4.

Table 4: Systemic Exposure on Day 1 and Day 14.

Dose Level (mg/kg/day)	AUC ₀₋₂₄ µM.h		C _{max0-24} µM	
	Day 1	Day 14	Day 1	Day 14
Unstressed Vehicle				
63	M:771 F:3700	M:310 F:472	M:122 F:350	M:67 F:68
125	M:1460 F:5400	M:360 F:730	M:154 F:374	M:58 F:102
400	M:5060 F:7970	M:1230 F:2200	M:325 F:430	M:123 F:201
Stressed Vehicle				
63	M:620 F:2140	M:270 F:338	M:88 F:194	M:43 F:58
125	M:2260 F:4900	M:501 F:830	M:206 F:353	M:72 F:129
400	M:4000 F:8100	M:1170 F:1780	M:262 F:433	M:88 F:204

— stress of the formulation had no effect on the overall exposure of animals to TPV. For both unstressed and stressed formulations, exposure was greater for females than for males. This difference was greater on Day 1 than on Day 14. Exposure to TPV was decreased for both genders after multiple days of dosing, indicating that enzyme induction resulted in increased clearance. At steady-state, exposure increased with dose but was not dose proportional.

Reviewer:

NDA No.

	Lot C		Lot B	
	Males	Females	Males	Females
HEMATOLOGY				
Prothrombin Time	+28	+5	+29	+8
APTT	+17	+18	+39	+8
RBC Count	-12	-1	-7	-10
Hemoglobin	-9	-2	-3	-9
Hematocrit	-4	-2	-1	-5
Mean Cell Hemoglobin Concentration	+1	-1	-6	+1
Reticulocyte Count	0	+27	+13	+25
Platelet Count	+10	+24	+12	+12
White Blood Cell Count	-34	+11	-5	+8
CLINICAL CHEMISTRY				
Total Protein	+7	+10	+9	+10
Albumin	+4	+3	+7	+3
Globulin	+7	+17	+11	+17
Alanine Aminotransferase	+60	+14	+23	+3
Aspartate Aminotransferase	-15	-23	-15	-13
Alkaline Phosphatase	-14	-25	-25	-16
Creatine Kinase	-33	-56	-33	-40
Triglycerides	-26	-9	-30	-3
Glucose	-9	0	-11	-2
Blood Urea Nitrogen	+8	0	+18	+8
Cholesterol	-11	0	-2	-3
Thyroxine	-9	+5	-9	+2
Total T3	-7	-11	-4	-8
TSH	+196	+306	+77	+275

C. Anita H. Bigger, Ph.D.
Pharmacologist

Reviewer:

NDA No.

Concurrences :

HFD-530/JFarrelly
HFD-530/ABigger

Disk:

HFD-530/JFarrelly

cc:

HFD-530 Original IND
HFD-530 Division File
HFD-340
HFD-530/JToerner
HFD-530/Microbiology
HFD-530/Chemistry
HFD-530/ABigger
HFD-530/LStephens

(5) IND 51,979 (217, SX)

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals**

Application (IND/NDA) number: IND 51,979
Submission date and number: March 24, 2003/217 (SX)
Division: Division of Antiviral Drug Products, HFD-530
Project manager: Virginia Yoerg
CAS#:
Drug name: Tipranavir
Pharmacological Classification: HIV protease inhibitor
Sponsor/Applicant: Nancy McKay
Sponsor/Applicant contact name: 203-791-6759
Sponsor/Applicant telephone and fax number: 203-791-6262
Date submitted (stamp date): March 25, 2003
45-day date (from submission stamp date): May 8, 2003
P/T Reviewer(s): Anita Bigger
Date Review Completed: April 30, 2003
Date of CAC review: May 6, 2003
CAC members: Abby Jacobs (acting chair), John Leighton, and David Jacobson-Kram

Summary of Proposal for Review:

Tipranavir (TPV) will be given in combination with ritonavir (RTV) in the clinic. The proposed maximum human dose is 450 TPV/200 mg RTV BID. Therefore, the proposed mouse carcinogenicity protocol includes TPV/RTV (3.75:1) and RTV only arms.

Species/strain:	CD-1(ICBR) VAF+ mice	
Number/sex/dose:	75	
Route:	Oral gavage	
	male	female
Doses proposed (mg/kg/day): TPV	0, 30, 150, 300	0, 30, 150, 300
TPV/RTV	300/80	300/80
RTV	80	80
Basis of dose selection:		
MTD	x	x
AUC ratio	_____	_____
saturation	_____	_____
MFD	_____	_____
PD	_____	_____
other	_____	_____
Kinetics submitted:	rodent	human
pharmacokinetics	x	_____
x	_____	_____
metabolism	_____	_____
protein binding	_____	_____

Notable design features: RTV in propylene glycol at 80 mg/kg/day (5 ml/kg) will be administered, followed approximately 1 hour later by TPV in aqueous solution pH 10.5 at 150 mg/kg (10 ml/kg). Approximately 4 hours later animals will receive TPV at 150 mg/kg (10 ml/kg) a second time. Other TPV only groups will receive doses BID (approximately 4 hours apart). In the dose-range finding study, single daily dosing was used.

Summary of Recommendations to CAC

Dose levels were selected based on results of a 13-week study in CD-1 mice with TPV and a 4-week study in CD-1 mice with TPV/RTV. Supplemental information from 4-week studies with TPV in FVB/N and CF-1 mice was used also.

A dose of 300 mg/kg/day TPV was chosen as the high-dose. This dose selection is based on mean body weight decreases due to decreased body weight gain observed in the 13-week study in CD-1 mice (4 – 9% in males/females) and 4-week studies in FVB/N and CF-1 mice (6 – 8% in males/females and 10 –14% in males, respectively), coagulation effects (increased APTT and fibrinogen \geq 400 mg/kg/day) observed in a 4-week study in CF-1 mice, as well as liver changes (increased ALT, increased liver weights, hepatocellular hypertrophy, vacuolation and necrosis) observed in all studies in mice. It

is anticipated that this dose level would result in weight loss of less than 10% over a period of 2 years, but liver toxicity in the form of moderate to marked necrosis would be evident. It is not anticipated that increases in coagulation parameters at this dose would be such that increases in mortality would be caused by excessive hemorrhage.

A TPV/RTV co-administration dose of 300/80 mg/kg/day and a RTV dose of 80 mg/kg/day have been chosen based on the 4-week study in CD-1 mice with TPV/RTV. Dose levels of 150/40, 300/80 and 600/160 mg/kg/day TPV/RTV tested in a 4-week study resulted in hepatocellular hypertrophy, hepatocellular vacuolation, hepatocellular necrosis, lymphoid follicular hyperplasia in the spleen and/or increased extramedullary hematopoiesis, with incidence and severity of these findings increasing in a dose-related fashion. Animals treated at the highest dose also displayed hypertrophy of the adrenal gland zona fasciculata in 7/10 males. It is anticipated that over two years, a dose level of 300/80 mg/kg/day TPV/RTV would result in similar changes of a greater magnitude and this dose level has been chosen as the high dose for the 2-year carcinogenicity study. A RTV alone dose level of 80 mg/kg/day was selected to correspond to the 300/80 mg/kg/day TPV/RTV dose level.

Mid-dose and low-dose TPV levels have been chosen at 150 mg/kg/day and 30 mg/kg/day. The low-dose of 30 mg/kg/day TPV is expected to be a NOEL over a 2-year period.

Co-administration of TPV and RTV greatly increases exposure to TPV. The only data from the 4-week range finding study to allow a comparison of this effect are 8 hours post-dose plasma concentrations for the 600 TPV and 600/160 TPV/RTV mg/kg/day doses. Without RTV, TPV plasma concentrations (μM) are 6 (M) and 13 (F); with RTV, they are 126 (M) and 151 (F). Plasma concentrations for the 300/80 TPV/RTV dose in this study are 73 (M) and 282 (F). Plasma concentrations for the mid-dose and low dose TPV only levels are expected to be around 3 μM and 1 μM , respectively.

Questions for the CAC:

1. Is the 4-week TPV/RTV dose-range finding study in CD-1 mice adequate for determination of dose levels?
2. Is it appropriate to use the "BID" dosing proposed when single daily dosing was used in the dose-range finding studies?
3. Is it realistic to assume based on a 4-week study that the high dose TPV/RTV animals will survive to the end of the 2-year carcinogenicity study?

**Executive CAC
May 6, 2003**

Committee: Abby Jacobs, Ph.D., HFD-540, Acting Chair
John Leighton, Ph.D., HFD-150, Alternate Member
David Jacobson-Kram, Ph.D., HFD-024, Alternate Member
James Farrelly, Ph.D., HFD-530, Team Leader
Anita Bigger, Ph.D., HFD-530, Presenting Reviewer

Author of Draft: Anita Bigger

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The Committee recommended that the sponsor consider evaluating the various dosage groups by pairwise comparison with the negative control. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND 51,979

Drug Name: Tipranavir

Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.

Sponsor's Mouse Carcinogenicity Study Protocol and Dose Selection:

Tipranavir 0, 30, 150 and 300 mg/kg/day
Tipranavir/Ritonavir 300/80 mg/kg/day
Ritonavir 80 mg/kg/day

Background:

Tipranavir (TPV) will be given in combination with ritonavir (RTV) in the clinic. The proposed maximum human dose is 450 mg TPV/200 mg RTV BID. Therefore, the proposed mouse carcinogenicity protocol includes TPV/RTV (3.75:1) and RTV only arms.

Sponsor's Proposal:

RTV in propylene glycol at 80 mg/kg/day (5 ml/kg) will be administered to \bar{C} 1 CD-1(ICBR) VAF+ mice (75/sex/dose), followed approximately 1 hour later by TPV in aqueous solution (pH 10.5) at 150 mg/kg (10 ml/kg). Approximately 4 hours later animals will receive TPV at 150 mg/kg (10 ml/kg) a second time. TPV alone in aqueous solution (pH 10.5) at 30, 150 or 300 mg/kg/day (10 ml/kg) will be administered BID (approximately 4 hours apart). RTV alone in propylene glycol at 80 mg/kg/day (5 ml/kg) will be administered QD.

Dose levels were selected based on results of a 13-week study in CD-1 mice with TPV and a 4-week study in CD-1 mice with TPV/RTV. Supplemental information from 4-week studies with TPV in FVB/N and CF-1 mice was used also.

Mid-dose and low-dose TPV levels have been chosen at 150 mg/kg/day and 30 mg/kg/day. The low-dose of 30 mg/kg/day TPV is expected to be a NOEL over a 2-year period.

Executive CAC Recommendations and Conclusions:

* The Committee recommended the following doses and dosing regimens:

- TPV: 300, 150, 30, 0 mg/kg/day given with BID dosing, i.e. 15, 75 and 150 mg/kg BID.
(The Committee concurred with the doses proposed by the sponsor; the high dose was based on liver necrosis.)
- TPV/RTV: 150/40 (fixed ratio) given with QD dosing for each drug.
(The Committee recommended a lower dose than the sponsor because 300/80 may be too high for survival based on the incidence and severity of liver vacuolation and necrosis in the 4-week dose range finding study in CD-1 mice.)
- RTV 40 mg/kg/day given with QD dosing (The Committee recommended this dose to be the same as the RTV dose in the TPV/RTV fixed combination.)

* The sponsor should consider that they may need more than 75 animals/sex/dose to allow for dosing errors, based on data provided showing a high level of gavage errors, particularly in the early weeks.

* It is recommended that the sponsor consider evaluating the outcomes of the various dosage groups by pairwise comparison with the negative control group.

Abby Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\n
/Division File, HFD-530
/JFarrelly, HFD-530
/ABigger, HFD-530
/VYoerg, HFD-530
/ASeifried, HFD-024

(6) IND 51,979 (474) Summary of Immunotoxicity Findings for TipranavirMemo to the File
Summary of Immunotoxicity Findings for Tipranavir

IND 51979: Tipranavir

Reviewer: Anita Bigger, PhD

Division of Antiviral Drug Products, HFD-530

Date: August 24, 2004

Relevant Submissions: N-050, N-185, N-317, N-318, N-324, N-474

The initial request (N-318) by the sponsor for FDA commentary on the adequacy of immunotoxicology studies was received in February 2004. However, not all of the studies cited had been submitted for review to the Agency. At that time, we replied that we would review the issue once all reports had been submitted and reviewed. The final report was received in July, 2004. The sponsor states in N-318 that tipranavir (TPV) might be immunostimulatory and immunosuppressive but that they don't think there is any direct immunomodulatory potential, that these changes are secondary to the related stress of multi-organ toxicities from drug administration. Five studies are cited:

1. Submission 050 (13 week mouse study TPV alone) and submission 317 (4 week interaction/toxicity study in the mouse on TPV and ritonavir (RTV)) show two immunostimulatory findings, i.e. exacerbation of granulocytic hyperplasia in bone marrow and exacerbation of lymphoid follicular hyperplasia.
2. In a mouse immune function study (185) a single dose of TPV inhibited anti-CD3-dependent T-cell stimulation as measured by IL-2 concentrations. Also a minimal, albeit statistically significant, T cell-driven delayed type hypersensitivity (DTH) was observed as measured by [unclear].
3. In a 26 week rat study (474), evidence of immunostimulation (reticuloendothelial cell hyperplasia in the mesenteric lymph node) and conversely immunosuppression (lymphoid depletion in lymph nodes, spleen and/or thymus & lymphocytolysis in the thymus) were observed in the TPV + RTV dose groups.
4. In a 26 week dog study (324), evidence of immunostimulation (granulocytic hyperplasia in the bone marrow) was observed in the high-dose TPV alone group and evidence of immunosuppression (depletion of lymphocytes in the spleen) was observed in the high-dose RTV alone group.

Consultations with immunotoxicology experts Shukal Bala, PhD, and Steven Kunder, PhD, (Division of Special Pathogen and Immunologic Drug Products, HFD-590) were obtained. The above studies were reviewed with attention to the indicators of immunotoxicity as described in Guidance for Industry Immunotoxicology Evaluation of Investigational new Drugs (October 2002):

1. Unexplained changes in hematological parameters;
2. Unexplained changes in serum globulin or total protein/serum globulin ratios;
3. Gross pathology findings (e.g. changes in thymus, spleen, lymph nodes or bone marrow);
4. Weight and/or histological changes in lymphoid tissues;
5. Increased incidence of infections or tumors.

The relevant data are reviewed in summary form below.

Submission N-050: PNU-140690E 13-week oral toxicity study in the mouse (dose range finding).

Doses: 40, 120 and 360 mg/kg/day (expressed as free acid equivalents)

Species/strain: CD-1 mice

Number/sex/group or time point (main study): 20

Route, formulation, volume, and infusion rate: Purified water adjusted to pH 10.5 with sodium hydroxide diluted aqueous solution; 12 ml/kg/day; treatment given in two equally divided doses (20, 60 and 180 mg/kg/dose) at an interval of eight hours.

Satellite groups used for toxicokinetics or recovery: 15/sex/group for toxicokinetics

Hematology: No treatment related changes were seen in hematological parameters or in lymphocyte subsets.

Clinical chemistry: There was a statistically significant ($P=0.05$) increase in globulin in the high dose male group (3.1 g/dL versus 2.6 g/dL in control group) and decrease in albumin/globulin ratio in the same group (0.8 versus 1.1 in control group).

Gross pathology: Gross pathology changes which occurred in single instances in treated animals were sporadic in nature and not treatment-related and consisted of enlarged spleen (1/20 high dose male and 1/20 low dose female) and lymph nodes (1/20 low dose female).

Organ weights: There were no statistically significant differences in absolute or relative organ weights for spleen and thymus.

Histopathology:

Histopathology Findings (N-050) Relating to Immunotoxicity in the Mouse:

Group: Dose (mg/kg/day): Test Article: Formulation: Aqueous pH 10.5	Control 0		Low-Dose 40 TPV		Mid-Dose 120 TPV		High-Dose 360 TPV	
	M	F	M	F	M	F	M	F
No. in Group	20	20	20	20	20	20	20	20
Mandibular Lymph Node Lymphoid Hyperplasia	4/19 +	6/19 +	0/0	1/1 ++	0/0	0/0	7/20 +	9/20 + 1/20 +++
Mandibular Lymph Node Lymphoid Depletion	0/19	0/19	0/0	0/1	0/0	0/0	0/20	1/20 ++
Mesenteric Lymph Node Lymphoid Hyperplasia	2/20 +	3/20 + 1/20 ++	0/0	0/0	0/0	0/0	4/20 +	3/20 +

Number affected/Number examined.

+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Infections/Tumors: No increased incidence of infections or tumors was noted. Loss of general condition, without a dose response relationship, was observed in two males and nine females in treated groups. No explanation for this was given.

Toxicokinetics:

Dose (mg/kg/day)	Gender	Study Day	C _{2h} (•M)	C _{10h} (•M)	AUC ₀₋₂₄ (•M.h)
40	F	30	nd	0.43	nd
		93	0.31	0.28	2.42
	M	30	nd	0.22	nd
		93	0.287	0.28	1.99
120	F	30	nd	0.91	nd
		93	0.99	1.16	9.31
	M	30	nd	0.66	nd
		93	0.80	0.74	6.48
360	F	30	nd	3.8	nd
		93	1.90	3.53	36.1
	M	30	nd	2.9	nd
		93	1.49	2.5	19.3

C = concentration at indicated time. C at 2 h and 10 h correspond to the estimated maximum plasma concentrations for the first and second daily dose, respectively. nd = not determined.

Submission N-317: 4-Week Oral (Gavage) Interaction/Toxicity Study in the CD-1 Mouse on Tipranavir and Ritonavir.

Doses: 0, 150/40, 300/80 and 600/160 mg/kg/day TPV/RTV, 600 mg/kg/day TPV or 160 mg/kg/day RTV

Species/strain: CD-1(ICRBR) VAF+ mice

Number/sex/group or time point (main study): 15

Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 10 ml/kg for tipranavir and 5 ml/kg for ritonavir; animals receiving TPV and RTV were dosed first with a single dose of RTV, followed 1 hour later by a single dose of TPV.

Satellite groups used for toxicokinetics or recovery: last 5/sex/group for toxicokinetics.

Hematology: Hematology parameters were not determined in this study.

Clinical chemistry: Clinical chemistry parameters were not determined in this study.

Gross pathology: There were no gross pathology findings suggestive of treatment-related immunotoxicity, except 1/15 females in the high dose TPV/RTV group had a thymus of diminished size.

Organ weights: Organ weight for spleen and thymus was not determined.

Histopathology:

Histopathology Findings (N-317) Related to Immunotoxicity in the Mouse:

Group: Dose (mg/kg/day): Test Article: Formulation: TPV Aqueous pH 10.5/RTV Propylene glycol	Control		Low-Dose TPV/RTV		Mid-Dose TPV/RTV		High-Dose TPV/RTV		High TPV		High RTV	
	0		150/40 TPV/RTV		300/80 TPV/RTV		600/160 TPV/RTV		600/0 TPV		0/160 RTV	
Gender:	M	F	M	F	M	F	M	F	M	F	M	F
No. in Group	15	15	15	15	15	15	15	15	15	15	15	15
Bone Marrow, Femur Granulocytic Hyperplasia	1/15 + 1/15 ++	1/15 ++	1/15 + 1/15 ++	0/15	1/15 + 1/15 ++	1/15 +	1/15 + 1/15 ++	2/15 +	3/15 + 2/15 ++	1/15 +	1/15 +	0/15
Bone Marrow, Sternum Granulocytic Hyperplasia	1/15 + 1/15 ++	1/15 ++	0/0	0/0	0/0	0/0	1/15 + 1/15 ++	2/15 +	3/15 + 2/15 ++	1/15 +	1/15 +	0/15
Lymph Node, Mandibular Lymphoid Hyperplasia, Paracourt	1/15 +	0/15	0/0	0/0	0/0	0/0	1/15 +	0/15	0/15	0/15	1/15 +	0/15
Lymph Node, Mandibular Lymphoid Hyperplasia, Paracourt	0/15	1/15 ++	0/15	2/15 + 1/15 ++	0/15	1/15 +	0/15	2/15 +	1/15 +	1/15 +	1/15 +	2/15 + 1/15 ++

Spleen Lymphoid follicular hyperplasia Focal	0/15	1/15 +	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	0/15	0/15	0/15	2/15 + 1/15 ++	0/15	3/15 +	4/15 +	1/15 + 1/15 ++	4/15 +	1/15 +	3/15 +	2/15 +
Thymus Lymphoid Cortical Depletion	0/15	0/15	0/15	1/15 +++ +	0/15	0/15	0/15	1/15 +++	0/15	0/15	0/15	1/15 ++

Number affected/Number examined.

+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Infections/Tumors: No increased incidence of infections or tumors was noted.

Toxicokinetics:

Plasma Concentrations of TPV and RTV on Drug Day 28 at 8 hours after TPV dose:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV (ng/ml)	TPV (• M)	RTV (ng/ml)
150/40	F	32,444	53.8	925
	M	8,444	14.0	387
300/80	F	169,686	282	2,812
	M	44,213	73.4	636
600/160	F	90,707	151	850
	M	76,067	126	801
600/0	F	7,512	12.5	0
	M	3,527	5.85	0
0/160	F	0	0	5,173
	F	0	0	2,774

Submission N-185: In vivo assessment of Tipranavir (EXRS 1406 XX) effects on murine immune function.

Key study findings: Tipranavir showed modest to marginal effects at oral doses up to 300 mg/kg on assays designed to examine T cell, B cell and complex inflammatory responses.

1. In Vivo Anti-CD3 Assay

A single dose (30, 100 or 300 mg/kg) of tipranavir was administered orally to female BALB/c mice prior to injection with a monoclonal antibody to mouse CD3 to measure the direct effects of the compound on T cell activation as assessed by plasma IL-2 levels.

In the first assay IL-2 was inhibited 46% by tipranavir at 300 mg/kg. These mice had been fasted overnight and exhibited marked diarrhea at the high dose. Since diarrhea can impair immune responses in this model, the assay was repeated using unfasted mice. In the repeat assay, IL-2 was inhibited 39%, 25% and 27% by 300, 100 and 30 mg/kg of tipranavir, respectively.

2. In Vivo Delayed Type Hypersensitivity (DTH)

Male BALB/c mice were immunized intradermally at the tail base.

Seven days later, DTH responses were elicited by subcutaneous injection of antigen into the dorsal surfaces of each ear, eliciting the maximal swelling response at 24 hours post-injection. In the treatment groups, a single dose (30, 100 or 300 mg/kg) was administered 1 hour prior to ear injection. Ear thickness is measured before and 24 hours after the injection. The change in ear thickness is calculated as a measure of the DTH response.

There was a statistically significant effect at the high dose. The sponsor notes that the mice were still able to mount a robust T cell and inflammatory response even at the high dose.

3. In Vivo T Cell Independent B Cell Activation

Trinitrophenyl (TNP)-conjugated lipopolysaccharide (LPS) was injected into mice to stimulate the polyclonal activation of B cells. Upon activation, these B cells produce TNP-specific IgM at levels which can be detected in the plasma by day 3. Tipranavir (30, 100 or 300 mg/kg) was administered orally 1 hour prior to TNP-LPS injection and 24 hours later. Tipranavir showed no detectable effects on B cell activation.

4. Efforts to address the ability of tipranavir to induce compound-specific immune response (i.e. allergic response to the compound itself) were hindered due to solubility and irritation. In the popliteal lymph node assay, five mg of soluble compounds are injected into the footpads of mice and seven days later the increase in draining lymph node size is measured as an indication of compound-triggered immune responses. Only 3 mg of tipranavir could be injected per paw. Even at this level, the paws were extremely swollen and showed evidence of scabbing, suggesting an irritant response. Thus, immunogenicity could not be adequately assessed for tipranavir.

Submission N-474: 26-Week oral (gavage) interaction/toxicity study in the rat on tipranavir and ritonavir.

Doses: 0, 120/32, 600/160 and 1200/320 mg/kg/day TPV/RTV, 1200 mg/kg/day TPV or 160 mg/kg/day RTV

Species/strain: CD(SD)IGS BR Sprague Dawley VAF+ albino rats.

Number/sex/group or time point (main study): 20

Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 15 ml/kg for tipranavir and 5 ml/kg for ritonavir; animals receiving TPV and RTV were dosed first with a single dose of RTV, followed immediately by a single dose of TPV in the first six weeks of the study. Due to difficulties with the bitter taste of TPV and struggling animals, beginning in Drug Week 7 animals were dosed with RTV followed 1 hour later by a single dose of TPV.

Satellite groups used for toxicokinetics or recovery: last 5/sex/group for toxicokinetics

Hematology: No explicit changes in hematology parameters indicative of immunotoxicity were observed.

Clinical chemistry: Changes noted in groups treated with both TPV/RTV were elevations in globulin (25 - 34%) in females, similar changes in animals treated only with TPV and elevations in globulin (44%) in females on RTV alone.

Gross pathology: Diminished size of the spleen and thymus in males and females in the TPV/RTV, TPV and RTV groups was observed.

Organ weights: Spleen and thymus weights were not determined.

Histopathology:

Histopathology Findings Related to Immunotoxicity in the Rat:

Group: Dose (mg/kg/day): Test Article: Formulation: TPV Aqueous pH 10.5/RTV Propylene glycol	Control		Low-Dose TPV/RTV		Mid-Dose TPV/RTV		High-Dose TPV/RTV		High TPV		High RTV	
	0		120/32 TPV/RTV		600/160 TPV/RTV		1200/320 TPV/RTV		1200/0 TPV		0/160 RTV	
Gender:	M	F	M	F	M	F	M	F	M	F	M	F
No. in Group	20	20	20	20	20	20	20	20	20	20	20	20
Lymphoid Depletion (no. animals affected) Lymph Nodes, Spleen, Thymus	0	0	0	1 +++ to +++ +	1 ++	1 ++	6 +	3 +	8 +	8 +	0	3 ++ to +++ +
Mesenteric Lymph Node Reticuloendothelial Cell Hyperplasia	0/15	0/15	0/15	0/15	0/15	1/15 +++ +	1/15 +++	0/15	0/15	0/15	0.15	1/15 +++
Thymus Lymphocytolysis	0/15	0/15	0/15	0/15	1/15 +++ +	0/15	3/15 +++ +	2/14 +++ +	3/15 +++ to +++ +	3/15 +++ to +++	0/15	1/15 +++ +

Number affected/Number examined.

+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Infections/Tumors: No increased incidence of infections or tumors was noted.

Toxicokinetics:

Plasma Concentrations of TPV and RTV on Drug Week 26 at 8 hours after TPV dose and 9 hours after RTV dose:

Dose Level TPV/RTV (mg/kg/day)	Gender	TPV (• M)	RTV (ng/ml)
120/32	F	133 (Week 14)	1882 (higher than expected - protocol deviation)
	M	198	585
600/160	F	406	418
	M	303	141
1200/320	F	488	462
	M	344	0 (below 10)
1200/0	F	361	0
	M	290	0
0/160	F	0	1689
	F	0	1555

Submission N-324: 26-Week oral (gavage) interaction/toxicity study in the Beagle dog on tipranavir and ritonavir.

Doses: Initial doses were 0, 15/4, 37.5/10 and 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV. These doses continued to the end of Drug Week 12 due to a high incidence of emesis. Doses were escalated in Drug Week 13 from 75/20 mg/kg/day TPV/RTV, 75 mg/kg/day TPV or 20 mg/kg/day RTV to 150/40, 150 and 40, respectively.

Species/strain: Beagle dogs □

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Number/sex/group or time point (main study): 3

Route, formulation, volume, and infusion rate: Tipranavir in aqueous solution, pH 10.5; ritonavir in propylene glycol. 2 ml/kg for tipranavir and 2ml/kg for ritonavir; animals received RTV first followed by TPV. Due to the bitter taste of TPV a cherry syrup wash was added in Drug Weeks 7 and 8.

Hematology: There were no drug-related changes in hematology parameters.

Clinical chemistry: There were no drug-related changes in clinical chemistry parameters indicative of immunotoxicity.

Gross pathology: There were no drug-related gross pathology changes indicative of immunotoxicity.

Organ weights: Spleen and thymus weights were not determined.

Histopathology:

Histopathology Findings Related to Immunotoxicity in the Dog:

Group: Dose (mg/kg/day): Test Article: Formulation: TPV Aqueous pH 10.5/RTV Propylene glycol	Control		Low-Dose TPV/RTV		Mid-Dose TPV/RTV		High-Dose TPV/RTV		High TPV		High RTV		
	0		15/4 TPV/RTV		37.5/10 TPV/RTV		75/20 150/40 TPV/RTV		75/0 150/0 TPV		0/20 0/40 RTV		
Gender:	M	F	M	F	M	F	M	F	M	F	M	F	
No. in Group	3	3	3	3	3	3	3	3	3	3	3	3	
Bone Marrow, Rib Granulocytic hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 +++	1/3 ++	0/3	0/3	
Bone Marrow, Sternum Granulocytic hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	1/3 ++	0/3	0/3	
Lymph Node Lymphoid hyperplasia	0/3	0/3	0/3	1/3 + 1/3 ++	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3 +++	
Mesenteric Lymph Node Lymphocytic depletion, cortical	0/3	0/3	1/3 ++	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	0/3	0/3	
Spleen Lymphocytic depletion	0/3	0/3	0/3	0/3	0/3	0/3	1/3 ++	0/3	0/3	0/3	1/3 ++ 1/3 +++	1/3 +	
Thymus Lymphocytic depletion, cortical	1/3 ++ 1/3 +++	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 + 1/3 +++ 1/3 +++ +	3/3 ++	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 + 1/3 ++ 1/3 +++ +	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 + 2/3 +++ 1/3 +++ +	1/3 ++ 1/3 +++ 1/3 +++ +	1/3 ++ 2/3 +++ 1/3 +++ +	1/3 +++

Number affected/Number examined.

+ = Minimal; ++ = Mild; +++ = Moderate; ++++ = Marked

Infections/Tumors: No increased incidence of infections or tumors was noted.

Toxicokinetics:

C_{max} and AUC of TPV and RTV on Drug Week 26:

Dose Level TPV/RTV	Gender	TPV	RTV
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(mg/kg/day)		C_{max} (• M)	AUC_{0-24h} (• M.h)	C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)
15/4	F	34	157	554	1,603
	M	19	72	61	274
37.5/10	F	41	217	438	2,031
	M	37	236	372	2,014
75-150/20-40	F	64	598	416	5,121
	M	66	722	852	7,780
75-150/0	F	16	74	6	18
	M	40	346	0	0
0/20-40	F	0	0	10,970	97,340
	F	0	0	10,574	79,014

Conclusions and Recommendations:

Drs. Bala and Kunder agreed that there is some evidence suggestive of tipranavir-induced immunosuppression and immunostimulation. There are no animal models for exploration of immunostimulation. However, there are animal models for exploration of immunosuppression. Dr. Kunder suggests that the sponsor be asked to perform the Sheep Red Blood Cell Plaque Forming Assay, a T-cell dependent antigen assay. This assay will give an overall view of immune function since it demonstrates most components of the classical immune response. The assay can be done in vitro or in vivo but the in vivo version is the simplest. The assay should be performed with three arms: 1) tipranavir alone; 2) ritonavir alone and 3) tipranavir and ritonavir in combination.

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