CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-977
20-978

PHARMACOLOGY REVIEW(S)

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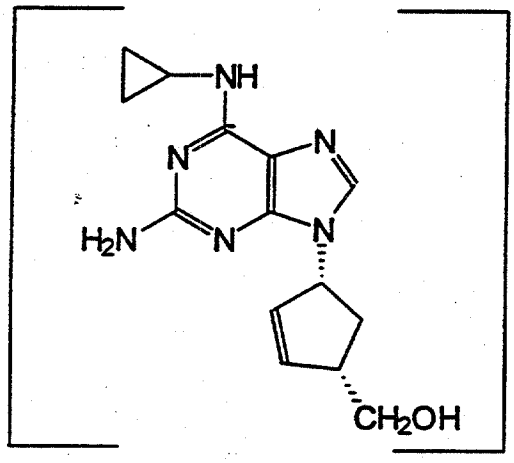
HFD-530

Sponsor: Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park
North Carolina 27709

Drug: ZIAGEN tablets for oral administration, **ZIAGEN** solution for oral administration.



$\cdot H_2SO_4$

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Other names: Abacavir,1592U89 sulfate
Chemical name: [(1S, *cis*)-4-[2-amino-6-(cyclopropylamino) -9H-purin-9-yl] -2-cyclopentene-1-methanol sulfate.(salt) 2:1

Empirical formula: $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$
Molecular weight: 670.76

Formulation: ZIAGEN Tablets for oral administration contain abacavir sulfate equivalent to 300 mg of abacavir. Inactive ingredients include colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate.
ZIAGEN Oral Solution. One milliliter of ZIAGEN Oral Solution contains abacavir sulfate equivalent to 20 mg of abacavir (20 mg/mL) in an aqueous solution. Inactive ingredients include artificial strawberry and banana flavors, citric acid (anhydrous), methylparaben, propylene glycol, propylparaben, saccharin sodium, sodium citrate (dihydrate), and sorbitol solution.

Related IND's:

Indication: Treatment of HIV infection.

Introduction:

Abacavir is a carboxylic nucleoside analog which has *in vitro* activity against HIV. 1592U89 inhibits HIV 1 (strain m B) in peripheral blood lymphocytes, macrophages and MT4 cells (a human leukemia cell line transformed with HTLV-l) and has an IC_{50} of 0.26 μM against fresh HIV isolates cultured in peripheral blood lymphocytes.

The active drug seems to be the triphosphate derivative of [redacted], a potent inhibitor of HIV reverse transcriptase *in vitro*. The putative active form of the drug, [redacted] inhibits reverse transcriptase at concentrations 300-fold less than those needed to inhibit human HeLaDNA polymerases α, β, γ and ε.

Abacavir is extensively metabolized and in man only 3 % of the administered dose is renally excreted unchanged. The primary pathways for metabolism in man are by alcohol dehydrogenase and by UDP glucuronyl transferase to produce the 5' carboxylate (2269W93) and the 5' glucuronide (361W94) metabolites. In humans, 83 % of a dose is recovered in the urine while 16 % of the dose is recovered in feces. Of the 83 % in the urine, < 2% exists as unchanged abacavir, 30 % as the carboxylate metabolite, 36 % as the glucuronide metabolite and 15 % is presumably minor metabolites. Abacavir is rapidly absorbed after oral administration, with peak concentrations occurring within 1 to 2 hours after dosing with tablets and within 0.5 to 1 hr after oral solution. The mean terminal half-life is approximately 1.5 hours. At therapeutic doses, (300 mg twice daily as a tablet) the steady-state peak plasma concentration of abacavir was 3.0 μg/ml and the overall exposure (AUC_{0-12h}) was 6 μg*hr/ml.

Toxicology Studies Summary

Acute studies- Mice and Rats

1. An acute oral toxicity study in the mouse with 1592U89. Report TTEP/93/0018.
2. An acute oral toxicity study of 1592U89 succinate in CD rats. Report TTEP/93/0016.
3. An acute intravenous toxicity study in CD-1 mice. Report TTEP/93/0019.
4. An acute intravenous toxicity study (limit test) in CD rats. Report TTEP/93/0017.

Repeat Dose Studies-Mice

5. A three day oral dose range finding toxicity/pharmacokinetic study in Charles River CD-1 mice given 1592U89 succinate. Report TTDR/92/0036.

6. A 21 day oral dose range finding study in Charles river DC-1 mice given 1592U89 succinate. Report TTDR/92/0035.
7. A 30-day oral toxicity study in CD-1 mice. Report TTEP/94/0006.
8. A 30-day oral toxicity study of 1592U89 succinate (with "diol method" impurities) in the CD-1 mouse. Report TTEP/95/0015.
9. A three-month oral toxicity study in mice given 1592U89 succinate. Report TTEP/94/0035.
10. A six-month oral toxicity study in mice given 1592U89 succinate. Report RD 1996/00245/01.

Repeat Dose Studies-Rats

11. A 21-day oral range finding toxicity study in rats given 1592U89. Report TTDR/92/0014.
12. A 30-day oral range finding toxicity study in rats given 1592U89 dihydrochloride. Report TTDR/91/0024.
13. 1592U89: A 90-day oral dose range finding study in Han Wistar rats. Report RD1997/03595/00.

Repeat –Dose Studies –Monkeys

14. A two-phase oral range-finding study with 1592U89 succinate in Cynomolgus monkeys. Report TTDR/93/0001.
15. A 30-day oral, dose range finding study in Cynomolgus monkeys with 1592U89 (base). Report TTDR/91/0030.
16. A 28-day, oral toxicity study in cynomolgus monkeys Report TTEP/94/0007.
17. A three-month oral toxicity study in cynomolgus monkeys given 1592U89 succinate. Report TTEP/94/0047.
18. A 12-month repeated dose oral toxicity study with a six month interim sacrifice in Cynomolgus monkeys given 1592U89 succinate. Report RD1996/00310/01.

Reproductive Toxicology Studies

19. Preliminary Dose-range Finding embryo-fetal development study of 1592U89 in the CD rat. Report TTDR/96/0005.
20. Oral toxicokinetic study in pregnant CD rats given 1592U89. Report RD1997/01057/00.
21. Dose range finding study of 1592U89 succinate administered by gavage to nulliparous New Zealand White rabbits. Report RD1996/00156.
22. Dose range finding study of 1592U89 succinate administered by gavage to timed mated New Zealand White rabbits. Report RD1996/00155.
23. Oral embryo fetal developmental study of 1592U89 succinate in pregnant New Zealand White rabbits. Report RD1997/01058/00.

24. Oral toxicokinetics study of 1592U89 succinate in pregnant New Zealand White rabbits. Report RD1997/01059/00.
25. Oral pre and post-natal developmental toxicology study of 1592U89 hemisulfate in CD-1 rats. Segment III. Report RD1997/040233.

Mutagenecity/genotoxicity Studies

26. Salmonella/Mammalian Mutagenicity Assay Report TTEP/93/0034.
27. 1592U89 succinate GI265235A): *In vitro* assay for chromosomal aberrations in cultured human whole blood lymphocytes. Report UTX/95/126.
28. Bone marrow micronucleus assay in male and female CD-1 mice dosed orally with 1592U89 succinate. Report TTEP/95/0073.

Toxicology Studies Review

1. An acute oral toxicity study in the mouse with 1592U89. Report TTEP/93/0018.

[redacted]. December 1992

Groups of CD-1 mice (5/sex/dose) were given single doses of 1592U89 at 1500, 1700 or 1900 mg/kg/day. Abacavir was dissolved in 0.5 % methylcellulose and administered to mice by oral gavage. For the next 14 days animals were monitored for clinical signs, body weights and mortality. Animals found dead during the postdose period were subjected to gross examination and discarded while surviving mice were sacrificed on postdose day 14 and gross necropsies were performed.

Deaths occurring during the study are shown in Table 2. Clinical signs were found at all doses in males and included ptosis, decreased activity, dehydration, coolness to touch and body tremors. At 1700 mg/kg and 1900 mg/kg, 1 (of 5) and 5 (of 5) male mice respectively showed labored breathing. In females, decreased activity or ptosis were observed, and mice treated at the two higher doses, weighed less on day 13 than mice treated with 1500 mg/kg 1592U89. No drug-related gross findings were detected at the postday 14 sacrifice.

Table 1. Mortality associated with oral dosing with 1592U89 in mice.

Dose group	Number of deaths	Day of death
1500	2 males	2 days postdose
1700	1 male	2 days postdose
1900	1 male	day 1 of dosing
	3 males	1 day postdose

The minimum lethal oral dose of 1592U89 was 1500 mg/kg, equivalent to a dose of 121.5 mg/kg/day based on body surface area conversions.

2. An acute oral toxicity study of 1592U89 succinate in CD rats. Report TTEP/93/0016. Study ACU 521. Toxicology a nd Pathology, December 199[illegible]

This study was designed to determine the acute toxic effects of orally administered 1592U89 (dissolved in methyl cellulose) in CD rats. Two groups of rats, 5 rats/sex /group, were given single oral doses of 1800 or 2000 mg/kg of 1592U89 succinate. During a 14-day observation period, clinical signs and body weights were monitored. All animals were sacrificed and examined on post-dose day 14.

One female in each of the dosage groups died but there were no deaths among the males. Clinical signs noted before deaths in the females included decreased activity, lacrimation, ptosis and salivation. There were no drug related adverse events noted in the post-dosing period and no treatment-related gross findings at the postday-14 sacrifice.

Minimum lethal oral dose was 1800 mg/kg which was equivalent to a human dose of 286 mg/kg/day based on body surface area conversions.

3. An acute intravenous toxicity study in CD-1 mice (TTEP/93/0019)

December 1992.

Two groups of mice, 5 males and 5 females per group, were treated with one intravenous dose of 195 or 260 mg/kg of 1592U89 succinate (dissolved in saline). Mice were observed for a 14-day period during which clinical signs and body weights were recorded. On day 14 postdose, all animals were sacrificed and subjected to necropsy.

There were no deaths in any of the treated groups and so the minimum lethal intravenous dose was determined to be > 260 mg/kg. Clinical signs commonly observed

at both doses included decreased activity, rapid breathing and ptosis. Ataxia was observed in males given 260 mg/kg 1592U89 succinate. There were no other remarkable findings.

Since all dose groups experienced adverse effects, a NOAEL could not be determined. Also since there were no deaths in any of the treated groups, the minimum lethal intravenous dose was determined to be > 260 mg/kg.

4. An acute intravenous toxicity study (limit test) in CD rats.

[redacted]
December 1992.

Five male and five female Charles River CD rats were treated with one intravenous dose of 260 mg/kg 1592U89 succinate (in saline). This was the maximum dose that could be given considering the solubility of 1592U89 succinate and the maximum practical intravenous dose in rats. Clinical signs and body weights were monitored over the next two weeks and, on postdose day 14, the animals were sacrificed and examined grossly.

There were no deaths among the treated animals. Clinical signs included decreased activity in all animals, ataxia in most males and some females, and rapid breathing in some males. No other significant findings were observed.

Since all animals experienced adverse effects, no NOAEL could be accurately determined. Minimum lethal intravenous dose in rats was estimated at > 260 mg/kg/day; equivalent human dose > 41.3 mg/kg/day based on body surface area considerations.

Repeat dose studies

5. A three day oral dose range finding toxicity/pharmacokinetic study in Charles River CD-1 mice given 1592U89 succinate. Report # TTDR/92/0036. Study # DRF

[redacted]

This study was designed to characterize the toxic effects and pharmacokinetic characteristics of 1592U89 succinate, when given orally by gavage to mice for three days. Groups of male mice (15 mice/dose group) were dosed for three days with 1592U89 succinate at 276, 440, 700 and 1120 mg/kg/day in equally divided doses, with 6 hours between doses. Daily doses were equivalent to 195, 312, 496 and 793 mg 1592U89 base/kg/day. Records were kept of daily clinical findings. Pharmacokinetic parameters were measured at 20 mins, 1, 2, and 4 hours after the second dose on day 3.

Results

No clinical signs were observed. Peak plasma drug levels were generally recorded in the first sample taken (20 minutes after dosing) and increased with dose. The AUC values for the two major metabolites, the glucuronide and the carboxylate also increased with dose. The AUC of the glucuronide was approximately 20 % of the AUC of the 1592U89 and the AUC of the carboxylate increased with dose between 25 and 42 %.

Table 2. AUC values in mice following oral gavage administration of 1592U89 succinate for three days.

Dose of 1592U89 (mg/kg/day)	AUC_{0-4h} (μM.h) (1592U89)	AUC_{0-4h} (μM.h) (Glucuronide)	AUC_{0-4h} (μM.h) (Carboxylate)
276	110	27	23
440	125	41	25
700	169	56	34
1120	344	145	71

Conclusions

Plasma AUC levels of 1592U89 increase with dose but the increase is less than dose proportional. The metabolism of 1592U89 in mice is similar to that in man with the two major metabolites being a glucuronide and a carboxylate. The carboxylate AUC was generally 20 to 21 % of the AUC for the parent compound, while the relative AUC for the glucuronide increased with dose from 25 to 42 % of the AUC for parent as the dose increased. Given that the major metabolites in the human are also the carboxylate and glucuronide, the mouse is clearly a relevant species in which to study the toxicity of 1592U89.

6. A

This study was designed to characterize the toxicity of oral 1592U89 in mice treated for 21 days by gavage. Groups of mice (9/sex/dose group) were treated with 1592U89 succinate (dissolved in 0.5 % methyl cellulose) at doses of 700, 910 or 1120 mg/kg/day. Total daily doses were equivalent to 500, 650 and 800 mg/kg/day free base and doses were administered in two equally divided daily doses spaced 6 hours apart. Records were kept of mortality, clinical signs, bodyweights, clinical chemistry, plasma concentrations, necropsy findings, organ weights, gross pathology and histopathology.

Mortality

There were no clear dose related increases in deaths among treated animals compared to control animals. See Table 3.

Table 3: Mortality by week in CD-1 mice given 1592U89 succinate

Dose	Week 1	Week 2	Week 3	Week +1
Control	4	0	0	0
700	4	2	1	0
910	3	1	1	0
1120	5	0	0	0

Clinical signs were variable and there was no consistency across dosed groups. White blood cell counts were reduced in male mice to 37, 51 and 54 % of control levels and to 73, 82 and 90 % in female mice at low, medium and high doses respectively. Control males were 4.3 x 1000 per cmm, much higher than the level in control females (2.2 X 1000/cmm). Glucose levels were increased by up to 50 % in males (+ 30% in females). Triglyceride levels were increased by between 26 and 91 %.

1592U89 induced a number of liver changes, including increased liver weights (19, 30, and 32 % increases at low medium and high doses). Minimal to mild hepatocellular hypertrophy was observed in most animals in the mid and high dose groups and in some (7/19) animals at the low dose.

Table 4. Peak plasma concentrations of 1592U89 and metabolites in mice: Day 2

Dose	[1592U89] (μg/ml)	[1592 Gluc] (μg/ml)	[1592 Carb] (μg/ml)	[1144U88] (μg/ml)	[Free base] (μg/ml)
700 mg/kg	143	143	51	2.2	2.6
910 mg/kg	110	104	33	2.0	1.7
1120 mg/kg	125	101	38	1.9	1.6

Table 5. Peak plasma concentrations of 1592U89 and metabolites in mice: Day 21

Dose	1592U89	[1592 Gluc] (μg/ml)	[1592 carb] (μg/ml)	[1144U88] (μg/ml)	[free base] (μg/ml)
700 mg/kg	140	110	40	1.2	1.3
910 mg/kg	121	104	56	1.3	1.3
1120 mg/kg	193	121	66	1.4	1.5

There was no significant difference between the plasma levels of abacavir as the dose was increased between 700 and 1120 mg/kg/day. There was also no significant difference between levels measured on days 2 and 21. A large degree of variability existed between mice. The level of the major metabolite (1592 gluc, the 5' glucuronide, 1459U89) was close to the level of parent while the level of the carboxylate (1592 carb) was found at levels about 30 % that of the parent. The levels of carbovir (1144U88) and the free base, were on the order of 1% of the parent.

Since increases in glucose levels, liver weights and hepatocellular hypertrophy were observed at even the lowest dose, the NOAEL could not be determined.

7. A 30-day oral toxicity study in CD-1 mice

Four groups of mice, 16/sex/group, were treated with 1592U89 succinate orally, by gavage, at doses of 0, 110, 330 and 1000 mg/kg/day for 30 consecutive days. Drug was administered in two equal portions approximately 6 hours apart. Another similar group of animals were given the same drug regimen and used to determine plasma concentrations at various time points. Records were kept of clinical signs, body weights, food consumption, hematology, clinical chemistry, urinalysis and ophthalmology. On day 30, 8-10 males and females from each treatment group were sacrificed, while 6 males and 6 females per dose-group were observed for a 21-22 day drug-free recovery period before being sacrificed. At sacrifice, animals were subjected to gross examination, organs were weighed and tissues were removed for histopathological examination. Liver, skeletal muscle and cardiac muscle were also removed for microscopic examination.

A summary of the significant changes (these were reversible) observed in mice, are shown on Table 6 below. There were no gross hepatic alterations, ultrastructural changes, clinical signs, changes in body weight or food consumption, hematologic or ophthalmoscopic abnormalities or electron microscopic changes in skeletal or cardiac muscle.

Table 6. Adverse effects of 1592U89 in mice: relationship to dose and sex

Dose of 1592U89 (mg/kg)	Adverse effects			
	↑serum cholesterol	↑serum triglyceride	↑relative liver weight	↑ absolute liver weight
110				
330	F	F		
1000	M/F	M/F	M/F	M/F

M=male, F=female. Liver hypertrophy was also seen at 1000 mg/kg in males and females.

The NOAEL was 110 mg/kg/day which was equivalent to a human dose of 8.9 mg/kg/day for 30 days.

Table 7. Pharmacokinetics of 1592U89 after oral administration in mice on day 5.

	Male		Female	
Dose of 1592U89 (mg/kg/day)	C_{max} (µg/ml)	AUC0-6hr (µg/ml*hr)	C_{max} (µg/ml)	AUC0-6hr (µg/ml*hr)
110	4.8	5.5	6.0	6.9
330	16.4	24.6	9.9	22.3
1000	21.5	57.8	23	54.4

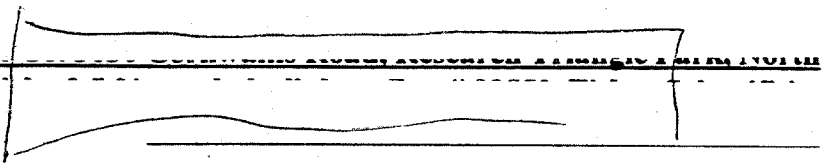
Table 8. Pharmacokinetics of 1592U89 after oral administration in mice on day 30.

	Male		Female	
Dose of 1592U89 (mg/kg/day)	C_{max} (µg/ml)	AUC0-6hr (µg/ml*hr)	C_{max} (µg/ml)	AUC_{0-6hr} (µg/ml*hr)
110	5.5	8.2	5.1	6.9
330	13.6	29.7	14.4	33.1
1000	26.5	85.9	28.0	78.9

Plasma concentrations of 1592U89 were measured following the first daily dose on days 5 and 30. Peak levels were observed 30 minutes after dosing (the first time point taken). See Tables 7 and 8. Drug exposure, as measured by the AUC, increased in near proportion to the dose and mean plasma levels were higher on day 30 compared to day 5.

No significant differences were observed between males and females except that at the 110 mg/kg/day dose, the AUC was slightly higher in males when compared to females.

8. A 30 day oral toxicity study of 1592U89 succinate (with "diol method" impurities) in the CD-1 mouse.
Burroughs Welle



The purpose of this study was to determine the toxicity of 1592U89 when synthesized by the "diol" route of synthesis. Groups of CD-1 mice, were treated with 1592U89 at 0 (Controls, Group 1, 25 animals/sex), 110 (Group 2, 10 animals/sex), 330 (Group 3, 10 animals/sex) and 1000 mg/kg/day (Group 4, 15 animals /sex). Animals were treated twice daily, six hours apart in equally divided doses. Ten animals from Group 1, were sacrificed predosing to obtain background clinical pathology data. Five animals from groups 1 and 4 were selected randomly for a two week post dosing recovery period. The main study animals therefore consisted of 10 animals per sex per dose group. Records were kept of clinical signs, bodyweights, food consumption, clinical pathology, urinalysis, ophthalmic examinations, organ weights, gross pathology findings and histopathology findings.

Mortality

Two animals died in the low dose group and three animals died from the high dose group. Deaths were caused by gavage accidents except in the case of one low dose and one high dose animal which were due to undetermined causes.

Abacavir administration was associated with increase in cholesterol (20 to 78 % increase between 110 and 1000 mg/kg/day) and increased triglycerides (males, (+83 %) and females (+30 %) at 1000 mg/kg). These changes were reversible. Abacavir's effects were generally most prominent in the liver, where abnormal coloration, increased relative liver weights (+ 29 %), hepatocellular hypertrophy and individual cell necrosis were observed in mice given 1000 mg/kg/day.

Conclusion

The toxic effects of abacavir, synthesized by the "diol" method did not produce any additional toxic effects not already known to be associated with abacavir.

9. A three month oral toxicity study in mice given 1592U89 succinate. Report # [redacted] 1993.

Male and female Charles River mice were assigned to treatment groups for treatment with 1592U89 orally, by gavage, for 96-97 days as described in the dosing design below in Table 9. Drug was dissolved in 0.5 % methyl cellulose and was administered in equal portions, twice daily, six hours apart. Ten animals/sex from group 1 were sacrificed prior to dosing for clinical pathology comparison. Five animals/sex/group were randomly selected prior to the start of dosing for the post-dose recovery period.

Table 9. Treatment protocol for 3 month oral dosing of mice with 1592U89

Group #	- Substance	Dose of 1592U89 (mg/kg/day)	Number of animals male/female
1	0.5% Methyl cellulose	0	26/25
2	1592U89 succinate	110 (55x2)	15/15
3	1592U89 succinate	330 (115x2)	15/15
4	1592U89 succinate	1000 (500x2)	15/15
5	1592U89 succinate	110 (55x2)	40/40
6	1592U89 succinate	330 (115x2)	40/40
7	1592U89 succinate	1000 (500x2)	40/40

Animals in groups 1-4 were evaluated for toxicology and pathology, while groups 5-7 were used for toxicokinetic determinations. Records were kept of clinical signs (daily), body weights (weekly), food consumption (weekly), hematology and clinical chemistry (days -2*, +1, +21 (males) and +22 (females), urinalysis (days -5 and 94) and ophthalmoscopic examination ((days -14 (males), -8 (females), 90 and +17). Animals were sacrificed on days +1, +21 (males) and +22 (females) and records kept of gross findings and organ weights. Histopathology (full panel) was examined in the control and high dose groups, as well as for all the animals that died or were sacrificed moribund. Representative portions of the liver, heart and skeletal muscle were taken from the first 3 mice/sex/group of groups 1-4 on postday 1 and from all control and high dose animals for electron microscopy. Plasma drug levels were measured in pharmacokinetics animals at 0, 0.5 1, 2, 4 and 6 hours after the first dose on days 2 and 91.

*Day -2 is used to designate two days before the initiation of dosing, day 90 is the 90 th day of dosing and day +17 is the 17th day after the end of dosing.

Mortality

Excess deaths were seen in the 1000 mg/kg/day group (see Table 10). The sponsor claims that the excess deaths seen in this study are due to gavage accidents. These are largely unsupported by necropsy findings and the sponsor provides no explanation for the increased number of gavage accidents at the highest dose level. Observations observed prior to deaths included distended abdomen, swelling of the neck and/or axillary area, decreased activity, yellow staining of the anogenital area, hypothermia, dehydration, mass on left side of head, body tremors, slowed righting reflex, rough coat, labored breathing, pale extremities, absent feces and urine and ptosis. There were no clear patterns to these findings.

Table 10. Mortality: 3 month oral dosing with 1592U89 in cynomolgus monkeys

Group	Dose (mg/kg)	Deaths/Sacrifices
1	0	1
2	110	1
3	330	2
4	1000	8

1592U89 was associated with increased cholesterol (21 to 30 % increase at 330 mg/kg and 61 to 129 % increase at 1000 mg/kg/day) , increased triglyceride (+ 59% at 1000 mg/kg) and increased ALT (+210 % at 1000 mg/kg).

In the liver there was individual cell necrosis and a dose related increase in absolute and relative liver weights in mice given 1592U89 (+42 % at 1000 mg/kg/day) which was associated ultrastructurally with an apparent increase smooth endoplasmic reticulum. There was also a decrease in thymus weights (approximately 20 to 40 % decreased at 1000 mg/kg/day).

Drug related histopathological findings were reversible and included minimal to mild hepatocellular hypertrophy, bile stasis (in one of 30 animals at 330 mg/kg/day and 11 of 30 animals at 1000 mg/kg/day).

On day 1, Cmax increased in an approximately dose-related fashion, while the increase in $AUC_{(0\text{-}6\ hours)}$ was superproportional to the increase in dose. On day 91, AUC values were less than proportional.

Table 11. Pharmacokinetics of 1592U89 in mouse: Day 2

Dose (mg/kg/day)	C_{max} (µg/ml)	$AUC_{0\text{-}6\ hr}$ (µg/ml*hr)
110	6	7
330	14	31
1000	41	100

Table 12. Pharmacokinetics of 1592U89 in mouse: Day 91

Dose (mg/kg/day)	C_{max} (µg/ml)	$AUC_{0\text{-}6\ hr}$ (µg/ml*hr)
110	5	6
330	10	23
1000	22	54

Summary

Administration of 1592U89 was associated with excess deaths at 1000 mg/kg and caused reversible liver changes and a variety of sporadic clinical changes. Drug exposure increased with dose, but the increase was less than proportional. On day 91, the exposure was less than on day 1 at 1000 mg/kg/day, indicating that the mice were eliminating the drug more quickly with repeated dosing. NOAEL level for this drug was 110 mg/kg/day for 91 days. The human exposure to drug at the recommended dose ($AUC_{0\text{-}12}$) is 6 hr•µg/ml. Thus at the NOEAL of 110 mg/kg/day, the exposure achieved in this mouse study ($AUC_{0\text{-}12}$, 12 hr•µg/ml) is equivalent an exposure twice that expected in a human at the recommended dose.

10. A six-month oral toxicity study in mice given 1592U89 succinate. RD 1996/00245/01. TOX 770. Medicines Safety Evaluation Division, Research Triangle Park, NC USA. October 1995. GLP Study. Drug reference number 94/5977-45

Charles River CD-1 mice were treated as described in Table 13 below. Records were kept of clinical signs, body weights, food consumption, clinical pathology, urinalysis, ophthalmic examinations, plasma drug levels, organ weights and necropsy findings. Portions of the liver, heart and skeletal muscle were taken from the first 3 mice/sex in groups 1 and 4 and examined by transmission electron microscopy. A full panel (see appendix #1) of histopathology was performed on all mice from groups 1 and 4 and all mice that died. Livers from animals in groups 2 and 3, and from recovery

animals in group 4 were also examined microscopically. Significant findings are discussed below.

Table 13: Treatment protocol for six month toxicology study in mice.

Group #	Dose of 1592U89 (mg/kg/day)	Number of animals male/female
1	0 (vehicle)*	45/45
2	55	30/30
3	110	30/30
4	330	35/35
5	55	40/40
6	110	40/40
7	330	40/40

*Control animals received 0.5 % methyl cellulose. Five animals/sex in groups 1 and 4 were randomly selected for a postdose recovery period of four weeks. Ten animals per sex were sacrificed before the start of dosing for pretest clinical pathology. Groups 5-7 were pharmacokinetics animals and were used for drug plasma level determinations.

Mortality

Mortality/moribund sacrifice was as follows: one (of 70) control animals, three (of 60) mice in the 55 mg/kg group, three (of 60) mice from the 110 mg/kg group and 2 (of 70) mice from the 330 mg/kg dose group. Two of the deaths (of three) at 55 and 110 mg/kg/day were a result of dosing accidents as indicated by perforated esophagus at necropsy. Cause of death in the other dosed animals did not show any patterns and included lymphoreticular sarcoma (55 mg/kg) and nephropathy (110 mg/kg/day). At 330 mg/kg/day, one animal was found to have distended intestines, while the other showed a transabdominal abscess. The deaths at 330 mg/kg/day are assumed to be drug-related.

Toxicity

Statistically significant toxic effects of the drug in mice included a slight, reversible increase in cholesterol in males at 110 mg/kg (+17 %) and in both sexes at 330 mg/kg/day (males, +24 % and females +43 %). Although changes are noted, much clinical chemistry data are missing because insufficient blood was available. On a number of occasions, group means are obtained from data from one animal.

At 330 mg/kg mice showed increased absolute and relative liver and thyroid weights. The increased liver weight was associated with hepatocellular hypertrophy, but there were no histopathological correlates with the increased thyroid weight. In the caecum (at 330 mg/kg), There was a very slight, reversible increase in crypt apoptosis and mixed submucosal inflammation. There was also a dose-related increase in diffuse, centrilobular and Kupffer cell pigment deposits in the liver at all doses. Other toxic effects observed were mild and/or reversible and did not show a dose-effect relationship.

Pharmacokinetics

As shown in Table 14 below, AUC values were similar on days 2 and 180, indicating that this drug does not accumulate under these experimental conditions. The increase in exposure was proportional to the increase in dose between 55 and 110 mg/kg, but, at 330 mg/kg the increase in exposure was disproportionately large when compared to the value expected based on the AUC at 110 mg/kg.

Table 14. Group mean AUC values (0-24 hours) (hr*µg/ml).

Dose	55 mg/kg	110 mg/kg	330 mg/kg
Day 2			
Male	7.2	15	100
Female	7.7	19	72
Day 180			
Male	6.8	15	47
Female	7.0.	16	80

While C_{max} increased with dose on both days, the increase was superproportional on day 2 between 110 and 330 mg/kg, but less than proportional on day 180 between 110 and 330 mg/kg (see Table 15).

Table 15. Group mean concentrations (C_{max}) of 1592U89 (µg/ml) in mice

Dose	55 mg/kg	110 mg/kg	330 mg/kg
Day 2			
Male	2.2	4.8	19
Female	2.9	6.1	22
Day 180			
Male	2.9	5.9	12
Female	3.2	5.6	11

Conclusion:

The NOAEL level for 1592U89 is 55 mg/kg/day for 6 months in mice. At this dose, the $AUC_{0\text{-}24h}$ value on day 180 (6.9 (µg/ml*hr) is about 60 % of the exposure obtained in humans receiving the recommended clinical dose.

11. A 21 day oral range finding toxicity study in rats given 1592U89 (free base). Report TTDR/92/0014. DRF 553. Division of Toxicology and Pathology, Burroughs Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709. Drug reference number 1592U89UK. Non GLP study. January 1992.

This study was designed to determine the toxicological effects of 1592U89 base dissolved in distilled water when dosed by oral gavage to Charles River CD rats. Rats (3/sex/dose group) were dosed at 0, 480, 640 or 800 mg/kg/day (in two equally divided doses, six hours apart) for 21 consecutive days. Records were kept of clinical signs, body weight, hematology, clinical chemistry, necropsy findings, organ weights, histopathology and toxicokinetics. Blood was drawn for drug level measurements at 30 minutes after the second dose on days 2 and 21.

No deaths occurred during this study. Drug related effects included post dose salivation, increased relative liver weight (+14 % at 480 mg/kg and +24 % at 640 and 800 mg/kg) and increased relative adrenal weights in males only (+ 14 %, + 26 % and + 34 % at low, mid and high doses). Relative testes weights were increased by 14 and 25 % at the mid- and high doses. Salivary gland weights were increased by 31 % in both mid and high dose groups. Hepatocellular hypertrophy was observed in all animals at 800 mg/kg/day. Platelet counts were decreased by 20 % at low and mid doses and by 30 % at the high dose.

Since adverse effects were observed at all doses tested, the NOAEL was estimated at < 480 mg/kg/day.

12. A 30 day oral range finding toxicity study in rats given 1592U89 dihydrochloride. Report TTDR/91/0024. DRF 511. Division of Toxicology and Pathology. Burroughs Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709. Drug reference number 1592U89UI2HCl. Non GLP study. January 1991.

Groups of rats, (6/sex/dose group) were treated with 1592U89 dihydrochloride at 0, 125, 250 and 500 mg/kg/day for 30 days. These doses were equivalent to 0, 100, 200 and 400 mg/kg/day 1592U89 base. Drug was dissolved in distilled water and administered orally in equally divided doses six hours apart. Records were kept of clinical signs, body weights, hematology, clinical pathology, plasma drug levels, necropsy findings, histopathology findings and electron microscopy findings in the liver.

Two high dose animals died in this study and the deaths were ascribed to dosing accidents. Apart from references to "observations (which) suggested that the most likely cause of death was misdosing," no evidence was supplied to support this conclusion.

1592U89 administration was associated with decreased red blood cells (8 to 14 % decreases at all doses on day 27) hematocrit (7-11 % decreases at all doses on day 27) and increased red blood cell distribution width (increased 14 and 15 % at the mid and high dose on day 27). Relative liver weights were increased by 9, 15 and 36 % at the low-, mid- and high doses. Relative thymus weights were decreased by 15 and 26 % at the mid- and high dose. Histological findings included hepatocellular hypertrophy in the mid- and high dose groups. Electron microscopy in the livers of the high dose groups revealed increases in rough endoplasmic reticulum, glycogen and numbers of lysosomes. Changes in amylase (increases up to 37 % at the high dose) and glucose (increases up to 14 % at the high dose) were confounded by large predose deviations in baseline values when drug treated groups were compared to controls.

Pharmacokinetics

Table 16. Mean plasma concentrations (µg/ml) observed in rats given 1592U89 dihydrochloride

Dose (mg/kg/day)	Day 2 (male)	Day 2 (female)	Day 24 (male)	Day 24 (female)
1	0	0	0	0
125	27	26	38	62
250	45	51	62	84
500	76	89	141	131

Mean plasma concentrations increased with dose in a linear fashion except in females on day 24 where the increase was less than dose proportional. Mean plasma concentrations were 50 to 90 % higher on day 24 than on day 2.

Administration of 1592U89 dihydrochloride in distilled water at 125 mg/kg day for 30 days produced minimal changes in rats.

13. 1592U89: A ninety day oral dose range finding study in Han Wistar rats. Report # RD1997/03595/00. Glaxo Wellcome Study # R40212. MPI Research, LLC, 54943 North Main St. Mattawan, MI 49071. MPI Research Study # 650-049. December 1996. Drug batch number R1128/111/5, R1128/133/1. Capps lot number. A96L630 and A96L702. GLP Study.

Male and female Han Wistar (Glx:Han:WlfBR) rats were treated with 1592U89 hemisulfate or vehicle as described in Table 17 below:

Table 17: Treatment protocol for 90 day toxicology study in rats.

Group	Dose of 1592U89 (mg/kg/day)	Number of animals/sex
1	0	5
2	35	5
3	135	5
4	530	5
5	35	12
6	135	12
7	530	12

*Control animals received 0.5 % methyl cellulose. Doses are expressed in terms of 1592U89 free base. Rats were dosed twice daily. The above doses represent the total daily doses. Groups 5-7 were pharmacokinetics animals and were used for drug plasma level determinations.

Records were kept of mortality, clinical signs, body weights, food consumption, clinical pathology, urinalysis, ophthalmic examinations, plasma drug levels, organ weights and necropsy findings. A full panel of histopathology was performed on all mice from groups 1 and 4 and all mice that died. Livers (males and females), testes, and thyroids (males) were examined microscopically from all dose groups.

Mortality

One animal in each of the two lower dose groups died on the study and their deaths were ascribed to gavage accidents. Both had corresponding esophageal inflammation. One female from the high dose group died and although the death was ascribed to a cage closure accident, no discussion of this accident is provided. The animal presented on autopsy with moderate, red lung discoloration in multiple lobes.

Toxicity

Intermittent increases in food consumption among female rats treated with 135 and 530 mg/kg/day were not considered to be of toxicological significance.

At 530 mg/kg/day, both males and female rats presented with decreased serum protein (-19 %), decreased serum albumin (-25 %), and increased serum cholesterol (+48 %). Serum globulin levels were also decreased in males (-22 %) and urea nitrogen was decreased in females (-31 %) at the 530 mg/kg/day dose.

Rats treated at 530 mg/kg/day had increases in liver weights, lung/mainstem bronchi weights (~ 20% , absolute and relative) and decreases in adrenal , thymus and thyroid/parathyroid weights.

Microscopically, diffuse, centrilobular hepatocellular hypertrophy was observed at the 530 and 135 mg/kg doses. Trace accumulation of brown pigment within the Kupffer cells (usually around the central vein) was observed at the 530 mg/kg/day dose. Thyroid hypertrophy was associated microscopically with an increase in the height of the follicular epithelium in several thyroid follicles. Germ cell loss was observed in the testes of 4/5 males treated at 530 mg/kg/day and was characterized by a lack of round and/or elongated spermatids in the seminiferous tubules.

Table 18. Group mean AUC values (0-24 hours) (hr*μg/ml).

Dose	35 mg/kg	135 mg/kg	530 mg/kg
Day 3			
Male	27	115	523
Female	20	93	298
Day 90			
Male	34	157	473
Female	25	131	424

Table 19. Group mean Cmax concentrations of 1592U89 (μg/ml) in rats

Dose	35 mg/kg	135 mg/kg	530 mg/kg
Day 2			
Male	2.6	9.4	31
Female	3.8	9.7	24
Day 90			
Male	4.9	16	27
Female	9.4	14	28

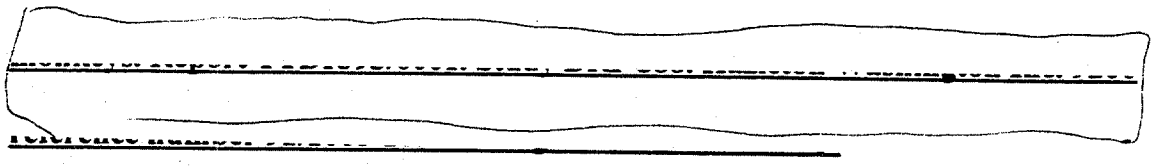
Group mean Cmax values increased with dose but were less than dose proportional. AUC values were generally dose proportional between 35 and 135 mg/kg, but accurate estimates were not obtained for the AUC values at the 530 mg/kg dose. The very high concentration of 1592U89 present at the last blood collection time (6h), precluded accurate estimation of the terminal elimination phase.

Comment

Blood samples were collected from 2 rats/sex/dose group on days 3 and 90 at time 0 (predose), 0.5, 1, 2, 4, and 6 hours following the first daily dose. The sponsor states that AUC over the plasma collection interval from time 0 to 6 hours was calculated by the trapezoid rule. The regression parameters generated by the analysis of the terminal stage of each curve were used to estimate AUC values out to 24 hours postdose. By the sponsor's own admission, "Plasma AUC_{0-24} exposure estimates of 1592U89 for all groups of rats dosed with 530 mg/kg/day of 1592U89 were greatly underestimated due to very high concentration of 1592U89 being present at the last blood collection time, 6h, thereby precluding accurate estimation of the terminal elimination phase of the pharmacokinetic profiles." Additional plasma collection times would have provided a more accurate estimate of the terminal elimination phase, and therefore AUC, after the second dose.

Conclusion:

In addition to characterizing the toxicity of 1592U89 in rats, this study was designed to help the sponsor select doses for the two-year carcinogenicity study. A dose of 530 mg/kg/day is likely to be tolerated by rats for the duration of a 104-week carcinogenicity study. Toxic effects seen with this drug, such as hepatocellular hypertrophy, hypertrophy of the thyroid, and germ cell loss in the testes are not expected to affect survival. The NOAEL level for 1592U89 is 35 mg/kg/day for 90 days in rats and this provides an exposure (AUC) which is about 2.5 times the AUC seen in humans at the recommended clinical dose.



This two-phase study was designed to characterize the acute effects of 1592U89 in cynomolgus monkeys. In Phase I, two male and two female monkeys were treated as follows: On days 1 and 2, monkeys were treated with 1592U89 succinate at 700 mg/kg. On day 3, monkeys received 560 mg/kg and on day 4, 420 mg/kg. In phase II, two male and two female monkeys were treated with 500 mg/kg 1592U89 succinate for 10 days. In phase I clinical signs were recorded. In phase II records were kept of clinical signs, hematology, clinical chemistry, plasma drug levels and CSF drug levels. Drug was

suspended in 0.5 % methyl cellulose and administered by gavage six hours apart in two equally divided doses.

Results

In phase I, vomiting was the only clinical sign observed and this occurred in a dose dependent fashion. A dose of 500 mg/kg was selected for use in phase II. In Phase II, all four animals had at least one episode of emesis, but there was no relationship between time of dosing and time of onset of emesis. The incidence of emesis was higher after the morning dose. There were no effects on body weights, hematology or clinical chemistry.

Table 20 Mean peak plasma concentrations (μM) measured 2 h post dose (C_{max})

Sex	1592U89		1592 Glucuronide		1592 carboxylate	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Male	85	172	68	208	4	14
Female	121	203	48	117	7	18

Mean plasma concentrations of 1592U89 and its major metabolites are shown above. The major metabolite was the 5' glucuronide (1459U89) and was present at levels similar to parent in males and at about 50 % of parent in females. Carboxylate levels were about 6 to 9 % of parent levels. Levels of parent (+85 %) and metabolites (2.5 fold to 3.7 fold) increased between day 1 and day 10. It is unclear why the drug levels are higher on day 10 since the drug does not seem to accumulate (predose levels, which would be high if the drug was accumulating, were low).

CSF levels of 1592U89 (measured 1hr post dose on day 11) were 16.4 and 23 μM for males and females respectively. These values averaged 26 % of plasma levels measured 1 hour after dosing on day 10.

15. A 30 day oral dose range finding study in Cynomolgus monkeys with 1592U89 (base).

1

Four groups of cynomolgus monkeys, *Macaca fasciculatis*, (two/sex/group) were treated with 0, 125, 250 or 500 mg/kg 1592U89 daily by oral gavage for 30 days. Drug was administered in two equally divided doses 6 hours apart. Monkeys were observed for clinical signs, body weight, hematology and clinical chemistry. Drug plasma levels were also measured one hour after the second dose on days 3 and 29. After necropsy, animals were examined grossly with selected organs being weighed and processed for histopathological examination.

Mortality

One male and one female from the high dose group did not survive the study. The female, which was found dead on day 6 had previously appeared thin and dehydrated and had had diahrrhea, but there were no abnormal macroscopic or microscopic pathological findings that would indicate the cause of death. The male was sacrificed for humane reasons after appearing weak and after blood was found in the urine and rectal area. Decreased sodium, BUN, creatinine and amylase were also noted in this animal. Severe pyelonephritis was observed upon microscopic examination of tissues post mortem and this was assumed to be the cause of the morbidity. Treatment at the 500 mg/kg dose was terminated following the death of this animal.

Toxicity

At 250 and 500 mg/kg/day, 1592U89 treatment was associated with reduced food intake, and emesis. BUN was reduced by about 50 % in the 250 mg/kg dose group on day 29 (no data were available at 500 mg/kg on day 29 since animals were no longer being dosed)

Table 21. Plasma levels of 1592U89 and its metabolites two hours after dosing.

Dose (mg/kg/day)	[1592U89]		1592 glucuronide		1592 Carboxylate	
	Day 3	Day 29	Day 3	Day 29	Day 3	Day 29
0	-	-	-	-	-	-
125	57	50	17	23	4	3
250	116	105	43	69	5	5
500	184	-	48	-	10	-

Plasma levels of 1592U89 increased with dose in a dose proportional fashion. On day 3, the level of 1592U89 glucuronide was approximately a third of the parent compound while carboxylate was about 5 %. On day 29 the glucuronide level was approximately one half the level of the parent while the carboxylate was about one tenth of the parent. Thus, the metabolites of 1592U89 seem to accumulate as dosing continues.

Conclusion

Mortality, vomiting, reduced food intake and kidney toxicity are the most prominent toxicities associated with dosing of 1592U89 base at doses up to 500 mg/kg/day. The toxicity of the 500 mg/kg dose was not adequately characterized since dosing in this group was halted due to excess mortality.

16. A 28 day oral toxicity study in cynomolgus monkeys (TTE)

[redacted]

Four groups of cynomolgus monkeys, Macaca fasciculatis, (five males and five females per group), were treated with 0, 50, 140 or 420 mg/kg 1592U89 daily by oral gavage for 28 days. Drug was administered in two equally divided doses 6 hours apart. Monkeys were observed for clinical signs, appetite, body weight, ophthalmology, electrocardiology, neurophysiology, hematology, clinical chemistry and urinalysis. Drug plasma levels were also measured. At the end of the 28-day dosing period, three monkeys/sex/group were sacrificed while two monkeys/sex/group were observed for an 18 day postdose drug-free recovery period before sacrifice. Animals were examined grossly with selected organs being weighed and processed for histopathological examination. Samples of liver, skeletal and cardiac muscle from control and high-dose animals were also examined ultrastructurally.

While a number of changes were noted in monkeys treated with 140 mg/kg and 420 mg/kg, all changes were reversible and there were no changes in urinalysis, ophthalmology, electrocardiography, neurophysiology, gross pathology or neuropathology. There were also no changes in cardiac or skeletal muscles when examined under the electron microscope. Adverse events at 420 mg/kg included increased liver weights, total erythrocyte counts, hemoglobin, hematocrit, serum triglycerides. At 140 mg/kg serum triglyceride levels were increased in males and females.

The NOAEL was 50 mg/kg/day for 28 days and was equivalent to a human dose of 16.15 mg/kg/day.

Plasma concentrations of 1592U89 were determined following the first daily dose on days 3 and 26 (see Table 22). Plasma levels were highly variable and the 4-hour post exposure interval was not long enough to adequately evaluate total exposure, since the Tmax. occasionally coincided with the last sampling time. AUC and Cmax values increased with increasing dose but differences were masked by the variability in the measured values. Systemic exposure, as measured by the AUC's appeared to be lower on day 26 relative to day 3.

Table 22. Pharmacokinetics of 1592U89 after oral administration in monkey, Macaca fascicularis

1592U89 dose mg/kg/day	Dose day	AUCO-4hr (hr.ug/ml)	Cmax(ug/ml)	Tmax (hr)
50	3	10.8	5.3	1.6
	26	4.3	2.3	2.4
140	3	35.4	13.3	2.2
	26	15.9	8.0	3.1
420	3	77	27	2.8
	26	38	19	31

Cerebrospinal fluid levels were variable but CSF ratios were constant within dose. The ratios (CSF:plasma) for 50, 140 and 420 mg/kg/day 1592U89 were 0.19, 0.17 and 0.16 respectively one hour after dosing on day 25.

17. A three month oral toxicity study in cynomolgus monkeys given 1592U89

1992. Study # HWA 509-220/BW-1592U89. December 1993. Drug lot number (not provided) GLP Study.

This study was designed to determine the toxic effects of 1592U89 succinate when given for three months to cynomolgus monkeys orally, by gavage. Drug was dissolved in 0.5 % methyl cellulose.

Groups of monkeys, 5/sex/dose group were treated with vehicle (group 1, control) or 50 mg/kg (group 2), 140 mg/kg (group 3) or 420 mg/kg (group 4) 1592U89 for three months, followed by a recovery period of three weeks. Animals were treated by nasogastric intubation twice daily for at least 3 months. Dosing occurred at least 6 hours apart except on toxicokinetics study days, when dosing was approximately 8 hours apart. The dosing volume was 5 ml/kg/dose or 10 ml/kg/day. Records were kept of clinical findings (twice daily), body weights (weekly), clinical pathology (prestudy, and study days 24, 52, 87 and 108 for hematology and clinical chemistry and pretest and study days 80 and 111 for urinalysis), electrocardiograms (prestudy and during weeks 1, 4 and 12), ophthalmic examinations (prestudy and during week 12), neurophysiologic examinations (prestudy in all animals and during weeks 1 and 4 during week 13), gross necropsy findings and organ weights after terminal sacrifice. Also examined were histopathology findings (full panel, all doses), electron microscopy (liver, left ventricle and biceps brachial muscle) and serum chemistries. Plasma drug levels were measured in blood samples taken on days 3 and 87, prior to the morning dose and 1, 2, 4, 6, and 8 hours following the morning dose.

Comment

The sponsor notes ( ), that "the animals were not fed until after the last collection interval (4 hours postdose)." It is unclear why the last collection interval is 4 hours postdose if samples were taken as described above (1, 2, 4, 6, and 8 hours following the morning dose.

Mortality

There were no deaths in this study.

Toxicity

Drug administration was associated with a dose related increase in emesis. A reduction in bodyweight in some animals in group 4 was slight and transient. Decreases in hemoglobin and hematocrit were transient, in the region of 10-20 % and seen in males only. Other changes were very mild, inconsistent and variable. SGOT levels showed no dramatic increases in males while SGPT levels increased dramatically (up to 10 times controls), albeit transiently in individual animals in groups 3 and 4. In females, SGOT and SGPT levels were not changed at the end of the study for group 3 animals but were increased at the end of the recovery period. (SGOT + 50%, SGPT +100 %). In group 4 females, SGOT and SGPT levels were increased at the end of the study (by 70 and 160 % respectively) and these levels remained elevated at the end of the recovery period.

Pharmacokinetics

Table 23. Pharmacokinetics of 1592U89 in cynomolgus monkey: 3 month dosing

	C_{max} (µg/ml)		$AUC_{0\text{-}8\ hr}$ (hr•µg/ml)		T_{max} (h)	
Dose (mg/kg/day)	Day 3	Day 87	Day 3	Day 87	Day 3	Day 87
50	2.0	2.2	7.2	6.8	1.1	1.1
140	7.2	6.2	28	22	1.2	1.6
420	24	18	138	93	2.8	1.9

Mean peak plasma levels of 1592U89 were observed between one and three hours post dose, and increased with increasing dose. Mean peak plasma concentrations were superproportional to dose. At the highest dose (420 mg/kg/day), mean peak plasma concentration on day 87, was lower than on day 3. Exposure, as measured by $AUC_{0\text{-}8\ hr}$ was approximately proportional to dose up to 140 mg/kg/day, but became superproportional to dose at 420 mg/kg/day.

Summary

Administration of 1592U89 was associated with increased liver enzymes in monkeys treated with this drug for three months. SGOT and SGPT levels were elevated, sometimes dramatically in individual monkeys at 140 or 420 mg/kg/day and these increases were not always reversible. Other changes were generally mild or reversible. The NOAEL level for this drug is 50 mg/kg for a 3 month administration. This is equivalent to a dose of 16 mg/kg/day for 3 months based on body surface area calculations.

18. A 1812 month repeated dose oral toxicity study with a six month interim

[redacted]

Study.

This study was designed to evaluate the effects of 1592U89, given twice daily, by oral gavage for twelve months in Cynomolgus monkeys. Drug was dissolved in 0.5 % methylcellulose and animals were treated with 0, 50, 140 or 300 mg/kg/day (9, 7, 7 and 9 animals/sex/dose group respectively). Control animals were given 10 ml/kg/day of 0.5 % methylcellulose.

Three animals/sex/group were sacrificed after 6 months of treatment. The dosage level for group 4 was initially 420 mg/kg/day from weeks 1 through 5, but was reduced to 300 mg/kg/day due to excessive toxicity. Records were kept of clinical observations, body weights, clinical pathology, electrocardiography, ophthalmoscopy, neurophysiology, gross necropsy and organ weights, neurohistopathology, histopathology and drug levels in blood.

Results

Mortality

Two group 4 animals did not survive this study: one died on day 32 and the other was sacrificed moribund on day 107. The first female became sick on day 22, with decreased appetite and soft stool. Eventually other symptoms evolved including emesis, diarrhea, dehydration inactivity and hunched posture. The animal died on day 32 and necropsy revealed enlarged kidneys with thickening of the renal pelvis as well as granular material in bladder, ureter and renal pelvis. There was also fluid in the thoracic and abdominal cavity. The second animal became sick on days 79-83 and was sacrificed moribund on day 107 after soft feces/ diarrhea, emesis and deterioration. Necropsy revealed gelatinous subcutaneous tissue in the central neck region and enlargement of the mesenteric lymph nodes and adrenals. Moderate typhilitis and colitis, with associated lymphoid depletion also contributed to the death of this animal.

Other findings included gastric submucosal edema, bone marrow hypoplasia and adrenal cortical hypertrophy.

Toxicity

High dose animals (which were initially dosed at 420 mg/kg/day) experienced sporadic emesis, and failure to gain weight. Other signs included hunched posture, hypoactivity, decreased appetite and fecal alterations. These toxic effects were also associated with decreased erythrocyte counts, hematocrit and hemoglobin at week 4. The mean $AUC_{0\text{-}24h}$ value was 478 h*μg/ml at 420 mg/kg/day, equivalent to 40 times the exposure expected in humans at the recommended dose. These adverse events led the sponsor to reduce the dose in the high dose group to 300 mg/kg/day for the rest of the study period . Ventricular extrasystoles were observed during week 13 in one 140 mg/kg male. This finding is not uncommon in monkeys and not dose-related therefore not assumed to be drug related.

Clinical pathology changes seen in group 4 animals during week 4 included lower RBC (-17%, females only), lower hemoglobin (-11%) and lower hematocrit (-15 %), higher reticulocyte counts (+140 %). Mean prothrombin times were lower in group 4 males at weeks 4, 9, 13 and 25, but mean activated partial prothrombin times were not affected. Mean platelet counts were higher in group 4 males at week 4. Except where otherwise mentioned, the aforementioned changes were usually transient and were not observed after the reduction of dose at week 5. Changes observed in group 3 animals included lower hemoglobin at week 25 in females (-10 %), lower hematocrit at weeks 9, 13 and 27 (<10 %), and increased lymphocytes (+53 %) in females at week 13. Drug administration was also associated with mild increases in SGPT at 140 and 300 mg/kg and minimal increases in triglycerides in male and female monkeys at 300 mg/kg/day.

Necropsy findings

Six month findings

Changes in organ weights, seen at the end of six months included increased adrenal weights, (group 3 females) decreased brain weights (groups 3, 4 males), and increased liver weights, (groups 2 and 4 females). These increases in absolute organ weights were not associated with changes in relative organ weights, were not dose related and tended to affect one sex or the other.

Histological examination revealed slight centrilobular hypertrophy in 5/6 300 mg/kg/day animals and one animal dosed at 140 mg/kg.

Twelve month findings

Toxic effects noted after twelve months of dosing with 1592 included increased alanine aminotransferase at 300 mg/kg/day (+ 80 % compared to controls), increased triglyceride levels at 140 mg/kg/day (+ 115 %, males only) and at 300 mg/kg (+ 123 % and + 66 %, males and females respectively) as well as increased relative liver weights (+ 40 %) and increased centrilobular hypertrophy (5/8 animals). Microscopic findings were reversible after a three-week drug-free period.

Pharmacokinetics

Table 24. The group mean AUC values for 1592U89 (hr μg/ml)

	50 mg/kg/day	140 mg/kf/day	300 mg/kg/day*
Day 3	21	93	477
Week 9	22	73	158

* Group 4 animals were initially treated at 420 mg/kg/day until after week 5 when the dose was reduced to 300 mg/kg/day. As such, the Day 3 data represents the results of 420 mg/kg dosing.

Plasma levels of 1592U89 increased proportionally with dose. There was little difference in pharmacokinetics when male and females were compared. There was also little change in the AUC values between day 3 and week 26 or 53. The drug does accumulate upon repeated dosing in monkeys at the tested dosing.

Since the drug produced toxic effects in the liver when administered for twelve months at the lowest dose studied, no NOAEL could be determined. The lowest dose produced an exposure about two times the exposure seen at the recommended clinical dose in humans.

Reproductive Toxicology Studies

19 Preliminary Dose-range Finding embryo-fetal development study of 1592U89 in

6479-300. Glaxo report # TTDR/96/0005. Glaxo Study # DRF 743. December 1995. Non GLP study.

1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to pregnant CD Sprague Dawley rats during organogenesis (gestation days 6 to 17). Doses were 0 (control), 500, 1000 and 1500 mg/kg/day 1592U89 succinate (324,

648 and 972 mg/kg 1592U89 base) and were administered in two equally divided doses, six hours apart. Control animals received vehicle (0.5 % aqueous methyl cellulose). Records were kept of body weights, food consumption, clinical signs. On gestation day 20, all dams were euthanized, weighed and necropsied. Ovarian corpora lutea were counted and uterine implantation sites were examined. Total non-live (early and late resorptions, dead fetuses) and live fetuses were noted and all live fetuses were weighed, examined for external fetal malformations, euthanized and discarded.

Dams treated with 1592U89 succinate at 1500 mg/kg/day showed gasping, lethargy, chromodacryorrhea, prone posture and rough coat while all treated animals showed piloerection, rooting post dose and salivation predose. Relative maternal liver weights were increased by 10, 17 and 28 % over control animals.

Bodyweight Gain

At 1500 mg/kg/day, 1592U89 produced a 16 % reduction in maternal bodyweight on gestational day 20 and a persistent reduction in body weight gain (27 to 75 % reduction) compared to control animals. At 1000 mg/kg/day, there was a reduction in body weight gain after dosing was stopped (27 % reduction in body weight gain between gestational days 17 and 20). Food consumption (measured in g/kg/day) was reduced by 12, 24 and 28 % in dams treated at 500, 1000 and 1500 mg/kg/day 1592U89 succinate on days 6-9. After dosing, there was an increase in food consumption (15, 10 and 28 % at low mid and high doses on gestational days 18 to 20). Thus, at 1000 and 1500 mg/kg/day, 1592U89 was associated with clear reductions in food consumption early in the dosing period and body weight gain after dosing was complete.

Gestational parameters

Dams treated at 1000 and 1500 mg/kg/day showed a 22 and 79 % decrease in gravid uterine weight at the termination of the study due to four fully resorped litters at 1500 mg/kg and reduced fetal weights of the live litters at 1000 and 1500 mg/kg/day. 1592U89 succinate administration was also associated with increased percentage preimplantation loss, percentage resorptions/litter, percent of litters with resorptions, percent nonlive implants (dead plus resorbed)/litter, percent litters with nonlive fetuses, as well as decreased fetal body weights and number of live fetuses/litter.