identified and gross evaluations of placentas were made. Males were sacrificed at the end of mating periods and epididymal spermatozoa were checked for motility, number of spermatid, sperm counts, and morphology.

For toxicokinetics studies, 2 ml of blood samples were collected from the orbital sinus, 2 hours after the first day of treatment, in the 6th week, and on the last day of treatment. Since previous studies showed, that there was a significant loss of activity, following thawing of samples, these sample have not been analyzed, till an appropriate method is developed. Therefore data for these studies are not available.

Results: One male from the control group died on day 39, the necropsy result showed uncollapsed lungs, frothy fluid in the trachea, and a mass at the infusion site. Another male rat, from 5 μg/kg/min group, died on the 8th day, during a repair surgery. A female rat, from 50 μg/kg/min group, died on the 20th day, it had dark fluid in the abdominal cavity, a pale liver and carcass, and a mass at the infusion site. However, none of these were attributed to the drug effect by the sponsor. Reddening of the skin, scab formation, fur staining, thin fur cover, and swelling, all were, a result of the surgical/infusion methods. In addition occasional clinical signs, such as blue discoloration of the skin, skin lesions and altered activity level were seen. No drug related changes in body weights or food consumptions were observed. No treatment related differences in gross pathology were observed with integrilin. The paternal reproductive performance was not affected, as there were no effects on male and female mating, and mating indices. The number of live/dead fetuses, resorptions, sex ratio, implantation sites, pre or post implantation losses were all unchanged. The testicular pathology or epididymal sperm factors (motility, count) were unaltered. There were no effects also on maternal reproductive performance of the treated females. Ovarian factors were unaffected, uterine findings indicated a higher corpora lutea counts at 1 and 5 μg/kg/min vs control (22.9, 23.4 resp vs 19.4), no effects were seen at a higher dose. The number of live/dead fetuses, resorptions, sex ratio, implantation sites, pre or post implantation losses were all unchanged. There were no changes in fetal weights or in external fetal morphology, Tables 12 to 14.

The results indicate that integrilin (1, 5, or 50 μg/kg/min) given to male and female rats by continuous iv infusion, before the matings, during the matings, and to females on days 0-6 postcoitus, had no effects on male or female fertility or general reproductive performance of animals.

Table 12. Effects of integrilin on fertility and general reproductive performance of male and female rats in Segment I. study.

Dose µg/kg/min	0	1	5	50
Males				
# of males paired	25	25	24	25
# failing to mate	0	0	0	0
Mean # of days to mating	3.2	3.4	2.5	2.7
pregnant females	19	25	23	22
Females				
# of females paired	24	25	23*	24
# failing to mate	0	0	0	0
Mean # of days to mating	1.9	3.9	4.7	3.1
pregnant females	22	18	20	20
conception rates (%)	91.7	72	87	87

* = 2 rats were excluded due to partial treatment during mating period.

Table 13. Effects of Integrilin on maternal and fetal parameters in Segment I. studies in rats.

Dose (μg/kg/min)	0	1	5	50
# of dams	24	25	23	24
Uterine findings				
# of corpora lutea/dam	19.4	22.9	23.4	21.6
total implantations/dam	15.8	15.6	15.8	14.5
male fetuses	7.6	7.8	6.8	6.6
female fetuses	7.1	6.6	7.2	7.0
live fetuses	14.7	14.4	14.0	13.7
sex ratio (%)	52.0	54.4	45.2	51.7
Dead fetuses	0	0	0	0
Early resorptions/dam	1.0	0.9	1.0	0.7
Middle resorptions/dam	0.1	0.1	0.2	0.1
Late resorptions/dam	0.1	0.1	0.4	0
Total resorptions/dam	1.1	1.1	1.7	0.8
preimplantation loss (%)	17.2	30.9	29.2	29.1
post-implantation loss(%)	8.3	7.5	13.8	5.0
Gravid uterus weight (g)	85.8	85.8	85.1	83.3
Mean fetal weight				
males	3.96	4.15	4.08	4.25
females	3.80	3.89	3.83	4.02

Table 14. Effects of Integrilin on paternal and fetal parameters in Segment I. studies in rats.

Dose (μg/kg/min)	0	1	5	50
# of dams	25	25	24	25
Epididymal sperm evaluation				
caudia epididymis weight (g)	0.29	0.29	0.29	0.30
spermatozoa count (10^6 /g)	812	856	844	832
% motility	80	81	81	81
Mean uterine findings in untreated females:				
Total # of corpora lutea	22.3	21.9	21.4	22.4
Total implantations sites	17.9	17.4	17.8	17.2
male fetuses	9.1	7.4	8.7	8.7
female fetuses	8.0	7.8	8.2	7.2
live fetuses	17.1	15.3	16.9	15.8
sex ratio (%)	53.3	49.0	52.0	54.4
Dead fetuses	0	0	0	0
Early resorptions/dam	1.0	1.5	0.8	1.1
Middle resorptions/dam	0	0.1	0.1	0.1
Late resorptions/dam	0	0	0	0.1
Total resorptions/dam	1.0	1.6	1.0	1.2
preimplantation loss (%)	16.8	20.5	14.8	22.2
post-implantation loss (%)	5.4	9.8	5.6	7.8
Gravid uterus weight (g)	97.3	89.2	98.0	91.1
Mean fetal weight				
males	3.9	4.0	4.0	4.0
females	3.7	3.8	3.8	3.8
total	3.8	3.9	3.9	3.9

Segment II. Effects of Integrilin (Continuous IV Infusion) on Teratogenicity in Rats
(Study # 95635)

Testing Laboratories:

Study Started: March 28, 1994

Study Completed: March 30, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Female Sprague Dawley rats (~ 87-95 days old, ~ 228-330 grams, Crl:CD(SD)BR),

Drug Batch No.: D0015A.

Methods; 4 groups of 30 mated female rats were given Integrilin by continuous iv infusion (by an indwelling catheter, placed in the right femoral vein), at doses of 0, 1, 5, 50 μg/kg/min (or 0, 1.44, 7.20, and 72 mg/kg/day), from days 6 to day 15 of gestation. The drug was administered at an infusion rate of 1.5 ml/kg/h. The dose selection was based on the availability of the highest dose of 2.0 mg/ml stock solution in a vehicle. Four females from each group were assigned to toxicokinetic studies. In all female (FO) rats, body weights were measured on days 0, 3, 6, 9, 12, 15, 18, and 20 PC. Food consumptions were recorded on days 0-6, 6-9, 9-12, 12-15 and 15-18 PC. Clinical evaluations were performed twice daily. The females were sacrificed on day 20 PC and necropsied. The corpora lutea were counted, the number of implants, the number of dead or resorbed fetuses (F1), the number of live fetuses were examined. All fetuses were individually weighed, sex identified, and examined for external anomalies. A detailed internal examinations were performed on half of fetuses, fetuses were then eviscerated and the heads of these fetuses were examined, using Wilson's technique. The other half of the fetuses were eviscerated, using Alizarin Red S. staining method and skeletons examined.

For toxicokinetic studies, 2 ml of blood samples were collected from the orbital sinus, on the 6th day of gestation, 2 hours after the initiation of treatment, and around the same time, on the 10th and 16th day of gestation. However, these samples have not been analyzed yet, till an appropriate method is developed, as mentioned earlier (Segment I. study).

Results: None of the females (F0) died in the study. The clinical observation included reddening of the skin, scab formation, fur staining, thin fur cover, and swelling due to the surgical/infusion methods. Other occasional clinical signs included blue discoloration of the skin, scabs and fur staining. No effects on changes in female body weights/food consumptions were observed. No drug related effects were observed at necropsy, except some secondary changes, such as dark areas in the lungs, lymph nodes, and thymus, and thickening at infusion site, due to experimental procedures. The pregnancy was not affected. There were no differences in the number of corpora lutea, implantation sites, in the number of live/dead fetuses, resorptions, sex ratios, pre or post-implantation losses, or uterine weights, except one dam in the control group was totally resorbed, Table 15.

The fetal weights, the incidence of major or minor skeletal deviations were not different. There were some differences in the incidences of fetuses affected, with visceral and external anomalies, or occasional minor skeletal irregularities, such as, irregular or reduced ossification of the interparietal bones, which was increased at low dose and decreased at mid dose (18 and 2 resp vs 11 in control), the irregular ossification of the supraoccipital bones was increased at 50 μg/kg/min (30 vs 12 in control), decreased ossification of the lumber vertebral arches, at mid and high doses (0 and 0 vs 9 in control), Table 16. However all of these were indicated to be due to biological diversities.

The results indicate that integrilin given to pregnant rats on days 6 to 15 PC, at concentrations of up to 50 μg/kg/min (72 mg/kg/day), was not teratogenic, as it did not cause any maternal or developmental toxicity, it had no effect on the course of pregnancy, or embryo/fetal viability.

Table 15. Effects of Integrilin on maternal and fetal parameters in Segment II. studies in rats

Dose (μg/kg/min)	0	1	5	50
<u>maternal performance</u>				
# of dams	30	30	30	30
# of pregnant rats	27	23	25	28
# of rats with total resorptions	1	0	0	0
# of rats littering, prior to planned cesarean	0	0	0	0
Pregnancy rate (%)	90.0	76.7	83.3	93.3
<u>Uterine findings</u>				
# of corpora lutea/dam	18.9	19.1	18.6	19.0
total implantations/dam	16.2	15.2	15.1	15.5
male fetuses	7.8	8.3	7.0	7.5
female fetuses	7.7	6.2	7.3	7.0
live fetuses	15.5	14.5	14.4	14.5
sex ratio (%)	48.3	56.7	48.5	51.1
Dead fetuses	0	0	0	0
Early resorptions/dam	0.7	0.6	0.7	1.0
Late resorptions/dam	0	0	0	0
Total resorptions/dam	0.7	0.6	0.7	1.0
preimplantation loss (%)	14.2	19.9	18.6	17.9
post-implantation loss(%)	8.0	4.3	4.5	7.1
Gravid uterus weight (g)	92.4	85.9	84.7	84.9
<u>Mean fetal weight</u>				
males	4.0	4.0	4.0	4.0
females	3.8	3.8	3.9	3.8

Table 16. Effects of Integrilin on mean incidence of minor external and visceral anomalies, and minor skeletal anomalies, in Segment II. toxicity studies in rats.

Dose (μg/kg/min)	0	1	5	50
Minor external and visceral anomalies (FA/LA)				
Litters examined for external and visceral anomalies resp	23 and 23	19 and 19	21 and 21	25 and 25
Fetuses examined for external and visceral anomalies resp	372 and 185	276 and 138	302 and 153	362 and 182
Eyes: Retina folded	0/0	1/1	0/0	3/2
Head: Hematoma adjacent to eye	0/0	2/1	2/1	2/2
Minor Skeletal anomalies (FA/LA)				
Litters examined	23	19	21	25
Fetuses examined	372	276	302	362
Skull: Interparietal:				
Irregular ossification	11/5	18/7	2/2	15/8
Reduced ossification	0/0	0/0	1/1	2/1
Supraoccipital:				
Irregular ossification	12/6	12/6	12/5	30/13
Reduced ossification	0/0	0/0	0/0	2/1
Vertebral column:				
Lumber centrum: Semi-bipartite	2/2	4/3	0/0	1/1
Lumber vertebral arches:				
Increased ossification	9/1	10/1	0/0	0/0
Caudal vertebra: Reduced no.	1/1	2/1	5/2 1/1	5/4 4/4
Ribs: Center	2/2	3/2	2/2	3/2
Rudimentary 14th rib	2/1	0/0		

FA = fetuses affected, LA = litters affected,

Segment II. Effects of Integrilin (Continuous IV Infusion) on Teratogenicity in Rabbits
(Study # 95636)

Testing Laboratories:

Study Started: April 25, 1994

Study Completed: August 25, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Female, New Zealand White rabbits (5-5 ½ months old, ~ 2.6-3.9 kilograms).

Drug Batch No.: E0019A.

Methods: 4 groups of mated female rabbits (22 animals in the first 3 groups, 24 animals in the last group,) were given Integrilin by continuous iv infusion, (by an indwelling catheter, placed in the right femoral vein), at doses of 0, 1, 5, 25 µg/kg/min (or 0, 1.44, 7.20, and 36 mg/kg/day), from days 6 to day 18 of gestation. The drug was administered at an infusion rate of 1.5 ml/kg/h. The stock solution was available at the highest dose of 2.0 mg/ml, in a vehicle. Four females from first 3 groups and 6 females from the last group (since 2 animals were sacrificed due to deteriorating conditions, in this group) were assigned to toxicokinetic studies. In all female (F0) rabbits, body weights were measured on days 0, 6, 9, 12, 15, 18, 24 and 29 PC, except for the rabbits in pharmacokinetics studies, which were sacrificed on the 19th day PC. Food consumptions were recorded daily, and clinical evaluations, twice daily. The females were sacrificed on day 29 PC and necropsied. The corpora lutea were counted, the number of implants, the number of dead or resorbed fetuses (F1), the number of live fetuses were examined. All fetuses were individually weighed, and examined for external anomalies. A detailed internal examinations were performed on all fetuses, fetuses were then eviscerated and the heads of 1/3 of these fetuses were examined, using Wilson's technique. The other 2/3 of the fetuses were eviscerated, using Alizarin Red S. staining method and skeletons examined.

For pharmacokinetics studies, ~ 2 ml of blood samples were collected, on the 6th day of gestation, 2 hours after the first treatment, and on the 12th and 19th day of gestation. However, due to sample collection techniques, there was a marked variability in these specimens, therefore, these will be reported separately in future.

Results: One rabbit given 1 μg/kg/min, died, pathology indicated multiple dark areas in the lung, with fluid accumulation in both lungs and trachea. Two rabbits from the pharmacokinetics group had to be sacrificed due to fractured vertebrae, none of these were considered treatment related. The clinical observation included reddening of the skin, scab formation, fur staining/thin fur cover, due to the surgical/infusion methods. Other occasional clinical signs included blue discoloration of the skin, scabs and fur staining, and soft/liquid feces. No effects on changes in female body weights/food consumptions were observed. At necropsy, 4/18 rabbits, from 1 μg/kg/min group, had pale foci in the endocardium or a dark area in the atrioventricular valve, the histopathology showed that 2 animals had slight myocardial fibrosis, and 1 a hematocyst in arterioventricular valve. At 5 μg/kg/min, 2/18 rabbits had similar dark area in the atrioventricular valve, and at 25 μg/kg/min, 4/20 rabbits had pale foci/area in the endocardium. All 6 rabbits showed myocardial fibrosis of varying degrees. However, none of these were attributed to the effects of the drug, as histopathology results from 150 control rabbits showed, an incidence of 24% and 49% of myocardial fibrosis in males and females resp. The pregnancy was not affected. There were no differences in the number of corpora lutea, implantation sites, in the number of live/dead fetuses, resorptions, sex ratios, pre or post-implantation losses, or uterine weights, except four dams (2 at 1 μg/kg/min, and 2 at 25 μg/kg/min) were totally resorbed. The fetal weights, the incidence of major or minor skeletal deviations were not different.

The results indicate that integrilin given to pregnant rabbits on days 6 to 18 PC, at concentrations of up to 25 μg/kg/min, did not cause any maternal/embryo/fetotoxicity or teratogenicity.

Table 17. Effects of Integrilin on maternal and fetal parameters in Segment II. studies in rabbits.

Dose (μg/kg/min)	0	1	5	25
Maternal performance				
# of rabbits inseminated	22	22	22	24
# of pregnant rabbits	17	20	19	20
# of rabbits with total resorptions	0	2	0	2
# of rabbits aborting, prior to planned cesarean delivery	1	0	0	0
Pregnancy rate (%)	77.3	90.9	86.4	83.3
Uterine findings				
Total # of corpora lutea	12.1	12.9	12.3	12.8
total implantation sites	7.3	7.6	8.2	6.8
male fetuses	4.1	3.2	4.2	3.6
female fetuses	2.5	3.8	3.3	3.1
live fetuses	6.6	6.9	7.5	6.3
sex ratio (%)	63.5	43.8	58.8	50.8
Dead fetuses	0	0	0	0.1
Early resorptions	0.4	0.6	0.6	0.5
Late resorptions	0.2	0.2	0	0
Total resorptions	0.6	0.6	0.7	0.5
preimplantation loss (%)	36.1	40.9	32.3	44.0
post-implantation loss(%)	8.8	3.6	8.4	14.9
Gravid uterus weight (g)	420.3	454.9	460.6	399.2
Mean fetal weight				
males	46.9	46.7	46.6	44.4
females	45.1	46.7	44.6	45.6

Table 18. Effects of Integrilin on mean incidence of major, minor external and visceral anomalies, and minor/common skeletal anomalies, in Segment II. toxicity studies in rabbits.

Dose (μg/kg/min)	0	1	5	25
Litters examined	14	16	16-17	15
Fetuses examined	93	118	122-127	100
Major malformations (FA/LA)				
Abdomen: gastroschisis	0/0	1/1	0/0	1/1
Minor external and visceral anomalies (FA/LA)				
Gallbladder: absent	0/0	0/0	1/1	3/2
Minor Skeletal anomalies (FA/LA)				
Extra pre-sacral vertebra	2/2	7/6	6/6	4/3
ossification center on 1st lumbar vertebra	1/1	2/2	1/1	2/2
caudal vertebra: reduced no.	3/2	7/4	13/5	5/4
Common skeletal variants: Ribs: (FA/LA)				
Unilateral 13th (%)	10.3	10.5	8.8	16.3
bilateral 13th (%)	25.0	31.7	25.6	29.7
Sternebrae (FA/LA) (%) (absent/reduced/irregular/ semi-bipartite/bipartite)	35.0	29.5	27.0	23.9

LA = litters affected, FA = fetuses affected.

Segment III, Effects of Integrilin (iv infusion) On Pre- and Post-Natal Development in Rats
(Study # 95629)

Testing Laboratories:

Study Started: March 12, 1994

Study Completed: August 25, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley rats (Crl:CD(SD)BR)
Animals were 80-85 days old (225-306 g).

Drug Batch No.: D0014A, E0019A

Methods: Four groups of 30 mated female (F0) rats were given Integrilin by continuous iv infusion (by an indwelling catheter, placed in the right femoral vein), at doses of 0, 1, 5, and 50 µg/kg/min (or 0, 1.44, 7.20, and 72 mg/kg/day), from day 6 of pregnancy, till day 20, of lactation. The drug was administered at an infusion rate of 1.5 ml/kg/h. Solutions of the vehicle (25 mM sodium citrate, pH 5.25) and the drug were used as provided for control and 50 µg/kg/min (group 1 and 4 resp), for the lower dose groups 2 and 3, the drug was diluted with sterile 0.9% saline. The dose selection was based on the availability of the highest dose of 2.0 mg/ml stock solution in a vehicle. This represented a volume of 36 ml/kg/day, in animals, which is ~ 50% of the blood volume.

F0 generation: In each animal, body weights were measured on days on days 0, 3, 6, 9, 12, 15, 18, and 20 PC, and on days 0, 4, 7, 14, 17, and 21 postpartum (PP). Food consumptions were recorded on days 0-6, 6-9, 9-12, 12-15, and 15-18 of gestation. At delivery, the pups were examined for malformations, sexed and the number of alive/dead pups were recorded. Clinical condition and body weights of pups were noted on days 4, 7, 14, and at 21 days after birth. Physical development of pups (such as pinna folding, tooth eruption, eye opening), righting reflex, auricular startle response, etc were examined. After the 21 days PP, the mothers were separated from their pups, and 1 male and 1 female from each litter were used to form F1 adult generation, the rest were sacrificed and received a complete gross pathological examinations.

F1 generation: Animals were examined for clinical signs. Body weights of mated female rats were recorded on day 0, 7, 14 and 21 PP. Physical development of F1 adults (vaginal opening, preputial separation), visual function, and behaviors (motor activity, etc) were evaluated. When these rats were ~85 days old, 1 male + 1 female, from the same dose group, were mated. F1 dams were observed from day 20 onwards, for signs and times of parturition. The F1 dams, F2 pups, and F2 litters were evaluated as described above for F0 dams, F1 pups, and F1 litters.

Remaining females from F0 and F1 generation, and F1 males after 3 weeks of mating period, were all subjected to complete necropsy. Similarly, pups from F1 and F2 generations were subjected to necropsy/internal-external gross examinations.

Results:

F0 generation adults

No drug related mortality was observed. The clinical observation included reddening of the skin, scab formation, fur staining/thin fur cover, and areas of swelling, due to the surgical/infusion methods. Other occasional clinical signs included blue discoloration of the skin, scabs and fur staining. No changes in body weights/food consumptions or gross pathology were observed except some infusion site lesions, and other incidental findings, such as dark areas or discoloration of lymph nodes, and thickening in subcutaneous tissue. Maternal performance was unchanged, as gestation index, duration of parturition, number of born live or dead pups or sex ratio were all normal, Table 19.

F1 generation pups

No clinical findings were seen in pups, the pup body weights, viability indices, survival, lactation, physical and reflex development (righting reflex, negative geotaxis and auricular startle) were all unchanged. Gross pathology was unchanged, except some incidental findings such as cyst and/or dilatation of the kidneys, pale or depressed area in the lungs, and thickening of the cecum, Table 20. In pups, days 0-7 PP, which were either dead or were dying during that period, one dead pup had anophthalmia, others had empty stomachs.

F1 generation adults

One male rat, at 1 μg/kg/min, was found dead in the 5th week, and one control female also died, both had no clinical signs and the pathology report was unable to find the cause of the death in both rats. One female rat at 5 μg/kg/min dose, had transient convulsions prior to death, which was the result of perforation of aortic arch. No changes in body weight (during gestation/ lactation/following weaning), physical development (vaginal opening, preputial separation), visual function, behavior, reproductive performance (fertility, gestation, number of live/dead fetuses) or gross pathology were noted. Paternal fertility/conception rate were unchanged, the mean day of mating was decreased compared to control at 50 μg/kg/min (1.80±2.34 vs 3.08±1.26 days), but was within the biological variation and not drug related. The maternal performance (gestation length, number of born live/dead pups or sex ratio) was normal, Table 21.

F2 generation pups

The viability of pups, survival, lactation, body weights, clinical or terminal findings were all unaffected, except some incidental findings, Table 22.

The results indicate that integrilin (1, 5, or 50 μg/kg/min) given to female rats during days 6 postcoitus to 20 days postpartum, had no effects on maternal toxicity. Similarly no effects were noted on F1 generation animals, i.e. post weaning behavior, physical/sensory development, reflexes, or on reproductive performance, and it was not teratogenic in F2 generation pups. Therefore, integrilin does not affect pre- or post-natal development in rats.

Table 19. Effects of Integrilin on maternal and neonate parameters in F0 generation rats, in segment III studies.

Dose (μg/kg/min)	0	1	5	50
# of animals examined	30	30	30	30
Pregnancy rate (%)	93.3	86.7	83.3	93.3
Length of gestation (days)	21.6	21.5	21.6	21.6
Sex ratio (%)	50.04	55.07	50.7	49.85
# of live pups at birth	15.3	13.1	13.8	14.7
# of dead pups	0.11	0.20	0.32	0.19
# of implant scars	16.3	14.8	15.0	16.6
Post implant loss (%)	5.8	20.2	7.3	14.1
Gross pathological findings in F0 generation rats:				
Infusion site:				
Mass	2	0	1	4
Thickening	0	0	1	0
Lymph nodes:				
Enlargement	1	0	0	7
Discoloration	1	0	0	1
Dark area	0	0	0	2
Subcutaneous tissue:				
Thickening	0	0	0	2

Table 20. Effects of Integrilin on F1 generation pups, in Segment III. studies in rats.

Dose (μg/kg/min)	0	1	5	50
Viability indices:				
Day 4 Viability index (%)	96.3	98.2	98.7	96.0
Day 14 survival index (%)	99.6	95.1	100.0	99.0
Day 21 Lactation index (%)	99.6	95.1	100.0	98.6
Litter size: 21 days post partum:				
Males	4.0	3.6	4.0	4.0
Females	3.9	3.7	3.9	3.9
Pup body weights: 21 days post partum:				
Males	52.9	55.7	57.0	53.8
Females	50.8	53.0	54.1	51.2
Gross pathological findings in F2 pups:				
Males				
# of animals/group	84	64	74	78
# of animals examined	84	64	74	78
Cecum: Thickening				
Kidney:				
Cyst	4	6	7	9
Dilatation	0	4	4	2
Lung:				
Dark areas	2	0	0	1
Uncollapsed	0	0	0	1
Females				
# of animals/group	81	66	72	76
# of animals examined	81	66	72	76
Cecum: Thickening				
Kidney:				
Cyst	4	9	7	2
Dilatation	2	0	1	2
Lung:				
Dark areas	1	0	0	0
Uncollapsed	0	0	0	1

Table 21. Effects of Integrilin on F1 generation adults, in Segment III. studies in rats.

Dose (μg/kg/min)	0	1	5	50
Body weights of females during gestation (21st day)	487.7	486	487.7	482.8
Body weights of females during lactation (21st day)	376.2	369.2	379.1	362.0
Paternal performance				
# placed for matings:				
Males	28	22	25	26
Females	28	22	25	26
# mated	25	18	23	25
Mean days to matings	3.1	2.0	3.0	1.8
# of pregnant rats	20	14	19	16
Conception rate (%)	80.0	77.8	82.6	64.0
maternal performance				
Length of gestation (days)	22	21.9	21.9	21.9
# of live pups	14.7	14.4	15.2	16.5
# of dead pups	0.47	0.14	0.16	0.25
Sex ratios (%)	48.0	52.9	54.7	58.0
# of implant sites	16.6	15.4	16.3	17.8
Post implantation loss (%)	11.0	6.5	7.1	7.2
Gross pathological findings in F1 rats:				
Males				
# of animals/group	28	23	25	26
# of animals examined	28	23	25	26
Kidney:				
Dilatation	6	4	4	5
Area decreased	1	0	2	0
Thymus:				
Dark foci	0	0	0	0
Dark area	1	3	3	0
Females				
# of animals/group	28	22	25	26
# of animals examined	28	22	25	26
Kidney:				
Dilatation	1	0	1	4
Area decreased	0	0	0	0
Thymus:				
Dark foci	1	2	0	2
Dark area	1	1	1	1

Table 22. Effects of Integrilin on F2 generation pups, in Segment III. studies in rats.

Dose (μg/kg/min)	0	1	5	50
Viability indices:				
Day 4 Viability index (%)	95.5	99.5	99.1	98.6
Day 14 survival index (%)	97.6	99.1	100.0	100.0
Day 21 Lactation index (%)	97.0	99.1	100.0	100.0
Litter size, 21 days post partum:				
Males	3.9	3.9	4.0	4.0
Females	3.7	3.7	3.8	4.0
Pup body weights, 21 days post partum:				
Males	57.9	56.5	62.1	59.0
Females	54.5	53.8	59.0	56.8
Gross pathological findings in F2 pups:				
Males				
# of animals/group	75	54	72	65
# of animals examined	75	54	72	65
Kidney:				
Cyst	7	2	7	5
Dilatation	1	1	4	1
Lung:				
Uncollapsed	0	1	0	5
Trachea:				
Fluid	0	1	0	5
Females				
# of animals/group	71	52	69	63
# of animals examined	71	52	69	63
Kidney:				
Cyst	6	3	2	5
Dilatation	3	1	0	1
Lung:				
Uncollapsed	1	1	0	4
Trachea:				
Fluid	1	1	0	4

SPECIAL TOXICITY STUDIES:

Following studies were submitted to amendment dated January 24, 1991, and were reviewed on 2/13/1991. These are reproduced below.

Antigenicity Study in Guinea Pigs (Study # PH-742-COR-001-90)

Testing Laboratories:

Study Started: August 8, 1990

Study Completed: August 30, 1990

Drug Batch No.: A0001A

Animals: Male and female Hartley guinea pigs (300-500 g)

Methods: Groups of 3 male and 3 female pigs were sensitized by s.c. injections of vehicle with 10% alum, C-68-22 with 10% alum or C68-22 without alum, once every week for three consecutive weeks. At week 4 the animals were challenged by intravenous administration of vehicle or test article. All animals were observed at 0, 1, 2, 4 and 24 hours after the challenge.

Results: There were no clinical signs or mortalities in any groups. Thus C68-22 has no immunogenic potential in the guinea pig. However, no positive control was included in this study. Sponsor should be asked to repeat this study with the inclusion of a positive control.

Induction of Delayed-Type Hypersensitivity (DTH)
(Study # PH 711-COR-001-90)

Testing Laboratories:

Study Started: August 14, 1990

Study Completed: August 22, 1990

Drug Batch No.: A0001A

Animals: Eight weeks old BALB/CBYJ female mice

Methods: Groups of 10 female mice were sensitized intradermally with 100 mcg of C68-22, 100 mcg of C68-22 plus complete Freund's adjuvant (CFA), 100 mcg of ovalbumin (OVA) plus CFA, or vehicle (A0002A). Seven days after the sensitization, the animals were challenged by each respective test material into the right foot pad. Left footpad of each mouse received the vehicle. Twenty-four hours after the challenge the hind paws of each mouse were measured.

Results: C68-22 did not induce delayed-type hypersensitivity in the mouse, while positive control gave expected result. Thus C68-22 has no immunogenic potential in this test.

Acute I.V. Irritation Study in Rabbits
(Study # PH-422-COR-001-90)

Testing Laboratories:

Study Started: August 27, 1990

Study Completed: August 30, 1990

Drug Batch No.: A0001A

Animals: Adult male and female Albino New Zealand White rabbit (2-3 kg)

Methods: Four male and 4 female rabbits were given i.v. infusion of C68-22 (10 mcg/kg/min for 15 min) in the right marginal ear veins, while the left marginal ear veins received the vehicle in the similar fashion.

All the animals were observed for clinical signs and mortality at 1, 4, 24, 48 and 72 hours after the start of injection. Two rabbit/sex were sacrificed at 24 and 72 hours after the drug administration, and ears were examined histologically.

Results: No clinical signs were seen in any animals, and no mortality was observed. Very slight to moderate erythema and very slight edema at the infusion site(s) were seen in all control and treated ears. Histological examination did not reveal a significant vascular tissue reaction. The vehicle alone caused vascular damages such as minimal to moderate vascular degeneration, vascular endothelial loss and formation of fibrin thrombi adjacent to these vascular changes.

3. Effect on Red Blood Cells Hemolysis: No significant hemolysis was seen when 20-200 mcg of C68-22 was added to 1 ml of whole blood.

4. Plasma Protein Flocculation Test: No significant amount of protein flocculation was seen when 20-200 mcg of C68-22 was added to 1 ml of human plasma.

Following studies were submitted to and were reviewed on 10/4/1990. These are reproduced below.

Immunomodulatory Properties

Delayed-Type Hypersensitivity to Oxazolone in Mice: C68-22 (300 mcg/kg, i.v.) had no effect on immune responses of oxazolone applied topically to mouse.

Antagonism Studies

C68-22 (300 mcg/kg, i.v.) had no effect on size of the wheal at the injection site after intradermally administration of histamine or platelet activating factor.

14-Day Continuous IV Infusion Toxicity Study of Degraded Integrilin in the Cynomolgus Monkey
(Study # BIO 54516)

Testing Laboratories:

Study Started: June 6, 1995

Study Completed: February 13, 1996

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Young adult male (3.2-4.0 Kg) and female (2.6-3.7 Kg) cynomolgus monkeys.

Drug Batch No.: E0028A. Degraded integrilin at 45^{0}C to 83.8% of initial conc.

Methods: The object of this study was to examine if degraded integrilin (83.8% purity) had toxic effects in monkeys.

The degradents include a major dimer product + other minor intermediary products. 4 Groups of (2 male and 2 female/group) monkeys were given continuous intravenous infusions of degraded integrilin at 1, 5, and 12.5 µg/kg/min (or 0, 1.44, 7.20, and 18 mg/kg/day) for 14 consecutive days. 1.5 ml/kg/hr of the drug was infused via an implanted catheter in the right femoral vein. Control groups received, 27 mM citrate solution, pH 5.45 (placebo). The dosing

solutions from the first and the last day were analyzed. All animals were sacrificed at the end of the study periods. Mortality and clinical signs were observed twice daily. Body weights were noted once weekly, food consumptions/appetence were determined daily. Ophthalmoscopic examinations were performed on all animals prior to treatment, and before the end of the treatment period, in the 2nd week. For hematology and clinical chemistry tests, blood was collected from the abdominal aorta of all animals, at baseline, and on day 14. Urinalysis was done prior to treatment and before sacrifice. Plasma drug conc were determined in a single sample at baseline, and on days 2 (~24 hrs after infusion), 7, and 14. Heart rates, blood pressures, and ECG were assessed at baseline and on day 11. Gross pathology as well as complete necropsy with histopathological examinations were carried out on all animals.

Results:

1. Observed Effects: No drug related clinical signs were observed.

2. Mortality: No drug related mortality was observed.

3. Body Weight/Food Consumption/Appetence: Body weights and food consumptions/appetence were all unaffected.

4. Hematology/Coagulation: No significant effects were noted. Platelet count in one female monkey (301 x 10^3), which had some unusual findings, was also not significantly different than the second monkey from the same group (310 x 10^3), or in 2 control female monkeys (609 and 371 x 10^3 resp).

5. Blood Chemistry/Urinalysis: No treatment related changes in blood chemistry or urinalysis were observed.

6. Plasma levels: A dose-related increase in plasma levels of the drug was seen between 5 and 12.5 μg/kg/min doses, whereas, concentrations at 1 μg/kg/min were below the level of quantitation. No sex differences were noted in plasma conc, Table 23.

7. Cardiovascular: The heart rates, blood pressures or ECG were unchanged.

8. Organ Weights: No drug related differences were found.

9. Gross Pathology: In both control and treated animals, there were constant findings of small testes, epididymides, prostate and thymus, as well as dark areas in the lungs.

9. Gross and Histopathology: No changes were seen here in both male monkeys, but in 1 of 2 high dosed female monkey had some unusual findings. In the bone marrow, moderate erythroid hypocellularity and enlarged megakaryocytes were noted. In the adrenal, a diffuse cortical hypertrophy with loss of normal cytoplasmic vacuolation and a mixed inflammatory cell infiltration was seen. In lungs, an intravascular infiltration of macrophages, and in splenic red pulp, an infiltration of histiocytes were observed. Other common findings at infusion site included thrombosis, intimal proliferation and inflammatory changes, which were suggested to be due to an experimental procedure. These observations were not seen in previous 14 and 28 day toxicity studies in monkeys.

These studies indicate that doses of degraded integrilin, up to 7.2 mg/kg/day (5 μg/kg/min), did not produce any toxicity in male or female monkeys, when given for 14 continuous days. At 18 mg/kg/day (12.5 μg/kg/min), no effects were noted in the male monkeys, but one of the 2 female monkeys, had several unusual findings in endocrine and hematopoietic systems, which may have been due to bacterial phlebitis seen at the infusion site or due to the effect of the drug itself. It would have been more useful, if a group of monkeys had received intact undegraded integrilin in this study, to compare the effects of the pure vs degraded one.

Table 23. Plasma concentrations of degraded integrilin in 2 male and 2 female monkeys.

On Days Determined	Plasma Concs of Integrilin (ng/ml)							
	Males (n = 2)				Females (n = 2)			
	5 μg/kg/min		12.5 μg/kg/min		5 μg/kg/min		12.5 μg/kg/min	
	# 1	# 2	# 1	# 2	# 1	# 2	# 1	# 2
2	237	104	814	726	247	406	702	908
7	340	250	733	527	300	237	∞	670
14	222	142	861	900	319	415	745	787

∞ = insufficient volume

GENOTOXICITY STUDIES:

Salmonella/Escherichia Coli Reverse Mutation Test: Ames test
(Study # HWA 16265-0-422)

Testing Laboratories:

Study Started: June 21, 1994

Study Completed: August 30, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Cells Employed: Salmonella typhimurium histadine auxotrophs strains TA98, TA100, TA1535, TA1537, and E.coli strain WP2uvrA.

Concentration Employed: 10, 33.3, 66.7, 100, 333, and 667 µg/ml of reaction mixture.

Basis of Dose Selection: The highest dose was based on the highest volume of the test substance, that can be used, without altering the test. This volume was 300 µl, which came from a stock of 2 mg/ml of integrilin, giving the maximum dose of 667 µg/ml of the incubation mixture.

Solvent Control: 25 mM citrate, pH 5.25.

positive Controls: 2-Aminoanthracene (3.8 µg/ml), sodium azide (3.1 µg/ml), 2-nitrofluorene (1.5 µg/ml), ICR-191 (3.1 µg/ml), 4-nitroquinoline-N-Oxide (1.5 µg/ml) and 2-aminoanthracene (38.5 µg/ml).

Source of Metabolic Activation: Rat liver microsome S9 fraction.

Drug Batch No: E0019A

Criteria of Genotoxic Effect: At least a 3-fold increase in the number of revertant colonies above the solvent control value for the strains TA1535, and TA1537, and at least a 2-fold increase in the number of revertant colonies above the solvent control value for the strains TA98, TA100, and WP2uvrA are considered as positive.

Results: Integrilin was not mutagenic in any of the tester strains at doses ranging from 10-667 μg/ml (plate), in the presence or absence of metabolic activation (S9 mix). A significant increase in mutant colonies was observed in all the microbial strains employed with positive controls (with or without S9 mix).

Salmonella/Escherichia Coli Reverse Mutation Test: Ames test
(Study # CHV 17110-0-422)

Testing Laboratories:

Study Started: November 3, 1995

Study Completed: January 19, 1996

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Cells Employed: Salmonella typhimurium histadine auxotrophs strains TA98, TA100, TA1535, TA1537, and E.coli strain WP2uvrA.

Concentration Employed: 10, 33.3, 66.7, 100, 333, and 667 μg/ml of reaction mixture.

Basis of Dose Selection: The highest dose was based on the highest volume of the test substance, that can be used, without altering the assay. This volume was 300 μl, which came from a stock of 2 mg/ml of integrilin, giving the maximum dose of 667 μg/ml of the incubation mixture.

Solvent Control: 25 mM citrate, pH 5.25.

Positive Controls: 2-Aminoanthracene (3.8 μg/ml), sodium azide (3.1 μg/ml), 2-nitrofluorene (1.5 μg/ml), ICR-191 (3.1 μg/ml), 4-nitroquinoline-N-Oxide (1.5 μg/ml) and 2-aminoanthracene (38.5 μg/ml).

Source of Metabolic Activation: Rat liver microsome S9 fraction.

Drug Batch No: E0028A (SCH 60936).

Criteria of Genotoxic Effect: At least a 3-fold increase in the number of revertant colonies above the solvent control value for the strains TA1535, and TA1537, and at least a 2-fold increase in the number of revertant colonies above the solvent control value for the strains TA98, TA100, and WP2uvrA are considered as positive.

Results: Integrilin was not mutagenic in any of the tester strains at doses ranging from 10-667 μg/ml (plate), in the presence or absence of metabolic activation (S9 mix). A significant increase in mutant colonies was noted with positive controls (with or without S9 mix).

Mouse Lymphoma Forward Gene Mutation Test of Integrilin
(Study # HWA 16265-0-431)

Testing Laboratories:

Study Started: June 15, 1994

Study Completed: August 23, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Cells Employed: Mouse lymphoma cell line,

Concentration Employed: 62.5, 125, 250, 500, 750, and 1000 μg/ml doses were used with or without metabolic activation.

Basis of Dose Selection: The highest dose was based on 1:2 dilution of the drug, as supplied by the sponsor.

Solvent Control: Two negative controls were used, 1:2 dilution of sterile water : RPMI culture medium, and 12.5 mM citrate buffer in RPMI culture medium.

Positive Controls: Methyl methane sulfonate (MMS), 10 and 15 ng/ml, was used without metabolic activation, and 3 methyl cholanthrene, 2 and 4 μg/ml, with metabolic activation.

Source of Metabolic Activation: Rat liver enzymes (S9) fraction.

Drug Batch No: E0019A

Criteria of Genotoxic Effect: If mutant frequency is ≥2-fold the mutant frequency of the vehicle control, and there is a dose dependent increase in mutant frequency with preferably, at least 3 doses of the test substance, the test is considered positive.

Method: Cells (6×10^6) with or without S9 activation mixture were incubated for ~4 hours, with the indicated concentrations of integrilin, along with the positive and negative controls (including an untreated control). At the end of the experiment (after 10 to 14 day), the mutant frequency and the viability of the cells (as % relative growth) was determined.

Results: Only one mutation assay was carried out, using 6 concentrations of integrilin. Doses, up to 1000 μg/ml, did not cause any toxicity, and all doses of integrilin, with or without metabolic activation, showed no significant differences in mutant frequencies, between vehicle control and the treated samples, in the mouse lymphoma cells. In contrast, a significant increase in the number of mutant frequency was observed in all the positive controls.

Effects of Integrilin on Chromosome Aberration In Human Peripheral Blood Lymphocytes
(Study # CHV 17110-0-449)

Testing Laboratories:

Study Started: August 30, 1995

Study Completed: January 17, 1996

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Cells Employed: Cultured peripheral human lymphocytes were prepared from two donors (1 male and 1 female).

Concentration Employed: 31.3, 62.5, 125, 250, 500, and 1000 µg/ml were used.

Basis of Dose Selection: The highest dose of 1000 µg/ml was based on the highest volume of the test substance, which could be used, without altering the assay. This dose was constituted from the stock of 2 mg/ml of integrilin.

Solvent Control: RPMI culture medium:25 mM citrate buffer, pH 5.25 (1:1 concentration) was used as a solvent control. RPMI medium alone was used as a negative control.

Positive Controls: Mitomycin C (0.1-1.5 µg/ml) was used in the absence of metabolic activation, and cyclophosphamide (20-150 µg/ml), in the presence of metabolic activation.

Source of Metabolic Activation: Rat liver S9 fraction.

Drug Batch No: E0028A

Criteria of Genotoxic Effect: If percentage of cells with aberrations are increased in a dose related manner, with one or more doses demonstrating statistically significant increase in relation to the vehicle or negative control, the test is considered positive. In the absence of dose relation, the significant results must be reproducible.

Results: Integrilin did not show any increase in structural chromosome aberrations, at any concentration tested, ranging from 31.3 to 1000 µg/ml, with or without metabolic activation. The concentrations of the drug analyzed, were within ±4% of their respective doses. Also the drug did not affect the pH, or the osmolality. The positive controls showed a highly significant increase in chromosome aberrations. Therefore, integrilin was not mutagenic in this assay.

In Vivo Micronucleus Test of Integrilin in Mice
(Study # HWA 16265-0-455)

Testing Laboratories:

Study Started: June 14, 1994

Study Completed: August 19, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Test Strain: Adult male (27.8-34.5 g) and female (23.2-30.1 g) ICR mice.

No. of Animals: 5 animals/sex/group/sacrifice time.

Route of Administration: IV

Concentration Employed: 10, 20, and 40 mg/kg body weight.

Basis of Dose Selection: Dose levels were selected by the sponsor, and the drug was supplied as 2 mg/ml of stock solution in 25 mM citrate buffer.

Solvent Control: 25 mM citrate buffer.

Positive Controls: Cyclophosphamide (CP), 80 mg/kg.

Drug Batch No: E0019A

Criteria of Genotoxic Effect: If a statistically significant dose related increase in the number of micronucleated polychromatic erythrocytes (PCE) was observed, compared to the vehicle control, or if a reproducible and significant positive response for detected for at least one dose, the test was considered positive.

Results: Integrilin did not cause any toxicity in any of the treated mice. It also did not induce an increase in micronucleated PCE, or the ratio of PCE to normochromatic erythrocytes (NCE), in either male or female mice bone marrow, at any dose level, or bone marrow collection time. In contrast, CP induced a significant increase in the micronucleated polychromatic erythrocytes in both male and female mice, compared to the vehicle control.

LABELING:

The indicated labeling of integrilin generally conforms to the format under CFR 21, 201.50 to 201.57, dated April 1, 1996. However, following changes are suggested.

Under 'Overdosage', the sponsor has proposed the following text:

> **OVERDOSAGE**
>
> While there have been no actual overdoses reported with INTEGRILIN™, eight patients in the IMPACT II study received bolus doses and/or infusion greater than called for by the protocol. In no case was the dose greater than double that called for. No serious adverse events or more than mild bleeding were noted in these patients.

The reviewer is suggesting the following contents for labeling.

Clinical signs of integrilin overdosage have not been determined. However, in rats, rabbits and monkeys, 500 μg/kg/min iv infusion doses of integrilin, for 90 min, were not lethal. Clinical signs of toxicity in rabbits (at 10, 50 and 500 μg/kg/min) included dose-related thrombocytopenia, and in monkeys (500 μg/kg/min), petechial hemorrhages in the abdominal and/or femoral regions, persisting for 1-3 days. No clinical signs of toxicity were noted in rats.

SUMMARY AND EVALUATIONS:

Integrilin is a selective inhibitor of platelet GP IIb/IIIa, and its proposed use is, to reduce the acute ischemic cardiac complications of coronary angioplasty. GP IIb/IIIa plays a primary role in both platelet adhesion, and thrombus formation at the site of vascular injury. On resting platelets, this complex is maintained in an inactive state, upon activation, it undergoes

conformational change and becomes a competent receptor for soluble fibrinogen, which promotes fibrinogen-GP IIb/IIIa clusters and platelet aggregation. During coronary angioplasty, atherosclerotic plaque is ruptured, and antithrombotic therapy is a normal practice, to prevent ischemic complications. Although, both aspirin and heparin are currently in use, they carry 2% to 10% risk of complication. Therefore, antiplatelet/antithrombotic agent, such as integrilin, may be useful in these surgical interventions.

Integrilin is indicated as an adjunct to aspirin and heparin in patients undergoing percutaneous transluminal coronary angioplasty (PTCA), for the prevention of acute cardiac ischemic complications (such as death, myocardial infarction, need for urgent intervention) related to abrupt closure of the treated coronary vessel. The recommended adult dose of integrilin is a 135 μg/kg bolus injection, followed by a 0.5 μg/kg/min infusion for a 20-24 hour period. It is supplied as a sterile solution, containing 20 mg/10 ml vial, and 75 mg/100 ml vial.

The sponsor has provided preclinical in vitro and in vivo pharmacology studies, pharmacokinetics, which include absorption, distribution, metabolism and excretion (ADME) of the drug in rats, and monkeys. Acute toxicity studies in rats, and monkeys. Subacute toxicity studies in rats (14 days, 28 days + 14 day recovery, 3-day toxicokinetics) and monkeys (14-days, 28 days + 14 day recovery, 3-day toxicokinetics). Segment I fertility studies in rats, Segment II teratology studies in rats and rabbits, Segment III peri and postnatal studies in rats, genotoxicity studies (Ames test, micronucleus test in mice, mouse lymphoma cell (TK locus) forward gene mutation test, and human lymphocyte chromosome aberration test). Also special toxicity studies (antigenicity in guinea pigs, acute irritation studies in rabbits, delayed type hypersensitivity, test for production of hemolysis and plasma protein flocculation, and 14-day iv toxicity of degraded integrilin in monkeys) are included.

Primary pharmacology studies indicate, that integrilin is a specific inhibitor of von Willebrand factor, and fibrinogen binding, to GP IIb/IIIa receptor, with IC_{50} values of <5 and 100-120 nM respectively. In a baboon dacron vascular graft model of thrombosis, 5-10 μg/kg/min of integrilin inhibit platelet aggregation, and are anti-thrombotic. In a Folt's dog model of thrombosis, integrilin inhibited platelet aggregation (IC_{50} of 1.9 μM), and thrombus formation (1-4 μg/kg/min). The secondary pharmacology studies in several in vivo and in vitro models show that it is devoid of action on CNS, renal, cardiovascular, GI systems, etc.

Since both aspirin and heparin are used in coronary artery syndromes, the interaction of integrilin, with these 2 drugs was examined in a healthy baboon model. Aspirin significantly increases the bleeding time of integrilin (from 7.3 to 14.0 min) and the combination of all 3 agents had additive effects on bleeding times (26.9 min), but no changes in thrombus formation were seen.

The pharmacokinetics of integrilin indicate that the half life of the drug in rats was 8 min, and in monkeys 17 min, after a single iv dose of 2 mg/kg. In rats, the plasma concs increased in proportion to the dose. In humans, the half-life of the drug was 2.2 hours, after 18-24 hour iv infusion, and the plasma drug conc increased in proportion to the dose (0.5-1.5 μg/kg/min). Integrilin was widely and rapidly distributed throughout the body, the peak tissue levels were attained within 0.1 hour after injection in to male rats, and decreased rapidly with time. By 48 hours, only <1% remained in tissues and carcass. Bladder, small intestine contents, and kidney had 2.1, 2.2, and 2.8 fold higher conc resp, than the plasma, at 0.25 hour. Integrilin does not cross the blood-brain barrier. Similar distribution of integrilin was also noted in healthy pregnant rats, at 19 days of gestation. The mean protein binding was 12.3% in rat, 14% in rabbit, 18.4% in monkey, and 24% in human plasma, suggesting that the drug is poorly bound to plasma proteins. The drug was extensively metabolized to deaminated product, and to 2-5 more polar metabolites in blood and urines of male rats, and monkeys. In plasma of both species, at 5-10 min after drug administration, only integrilin was present, but at 30-60 min, not only integrilin (31-40% of total radioactivity), but also deaminated metabolite (11-30%), and 2-3 polar metabolites were present. Similar metabolic patterns (unchanged drug, deaminated product, and 3-5 more polar metabolites) were observed in the urines of both species. In male rats, >90% of the radioactivity was excreted at 24 hours, 70-76% was seen in urine, 5-10% in feces, and 10% as CO_2. In monkeys, 25-51% was recovered in urine, and 6% in the feces. In rats, biliary excretion of integrilin indicates almost complete metabolism to its deaminated and polar metabolites in bile/urine, and 14% of the drug undergoes enterohepatic circulation. In humans, 50% of the administered dose is excreted in urine, both as a parent compound and deaminated integrilin. These studies indicate a similar pharmacokinetic drug dispositions in rats, monkeys and humans.

In acute toxicity studies, the highest single dose tested (500 μg/kg/min, by 90 min continuous iv infusion, with no bolus dose), was not lethal in any species (rats, rabbits or monkeys), as no mortalities were noted. At 10, 50 and 500 μg/ kg/min,

female rabbits showed dose-related thrombocytopenia (338 x $10^3/mm^3$, 263 x $10^3/mm^3$, 125 x $10^3/mm^3$, resp vs control 381 x $10^3/mm^3$), and 24 hours later, the platelet count improved to some extent in rabbits. Monkeys (2 of 3) had petechial hemorrhages in the abdominal and/or femoral regions, which persisted for 1-3 days, at 500 μg/kg/min.

In a 14-day continuous iv infusion toxicity study in rats (with no bolus dose), 2, 10, and 50 μg/kg/min doses were used. No toxicity was seen in any organ at the highest dose.

In a 28-day continuous iv toxicity study, with a 14-day recovery period in rats, doses of 1, 5, and 50 μg/kg/min (or 1.44, 7.20 and 72 mg/kg/day) were used. At the end of 28-day period, all animals were sacrificed, except half of the animals from control and a high dose group, which were kept for 14-day recovery periods. No drug related toxicity was seen in any organ. The 'no effect dose' in rats was 50 μg/kg/min.

In a 14-day continuous iv toxicity study in monkeys, 1, 5, and 50 μg/kg/min doses were used. Three monkeys, in a high dose group, died or were sacrificed due to deteriorating conditions, and the rest had continuous excessive bleeding and petechial hemorrhages resulting in severe anemia. At 5 μg/kg/min, 1 of 5 monkeys had focal hemorrhages in the kidney, and skeletal muscle. At 50 μg/kg/min, focal hemorrhages were noted in hearts (2 of 5 animals), lungs (1 of 5), subcutaneous tissue (1 of 5), and thymus (2 of 5). These studies indicate excessive bleeding, and focal hemorrhages in monkeys, at 5 and 50 μg/kg/min doses.

In a 28-day continuous iv toxicity study, with a 14-day recovery period in monkeys, doses of 1, 5, and 12.5 μg/kg/min (or 1.44, 7.20 and 18 mg/kg/day) were used. At the end of 28-day period, all animals were sacrificed, except 2 animals from control and a high dose group, which were kept for 14-day recovery periods. One of 3 male monkeys, at 12.5 μg/kg/min, had moderate hemorrhage in the skeletal muscle area, along the vena cava. In a previous study, a focal hemorrhage was noted in 1 of 5 monkeys at 5 μg/kg/min. No other drug related toxicity was noted in any organ. In this study, 5 μg/kg/min was a 'no effect dose' in monkeys.

In a segment I fertility study, in male and female rats, the animals were treated by continuous iv infusion of integrilin, at doses of 0, 1, 5, and 50 μg/kg/min. Females were given integrilin for 14 days prior to mating, throughout mating and from days 0 to 7 postcoitus, males were given the drug for 28 days prior to mating, during the matings, until their necropsy. No drug related effects were seen at any drug dose on male or female fertility, or general maternal/paternal reproductive performance of rats or on the progression of pregnancy.

In a segment II teratology study in rats and rabbits, rats were given continuous iv infusion of the drug, at doses of 0, 1, 5, and 50 μg/kg/min from days 6 to day 15 of gestation. Rabbits received the drug at doses of 1, 5, and 25 μg/kg/min from day 6 to day 18 of gestation. These doses did not cause any maternal/embryo/fetotoxicity or teratogenicity in either species.

In segment III study in rats, integrilin was given by a continuous iv infusion, at doses of 0, 1, 5, and 50 μg/kg/min from day 6 of pregnancy to through day 20 of lactation period. No effects on maternal toxicity or in F1 generation animals were seen, i.e. on post weaning behavior, physical/sensory development, reflexes. Similarly reproductive performance were all normal, at doses up to 50 μg/kg/min.

Antigenicity of integrilin was evaluated in guinea pigs, after subcutaneous injection, once/week, for 3 weeks. The drug was not antigenic to guinea pigs. Integrilin did not induce delayed-type hypersensitivity in mice.

Acute iv irritation of integrilin (10 μg/kg/min for 15 min) in rabbits, did not result in any significant erythema.

Integrilin (20-200 μg/kg/min) did not cause any red blood cell hemolysis or plasma protein flocculation, when added to 1 ml of whole blood or plasma resp.

To examine if degraded integrilin (83.8% pure) had any toxic effects in monkeys, it was given to 2 male and 2 female monkeys for 14 continuous days by iv infusion, at doses of 1, 5, and 12.5 μg/kg/min (or 1.44, 7.20 and 18 mg/kg/day). Animals were sacrificed at the end of 14-days. One of the 2 female monkeys, at 12.5 μg/kg/min had bacterial phlebitis at the infusion site.

No mutagenic potential of the drug was seen, when integrilin was tested in the following 4 different tests: Ames test, mouse lymphoma cell forward gene mutation test, human peripheral blood lymphocyte chromosome aberration test, and in vivo micronucleus test in mice.

The proposed dose for the marketing is 135 μg/kg bolus injection, followed by a 0.5 μg/kg/min for 20-24 hours, from a preclinical standpoint, this application is recommended for approval.

RECOMMENDATIONS:

From a preclinical standpoint, approval of this application is recommended.

The sponsor should be asked to make the changes in the 'Overdosage' section of the labeling, as per suggested in the review.

= -

2/25/97
Indra Antonipillai, Ph.D.
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CC:
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HFD-181/CSO
HFD-180/Dr. Antonipillai
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-345/Dr. Viswanathan

R/D Init.: J. Choudary 11/3/96

IA/hw/12/4/96 & 2/25/97

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(1) Noted.

(2) See the accompanying Pharmacology Team Leader's Addendum

2/28/97