CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

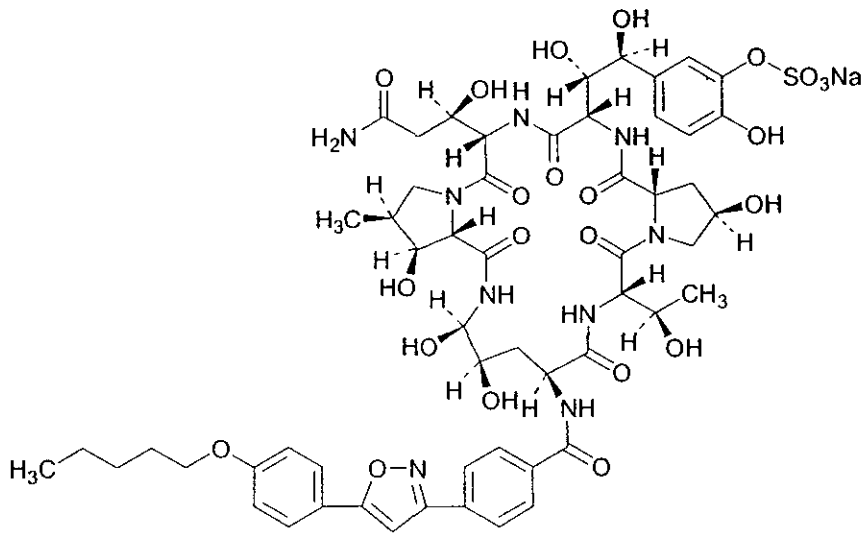
21-506

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA numbers: 21-754 and 21-506
Review number: 1
NDA submission # 000
Submission date: April 23, 2004
Information to sponsor: Yes
Sponsor: Fujisawa Healthcare, Three Parkway North, Deerfield, Illinois, 60015-2548
Manufacturer of drug substance: Fujisawa Pharmaceutical Company Ltd. 30, Ttoedesakae-machi, Tokaoka, Toyama 939-1118, Japan
Reviewer name: Owen McMaster, Ph.D.
Division of Special Pathogen and Immunologic Drug Products.
HFD-590
Review completion date: March 11, 2005
Drug Trade name: Mycamine™
Generic name: Micafungin
Code names: FK 463, FR 179463
Chemical name: *IUPAC:* Sodium 5-[(1*S*, 2*S*)-2-[(3*S*,6*S* ,9*S* ,11*R*, 15 *S* ,18 *S* ,20*R* ,21*R*, 24 *S* ,25*S*,26*S*)-3-[(R)-2-carbomoyl-1-hydroxyethyl]-11,20,21,25-tetrahydroxy-15-[(R)-1-hydroxylethyl]-26-methyl-2,5,8,14,17,23-hexaoxo-18-[4-[5-(4-pentyloxyphenyl)isoxazol-3-yl]benzoylamino]-1,4,7,13,16,22-hexaazatricyclo[22.3.0.$0^{9,13}$]heptacos-6-yl]-1,2-dihydroxyethyl]-2-hydroxyphenyl sulfate.

CAS registry number: 208538-73-2 (sodium salt)
Molecular formula: C_{56} H_{70} N_9 NaO_{23} S
Molecular weight: 1292.26
Structure:



Relevant INDs/NDAs/DMFs: IND — DMF — DMF — , NDA —

Drug class: Echinocandin

Indications: (1) Treatment of patients with esophageal candidiasis.
(2) Prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation.

Clinical formulation: Micafungin for injection is a sterile, non-pyrogenic lyophilized product for intravenous injection. The drug product is available in a 50 mg vial. Each vial contains micafungin (50 mg), lactose monohydrate (200 mg), anhydrous citric acid (for pH adjustment, pH 5- [illegible] sodium hydroxide (for pH adjustment, pH 5- [illegible]

Route of administration: Intravenous infusion (over 1 hour)

Proposed use: Treatment of patients with esophageal candidiasis and prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation.

Disclaimer: Tabular and graphical information are from the sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability

There are no safety issues that would preclude the approval of micafungin for injection for the treatment of systemic fungal infections at the proposed doses of 50 or 150 mg.

B. Recommendation for Nonclinical Studies

No additional preclinical studies are being recommended at this time.

C. Recommendations on Labeling

The label should include

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

The toxicology program for micafungin included studies in mice, rats, rabbits and dogs. The highest dose administered was 250 mg/kg (equivalent to a human dose of 40 mg/kg based on body surface area comparisons) as a single dose to rats and the longest studies were for 26 weeks in rats and 39 weeks in dogs. The minimum lethal dose was 8 times the highest recommended human dose (based on a body surface area comparison) using data from the more sensitive species, the rat. Using data from the dog, the minimum lethal dose was more than 43 times the maximum recommended human dose.

In animals, the target organs are the liver and the testes, with adverse effects also seen in clinical chemistry, spleen, and at the injection site. Liver toxicity included enlarged, discolored livers with centrilobular hypertrophy, single cell necrosis, acidophilic bodies, nuclear hypertrophy, vacuolation, bile duct proliferation and mitosis. In rats and dogs, micafungin administration produced testicular atrophy and decreased sperm count. Plasma AST, ALT, alkaline phosphatase and LDH were also increased. In the blood, there were decreased erythrocytes, hemoglobin and hematocrit as well as increased reticulocytes, serum total bilirubin and potassium. Congestion, pigmentation and increased weight were recorded in the spleen along with hypercellularity in the femoral bone marrow. Injection sites showed hemorrhage, and cellular infiltration of the perivascular tissue. Injection site irritation was less severe when the drug was infused over one hour compared to bolus injections.

Patients treated with micafungin at the highest recommended clinical dose (150 mg) show a Cmax of 16 mcg/ml and an $AUC_{0\text{-}24h}$ of 167 mcg*h/ml. Very high doses of micafungin, when administered for prolonged periods, produced irreversible changes to the liver. In a

26-week rat study (dosed to 10-times clinical C_{max}), with four- or 13-week recovery, colored patches/zones, multinucleated hepatocytes and altered cell foci remained at the end of the recovery period. In a similar 13 week dog study with 4 week recovery (dosed to seven times clinical exposure), liver discoloration, cellular infiltration and hypertrophy remained visible at the end of the 4-week recovery period.

B. Nonclinical Safety Issues Relevant to Clinical Use

The predominant safety concern regarding the use of micafungin is the potential for irreversible liver damage if patients are exposed to the drug at high doses for prolonged periods. Thorough, frequent monitoring of liver function should therefore be considered.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

IV. GENERAL TOXICOLOGY:

Toxicology Studies Summary

1. Single dose intravenous toxicity study of FR179463 in rats
2. Single dose intravenous toxicity study of FR179463 in dogs
3. Four week intravenous toxicity study of FR179463 in rats
4. Thirteen week intravenous toxicity study of FR179463 in rats
5. A 26-week intravenous toxicity study of FR179463 in rats
6. Four week intravenous toxicity study of FR179463 in dogs
7. Thirteen week intravenous toxicity study of FR179463 in dogs, (with a 4-week recovery).
8. A 39-week intravenous toxicity study of FR179463 in beagle dogs.
9. FR179463: Intravenous study for effects on Pre- and postnatal development including maternal function in rats.
10. Study of Fertility and early embryonic development up to implantation in rats treated intravenously with FR179463
11. Intravenous dosing study of FR179463 on embryo-fetal development in rats.
12. Intravenous dosing study of FR179463 on embryo-fetal development in rabbits.
13. Mutagenicity study of R179463: Reversion test with bacteria.
14. Mutagenicity study of R179463: Chromosomal aberration test with Chinese Hamster Lung cells in culture.
15. Mutagenicity study of R179463: Micronucleus test in mice
16. Safety Pharmacology Study of FR179463: Action Potential Measurement by Microelectrode Techniques
17. Safety Pharmacology Study of FR179463: hERG Assay.

1. SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: The minimum lethal dose was 125 mg/kg. This dose is equivalent to a human dose of 20 mg/kg, based on body surface area conversions.
Study no: GLR970113
Conducting laboratory and location: —

Date of study initiation: May 15, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot # 00105YL . — purity
Formulation: powder was dissolved in physiological saline

Three groups of SPF — CD(SD) strain rats, 5 per sex per dose group, were injected in the tail vein with micafungin at 62.5, 125 and 250 mg/kg. Drug was infused over 1 minute. Animals were observed for mortalities for 7 days after dosing. Animals were 6 weeks old and males weighed between 200 and 250 g while females weighed between 162 and 190 g.

All animals in the 250 mg/kg dose group died immediately following dosing. Two males in the 125 mg/kg group died the day after administration.

At 125 mg/kg, animals showed decreased spontaneous movement, dark red coloration of the ear auricle and extremities, swelling of the face, tachypnea and frequent approach to the water dispenser. Abnormal signs resolved two hours after dosing. Dark red spots were seen on the lungs of the males that died the day after administration. Mild congestion and perivascular edema and hemorrhage was noted in one and mild alveolar focal hemorrhage was noted in the other male that died.

Additional signs seen at 250 mg/kg included prone position, clonic convulsions, and oligopnea. Almost all these animals died within 5 minutes of dosing.

SUMMARY

The minimum lethal dose of FR179463 was 125 mg/kg, a dose equivalent to a human dose of 20 mg/kg based on body surface area conversions.

2. SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS

Key study findings: The minimum lethal dose was greater than 200 mg/kg a dose equivalent to a human dose of greater than 108 mg/kg based on body surface area conversions.
Study no: GLR970114
Conducting laboratory: —

Location: —

Date of study initiation: May 16, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 00105YL — purity
Formulation: Dissolved in physiological saline

Two groups of 8 kg, 5 month-old beagle dogs, one/sex/dose group were injected (bolus) with a micafungin at 100 or 200 mg/kg. Animals were observed twice a day for 14 days.

No animals died. At 100 mg/kg, clinical signs included reddened eyelids and extremities, increased total bilirubin, and increased leukocytes. Additional signs seen at 200 mg/kg included increased salivation, pale mouth and ear, reddened eyelids, ear, abdominal skin, decreased movements and sluggishness. The higher dose also caused decreased erythrocytes, hemoglobin and hematocrit and increased fibrinogen, segmented neutrophil rate, AST, LDH and reticulocytes.

SUMMARY

The minimum lethal dose was greater than 200 mg/kg a dose equivalent to a human dose of greater than 108 mg/kg or 6.5 grams for a 60 kg subject. The swollen eyelids and extremities and flushing suggest histamine release and the increased AST, LDH suggest that these high doses of micafungin result in damage to the liver

3. FOUR WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 32 mg/kg to rats for four weeks was associated with liver toxicity, hemolysis, bladder injury and irritation of injection site. Slight changes in the spleen, bone marrow and injection site were seen even at 3.2 mg/kg. AUC values for rats treated at 3.2 mg/kg were around 54 mcg*h/ml. Human subjects treated with micafungin at the highest dose of 150 mg demonstrated an AUC value around 167 mcg*h/ml. Thus the toxic effects, seen in this study, begin at systemic exposures one third of those seen at the highest recommended dose.

Study no: GLR970115
Conducting laboratory: —

Location: —
Date of study initiation: May 16, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 00105YL — purity
Formulation: Dissolved in physiological saline

Four groups of SPF — CD (SD) strain rats, 10 per sex per dose group, were injected once daily for four weeks, in the tail vein with micafungin at 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Drug was infused over 1 minute. Records were kept of general

signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and plasma drug concentrations. Animals were 4 weeks old, with males weighing between 190 and 230 g while females weighed between 142 and 192 g.

Animals treated at 3.2 mg/kg showed slight changes, consistent with the changes observed at the higher doses. These included decreased total protein (females), slight hematopoiesis in the spleen (one male), and mild hypercellularity in the bone marrow (4 of 20 animals).

Additional findings at 10 mg/kg, included increased total bilirubin and congestion of the spleen. At 32 mg/kg, changes included small round epithelial cells in the urinary sediment, and decreased erythrocytes and lymphocytes. Increases were recorded in MCV, MCH, MCHC, reticulocytes and segmented neutrophils. There was also a significant shortening of the partial thromboplastin time. Clinical chemistry changes included increased plasma AST, ALT and LDH, total bilirubin, potassium, chloride, BUN and α_2-globulin. Decreases were recorded in serum creatinine, α_1-globulin, and total protein.

Post mortem findings at 32 mg/kg included increased spleen weights and a slight increase in eosinophilic granules in the duct of the submandibular gland (8 of 10 males). In the liver, increased acidophilic bodies (secondary lysosomes), single cell necrosis, slight nuclear hypertrophy, hepatocellular vacuolation were observed and mild round cell infiltration/accumulation in the sinusoid (probably a reaction to necrosis). In the spleen, there was mild congestion of the red pulp (most animals), extramedullary hematopoiesis (2 females) and brown pigment in the red pulp (4 females). In the kidney, there was vacuolation in the pelvic mucosal epithelium (in 8/20 animals) and in the bladder there was vacuolation in the mucosal epithelium (all animals). Bone marrow hypercellularity was observed in most high dose group males. Slight focal atrophy of the seminiferous tubules was observed in one high-dose animal. Injection sites were characterized by slight or mild perivascular hemorrhage, cellular infiltration and fibrosis. Effects were more pronounced and frequent at the higher doses and so these effects were clearly drug related

Table 1. Pharmacokinetics: $AUC_{0\text{-}24h}$ values (mcg*h/ml) recorded after repeated micafungin dosing in rats.

FK463 Dose	$AUC_{0\text{-}24h}$ mcg*h/ml		
(mg/kg/day)	Day 1	Day 14	Day 28
3.2	54	53	56
10	180	203	223
32	573	680	853

Mean AUC values were proportional to dose on day 1, but increased over time and thus dose proportionality was not maintained

Table 2. Pharmacokinetics: $T_{½}$ values (h) recorded after repeated micafungin dosing in rats.

FK463 Dose	$T_{½\ values}$ (h)		
(mg/kg/day)	Day 1	Day 14	Day 28
3.2	4.8	5.3	5.2
10	5.7	6.5	6.2
32	6.8	7.7	7.5

Mean terminal half-life increased with dose.

SUMMARY

FK463 produced adverse effects at all doses beginning at 3.2 mg/kg when administered for 4 weeks. The effects seen at 3.2 mg/kg, though less severe, reflected the effects seen at higher doses. Therefore the lower total protein values, hematopoiesis, and bone marrow hypercellularity, are true toxic effects of FK463. At higher doses, effects on the liver, spleen, kidneys, bladder, testes and injection site became evident. AUC values for rats treated at 3.2 mg/kg were around 54 mcg*h/ml. Human subjects treated with micafungin at the highest dose of 150 mg demonstrated AUC values around 167 mcg*h/ml. Thus the toxic effects seen at the lowest dose in this study occur at systemic exposures one third of those seen at the highest recommended dose.

4. THIRTEEN-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 1.25 to 2.5 mg/kg to rats for thirteen weeks was associated with extramedullary hematopoiesis, irritation of injection site, decreased urine specific gravity, and increased total excreted potassium. Additional changes at 5 mg/kg included increased blood potassium levels, increased relative thyroid weight, a hematoma (one male) and a mild hemorrhage in the eyeball (one animal). At 10 mg/kg, animals exhibited increased total excreted sodium, potassium and chloride, increased urine volume, increased reticulocytes, blood potassium, total bilirubin, extramedullary hematopoiesis and injection site damage. Cmax values for rats treated at 2.5 mg/kg were around 9 mcg/ml. Human subjects treated with micafungin at the highest dose of 150 mg demonstrated a Cmax value around 16 mcg/ml. Thus, the toxic effects seen in this study at 10 mg/kg (Cmax values ranging from 22 to 40 mcg/ml over the study) could be expected at the recommended human doses. The NOAEL 2.5 mg/kg, occurs at a systemic exposure about 56 % of that expected at the highest recommended clinical dose.

Study no: GLR970291
Conducting laboratory: [redacted]

Location: [redacted]
Date of study initiation: August 26, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 002061L [redacted], purity

Formulation: Dissolved in physiological saline

Four groups of SPF — CD (SD) strain rats, (10/sex/dose group), were injected in the tail vein, once daily for thirteen weeks, with micafungin at 1.25, 2.5, 5 or 10 mg/kg. Control animals received physiological saline. A satellite group of 12 animals per dose was used to measure drug plasma levels. Drug was infused as a bolus. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and drug plasma concentrations. Animals were 6 weeks old and males weighed between 192 and 216 g while females weighed between 150 and 177 g.

Animals treated at 1.25 mg/kg FK463 for thirteen weeks showed slight extramedullary hematopoiesis (2/10 females) as well as injection site changes (perivascular hemorrhage, cellular infiltration, necrosis, fibrosis). The next higher dose, 2.5 mg/kg/day also produced decreased urine specific gravity and increased total excreted potassium per day.

At 5 mg/kg/day, animals showed extramedullary hematopoiesis, injection site damage, increased blood potassium, decreased relative thyroid weight, a hematoma (one male) and one animal showed a mild hemorrhage in the eyeball.

At 10 mg/kg, animals exhibited increased total excreted sodium, potassium and chloride, increased urine volume, increased reticulocytes, blood potassium, total bilirubin, decreased thyroid weight, extramedullary hematopoiesis and injection site damage. Isolated occurrences of thickening of the cecal wall, periarteritis in the cerebrum, myocarditis, granulomatous inflammation of the stomach and urinary bladder, cellular alteration of the adrenals may not have been related to drug.

Table 3. Pharmacokinetics: Mean peak plasma concentrations of FK463 after repeated intravenous injections

FK463 Dose	**Cmax (mcg/ml)**		
(mg/kg/day)	**Day 1**	**5 weeks**	**13 weeks**
1.25	2.5	3.2	4.1
2.5	4.9	6.7	8.9
5	11.0	14.8	18.9
10	22.3	31.7	40.0

Mean peak (15 minute) FK463 levels were approximately proportional to dose throughout the study. The mean peak drug levels increased over time.

Table 4. Pharmacokinetics: Mean trough plasma concentrations of FK463 after repeated intravenous injections

FK463 Dose	FK 463 trough levels (24 hours post dose) (mcg/ml)		
(mg/kg/day)	Day 1	5 weeks	13 weeks
1.25	0.03	0.09	0.20
2.5	0.14	0.25	0.52
5	0.36	0.74	1.39
10	1.02	1.89	3.84

Cmax values for rats treated at 2.5 mg/kg were around 9 mcg/ml. Human subjects treated with micafungin at the highest dose of 150 mg demonstrated a Cmax value around 16 mcg/ml. Thus, the toxic effects seen in this study at 10 mg/kg (Cmax values ranging from 22 to 40 mcg/ml over the study) may not be expected at the recommended human doses. The NOAEL, 2.5 mg/kg, produced systemic exposure about 56% of that expected at the highest recommended clinical dose.

5. A 26-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 1mg/kg to rats for twenty six weeks was associated with no drug-related findings. At 3.2 mg/kg, drug related findings included altered reticulocyte ratio, extramedullary hematopoiesis and smallness of testes/epididymis. At higher doses effects involved the liver, spleen, kidneys bladder, lungs, bone arrow and epididymis. Cmax values for rats treated at 10 mg/kg were between 20 and 45 mcg/ml. Human subjects treated with micafungin at the highest dose of 150 mg demonstrated a Cmax value around 16 mcg/ml. Thus the toxic effects seen in this study at 10 mg/kg may not be seen at the recommended human doses. The NOAEL, 1 mg/kg, produced an exposure (Cmax) about 27 % of that expected at the highest recommended clinical dose.

Study no: GLR010153
Conducting laboratory: —
Location: —
Date of study initiation: June 16, 1999
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L and 003062L — purity
Formulation: Dissolved in physiological saline

Four groups of SPF — CD (SD) strain rats, (14/sex/dose group), were injected in the tail vein, once daily with micafungin at 1.0, 3.2, 10 or 32 mg/kg. An additional group of control animals received physiological saline. A satellite group (4/sex/dose) was used to measure plasma drug levels. Drug was given as a bolus dose. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and plasma drug concentrations. Animals were 6

weeks old and males weighed between 192 and 216 g while females weighed between 150 and 177 g.

Administration of micafungin at 1.0 mg/kg to rats for twenty six weeks produced no drug related effects. At 3.2 mg/kg, toxic effects included increased reticulocytes, extramedullary hematopoiesis, and bilateral smallness of the testes and epididymis. Injection site damage at this dose was no more frequent than in control animals.

At 10 mg/kg animals showed significant increases in excretion of sodium, decreased red blood cell count, hemoglobin and hematocrit, a significant shortening of the activated partial prothrombin time and an increase in reticulocytes. Extramedullary hematopoiesis and relative spleen weights were also increased at this dose as was nuclear hypertrophy, vacuolation and round cell infiltration/accumulation in liver sinusoid.

At 32 mg/kg, the bodyweights of the males were about 10% lower than controls. Urinalysis changes included lowered urine pH, increased numbers of animals with small round epithelial cells, increased water intake and urine volume, low urine osmolarity and increases in excretion of sodium and chloride. Hematology findings (in addition to those seen at 10 mg/kg), included increased mean corpuscular hemoglobin concentration (MCHC) and platelet count and a shortening of the activated partial thromboplastin and prothrombin times. Blood chemistry changes reflected changes in the liver, and included increases in AST, ALT, ALP, total bilirubin, urea nitrogen, potassium and calcium and decreases in total cholesterol, triglycerides, phospholipids, glucose, creatinine and total proteins, increased A/G ration, albumin fraction and globulins fraction (α_2, β and γ) and decreases in the proportion of alpha-1 globulin.

Necropsy findings associated with micafungin administration at 32 mg/kg included increased relative liver, spleen, kidney, heart and lungs weights. There was also a significant decrease in the absolute weight of the testes at this dose. Histopathology examination of the liver revealed that 32 mg/kg micafungin was associated with nuclear hypertrophy, single cell necrosis, cytoplasmic acidophilic bodies in hepatocytes, mild round cell infiltration/accumulation in the sinusoid, vacuolation of the hepatocytes, mitosis in hepatocytes, multinucleated hepatocytes, swelling of sinusoidal cells and increased number of cells with altered cell foci. Extramedullary hematopoiesis was increased at this dose. In the kidney, deposition of a hemosiderin-like brown pigment was detected in the proximal tubular epithelium as well as mild dilatation of the collecting duct, swelling of the collecting ductal epithelium and vacuolation of the pelvic epithelium. In the bladder there was mild vacuolation and thickening of mucosal epithelium. Slight hypercellularity in the bone marrow was observed in the sternum and the femur. Osteochondroma was detected in the femur, tibia and rib of one male at 32 mg/kg. Slight atrophy of the epididymis was detected in 2 males and slight cellular infiltration of the Haderian gland was found in one female.

Table 5. Pharmacokinetics: Mean peak plasma concentrations of micafungin after repeated intravenous injections in rats

FK463 Dose	Cmax (mcg/ml)		
(mg/kg/day)	Day 1	13 weeks	26 weeks
1.0	2.4	3.6	4.3
3.2	6.2	11.5	14
10	20	38	45
32	76	118	159

Mean peak (15 minute) FK463 levels were approximately proportional to dose throughout the study. The mean peak drug levels increased over time.

Table 6. Pharmacokinetics: Mean trough plasma concentrations of micafungin after repeated intravenous injections in rats

FK463 Dose	FK 463 trough levels (24 hours post dose) (mcg/ml)		
(mg/kg/day)	Day 1	13 weeks	26 weeks
1.0	0.06	0.15	0.23
3.2	0.20	0.67	0.99
10	1.0	3.5	5.09
32	5.3	13.7	20.6

Trough levels of micafungin were proportional to the dose administered for the two lower doses but were superproportional to the dose above doses of 3.2 mg/kg. Also, trough levels increased as dosing duration increased.

6. FOUR WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS

Key study findings: The NOAEL for micafungin was determined to be 3.2 mg/kg. At 10 mg/kg, changes were detected in MCHC, calcium, A/G ratio and serum albumin rate. Exposure at 32 mg/kg produced adverse effects in the liver including centrilobular hypertrophy, increased size and weight and discoloration. Exposure at this dose (591 µg*h/ml) was about 3.5 times the exposure that would be experienced at the highest recommended human dose (167 µg*h/ml).

Study no: GLR970118
Conducting laboratory:

Location:

Date of study initiation: February 7, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )

Drug lots: 00105YL , purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 3 per sex per dose group, were given intravenous injections once daily for four weeks with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and drug plasma concentrations. Animals were 6 months old at the beginning of the study.

Dogs treated with micafungin at 3.2 mg/kg did not show any consistent drug related adverse effects. At 10 mg/kg, dogs showed reduced MCHC, increased calcium, A/G ratio and serum albumin. At the higher dose, additional findings included increased urea nitrogen and glucose, liver enlargement and increased liver weights, liver discoloration, centrilobular hypertrophy, increased smooth endoplasmic reticulum in the centrilobular hepatocytes, and mild myelinosomes in the centrilobular and mid lobular hepatocytes.

Table 7. Pharmacokinetics: $AUC_{0\text{-}24h}$ values (mcg*h/ml) recorded after repeated micafungin dosing in dogs.

Dose (mg/kg)	$AUC_{0\text{-}24h}$ (Day 1)	$AUC_{0\text{-}24h}$ (Week 4)
3.2	45	52
10	154	162
32	557	591

AUC values for micafungin were dose proportional and values on day One were similar to those after four weeks of dosing. Elimination half life was between 3.6 and 4.2 hours.

SUMMARY

The NOAEL for micafungin was determined to be 3.2 mg/kg. At 10 mg/kg, changes are detected in MCHC, calcium, A/G ratio and serum albumin. Exposure at 32 mg/kg resulted in adverse effects in the liver including centrilobular hypertrophy accompanied by increased size, weight, and discoloration. Exposure at this dose (591 mcg*h/ml) was about 3.5 times the exposure that would be experienced at the highest recommended human dose (167 mcg*h/ml).

7. THIRTEEN-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS WITH A 4-WEEK RECOVERY STUDY.

Key study findings: The NOAEL for micafungin was determined to be 10 mg/kg. At 32 mg/kg adverse effects observed in the liver included centrilobular hypertrophy, accompanied by increased size, weight, and discoloration. Exposure (Cmax) at this dose (105 mcg/ml) was about seven times the exposure that would be experienced at the highest recommended human dose (Cmax =16 mcg/ml).

Study no: GLR970292

Conducting laboratory: —

Location: —

Date of study initiation: June 28, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L. – . purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 3/sex/dose group, were given injections once daily with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Two additional groups of dogs, (2/sex/dose) were treated with saline or 32 mg/kg micafungin and allowed to remain untreated for four weeks after the end of dosing to assess the reversibility of any toxic effects. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and drug plasma concentrations. Animals were 6 months old and weighed between 6.5 and 9.6 kg at the start of the study.

There were no drug-related effects at doses below 32 mg/kg. Leukocytes were decreased and cholesterol and ALT levels were increased in the 32 mg/kg dose group. Changes in the liver included increased absolute and relative liver weights, enlarged, discolored livers and centrilobular hypertrophy. Spleen congestion and discoloration was found in one male animal.

In the recovery animals, discoloration of the liver was observed, as well as increased liver weight and centrilobular hypertrophy. Treatment with micafungin at 32 mg/kg for 13 weeks produced some changes in the dog which were not reversible after a four week recovery period.

Table 8. Pharmacokinetics: Mean peak plasma concentrations of micafungin after repeated intravenous injections in dogs

Dose (mg/kg)	Day 1	Week 13
3.2	10	9
10	31	30
32	102	105

Table 9. Pharmacokinetics: Mean trough plasma concentrations of micafungin after repeated intravenous injections in rats

Dose (mg/kg)	Day 1	Week 13
3.2	0.12	0.15
10	0.40	0.6
32	1.6	2.0

Irreversible changes were associated with micafungin, when administered at 32 mg/kg for 13 weeks in beagle dogs. This dose produced a peak plasma concentration that is 105 mcg/ml, almost seven times the peak plasma concentration observed in human subjects receiving the

maximum recommended dose of micafungin. Irreversible changes in human subjects likely would not occur under normal dosing conditions.

8. A 39-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN BEAGLE DOGS.

Key study findings: No NOAEL could be established for this study of intravenous micafungin using doses 3.2, 10 and 32 mg/kg. At the lowest dose administered, micafungin resulted in adverse effects on the liver, spleen and testes. At 32 mg/kg, effects included increased liver weights, swollen, discolored livers, centrilobular hypertrophy, increased total cholesterol, alpha$_2$ globulin ratio, platelet count, fibrinogen and phospholipids, high splenic weight, congestion of spleen and dark red coloration of the spleen. Seminiferous tubules showed bilateral atrophy and spermatogenic cells were decreased. Exposure AUC $_{0\text{-}24h}$ at 3.2 mg/kg, (95 mcg*h/ml) is less that the exposure that would be experienced at the highest recommended human dose AUC $_{0\text{-}24h}$, 167 µg*h/ml). As such the findings observed at the lowest dose in this study could be reasonably expected at the highest clinical dose after 39 weeks of dosing.

Study no: GLR000510
Conducting laboratory: —

Location: —

Date of study initiation: December 17, 1998.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L — , purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 4 per sex per dose group, were injected once daily for 39 weeks with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, ophthalmology, electrocardiography, blood pressure measurements, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and drug plasma concentrations. Animals were 6 months old and weighed between 7.3 and 10 kg.

Dogs treated at 3.2 mg/kg/day showed a number of toxic effects which were mirrored at the higher doses. These included increased liver weight, liver swelling, swollen spleen, hemorrhage in the spleen, white spots on lungs, and atrophy of the seminiferous tubules. At 10 mg/kg, animals also showed prolonged QRS interval (17 % increased), liver swelling, and high liver weight, small thyroid, bilateral atrophy of the seminiferous tubules, dilatation of the seminiferous tubules, stasis of spermatids and decreased sperm count.

At 32 mg/kg, livers were increased in weight, swollen, discolored and showed centrilobular hypertrophy and prolonged QRS interval (13 to 20% increased). One dog showed increased total cholesterol, alpha2 globulin ratio, platelet count, fibrinogen and phospholipids, high splenic weight, congestion of spleen and dark red coloration of the spleen. Seminiferous tubules showed bilateral atrophy which at times was severe and spermatogenic cells were decreased. In some animals, seminiferous tubules contained only Sertoli cells. Slight to mild

vacuolation of spermatogenic cells was observed in all animals of the 32 mg/kg group. Severe decreases in sperm count were observed at this dose and cell debris was observed in ¾ males. There was also mild cellular infiltration of the interstitium. These findings were thought to be related to the atrophy of the seminiferous tubules.

Table 10. Pharmacokinetics: Cmax and AUC_{0-24h} values (mcg*h/ml) on Day One of FK463 administration in dogs.

Dose (mg/kg)	AUC (mcg*h/ml)	Cmax
3.2	95	9.8
10	280	31
32	1028	106

AUC values were essentially the same as in week 39 (AUC values 114, 303 and 1132 mcg*h/ml)

9. FR179463: INTRAVENOUS STUDY FOR EFFECTS ON PRE- AND POSTNATAL DEVELOPMENT INCLUDING MATERNAL FUNCTION IN RATS.

Key study findings: At 10 mg/kg, the only abnormal findings related to drug were in one dam that lost its litter after a difficult delivery. Since this did not occur in any animals at the higher dose it was assumed that this effect was not drug related. At 32 mg/kg, dams showed effects on body weight, food consumption and injection site. Pups weighed slightly less than control pups. Thus the NOAEL for general toxicity of the dams and development of their offspring was 10 mg/kg. The NOAEL for reproductive function was 32 mg/kg.

Study no: GLR000640
Conducting laboratory: Toxicology Research Laboratories, Fujisawa Pharmaceutical Company Ltd. 1-5, Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan
Date of study initiation: December 17, 1998.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L —, purity
Formulation: Dissolved in physiological saline

Groups of ~~~.CD(SD) female rats (20/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg from day 7 of gestation through day 20 postpartum. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, physical development, litter data, internal and external abnormalities, locomotor coordination, emotional behavior (grooming, defecation). motor activity, avoidance test, mating performance, copulation and fertility indices.

Dams treated at 32 mg/kg experienced swollen snout, and black coloration at the injection site followed by tail loss. Body weight and food consumption were also reduced on certain days. Pups born to these mothers had slightly (about 5 %) lower weights than controls. One dam treated at 10 mg/kg showed paleness on delivery and prolongation of labor and this dam lost its entire litter on day 0 of gestation.

There were no other abnormal changes that could be attributed to micafungin.

SUMMARY

At 10 mg/kg, the only abnormal findings related to changes related to one dam that lost its litter after a difficult delivery. Since this did not occur in any animals at the higher dose it was assumed that this effect was not drug related. At 32 mg/kg, dams showed effects on body weight, food consumption and injection site. Pups weighed slightly less than control pups. Thus the NOAEL for general toxicity of the dams and development of their offspring was 10 mg/kg. The NOAEL for reproductive function was 32 mg/kg.

10. STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT UP TO IMPLANTATION IN RATS TREATED INTRAVENOUSLY WITH FR179463

Key study findings: Females treated with micafungin at 32 mg/kg showed temporary reductions in food consumption. Males showed vacuolation of the ductal epithelium of the caput epididymis at 10 and 32 mg/kg as well as reduced sperm count and increased epididymis weight. NOAEL for reproductive toxicity was therefore determined to be 3.2 mg/kg for males. For females the NOAEL was 10 mg/kg for general toxicity and 32 mg/kg for reproductive toxicity.

Study no: GLR970119
Conducting laboratory: —

Location: —
Date of study initiation: February 23 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 00105YL, — purity
Formulation: Dissolved in physiological saline

Groups of — CD(SD) rats (20/sex/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg. Drug was given to males from 9 weeks before mating through the mating period to the day before necropsy. Females were treated from 2 weeks before mating, through mating to 7 days after confirmed copulation. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, gross pathology of testes and epididymis, plasma ALT activity, sperm counts, number of estrus cycles before mating, litter data, internal and external abnormalities, mating performance, copulation and fertility indices.

Two males from the 10 mg/kg group died on days 71 and 76 of dosing. Foam was found in the renal vein and inferior vena cava of one animal, but cause of death was not determined in the second animal. There were no other abnormal clinical signs. Plasma ALT was significantly higher in the 32 mg/kg group.

In males in the 32 mg/kg group, epididymis weight was higher than in controls. In addition, the number of sperm cells was significantly lower than control even though there was no difference in sperm motility, viability or occurrence of abnormal sperm. Vacuolation of the ductal

epithelial cells was observed in almost all animals at 32 mg/kg and 3 animals from the 10 mg/kg group.

In females, there were temporary reductions in food consumption in the high dose group before mating. Copulation, fertility indices, insemination, number of corpora lutea, implantations, live embryos, implantation index or dead embryo rate were no different between treated and control animals.

SUMMARY

Females treated with micafungin at 32 mg/kg showed temporary reductions in food consumption. Males showed vacuolation of the ductal epithelium of the caput epididymis at 10 and 32 mg/kg as well as reduced sperm count and increased epididymis weight. NOAEL for reproductive toxicity was therefore determined to be 3.2 mg/kg for males. For females the NOAEL was 10 mg/kg for general toxicity and 32 mg/kg for reproductive toxicity.

11. STUDY TITLE: INTRAVENOUS DOSING STUDY OF FR179463 ON EMBRYO-FETAL DEVELOPMENT IN RATS.

Key study findings: The NOAEL for maternal effects was 10 mg/kg. The NOAEL for reproductive toxicity was 32 mg/kg since no adverse effects on reproductive function could be detected at this dose.
Study no: GLR970379
Conducting laboratory: —

Location: —
Date of study initiation: October 7, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L, — purity
Formulation: Dissolved in physiological saline

Groups of pregnant female — CD(SD) rats (20/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg from day 7 to day 17 of gestation. This period corresponds to the period from implantation to the closure of the hard palate. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, plasma ALT activity, gross pathology at cesarean section, litter data, internal and external abnormalities.

Plasma ALT levels were higher than control values in the 32 mg/kg group. There were no abnormal findings on cesarean section and no external visceral or skeletal abnormalities that could be ascribed to the drug.

The NOAEL for maternal effects was 10 mg/kg since ALT was increased at 32 mg/kg. The NOAEL for reproductive toxicity was 32 mg/kg since no adverse effects on reproductive function could be detected at this dose.

12 STUDY TITLE: INTRAVENOUS DOSING STUDY OF FR179463 ON EMBRYO-FETAL DEVELOPMENT IN RABBITS.

Key study findings: The NOAEL for maternal effects was 10 mg/kg since an abortion and numerous visceral abnormalities were observed at 32 mg/kg.
Study no: GLR970380
Conducting laboratory: —
—
Location: Kannami Laboratory, 1308 Sanbonmatsu, Kuwahara, Kannami-cho, Tagata-gun, Shizuoka-ken 412, Japan
Date of study initiation: August 1, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L, — purity
Formulation: Dissolved in physiological saline

Groups of pregnant female SPF New Zealand White (Kbl:NZW) strain rabbits (16-18/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg for 13 days from day 6 to day 18 of gestation. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, plasma ALT activity, gross pathology at cesarean section, litter data, internal and external abnormalities.

General signs observed at 32 mg/kg included a reduction in feces and one abortion. This abortion occurred in an animal which, upon gross pathological examination was shown to have a congenital absence of the right uterine horn.

The only effects which appeared to be related to drug were seen at 32 mg/kg and were increases in visceral abnormalities. Whereas only one fetus from one control dam showed visceral abnormalities, 10 fetuses from 5 treated (32mg/kg) dams showed visceral abnormalities. These included abnormal lobation of the lung in 6 fetuses, levocardia in 3 fetuses, retrocaval ureter in 3 fetuses, anomalous right subclavian artery in 1 fetus and dilatation of the ureter in 1 fetus.

SUMMARY

Dosing rabbits with FR 179463 at 32 mg/kg resulted in visceral abnormalities and abortion. This abortion is consistent with abortions observed in a preliminary study at 60 mg/kg. Although this animal also suffered from congenital absence of the right uterine horn, the relationship to drug cannot be ruled out. Visceral abnormalities included abnormal lobation of the lung, levocardia, retrocaval ureter, anomalous right subclavian artery, and dilatation of the ureter.

13. MUTAGENICITY STUDY OF R179463: REVERSION TEST WITH BACTERIA.

Key study findings: No evidence of potential mutagenic effects
Study no: GLR970122

Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Location: Toxicology Research Laboratories, Fujisawa Pharmaceutical Company Ltd. 1-5, Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan
Date of study initiation: May 17, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 00105YL — purity
Formulation: Dissolved in physiological saline

This study was designed to assess the ability of R179463 to produce mutations. In this model the mutagenicity is assessed by the drugs ability to increase the number of revertant colonies using *Salmonella typhimurium* TA100, TA98, TA1535 and TA1537 and *Escherischia Coli* WP2 uvrA as tester strains.

The test was performed after preincubation with or without metabolic activation (rat S9 mix) and drug levels ranged from 156 to 5000 µg/plate of FR179463. A positive result was taken as any condition under which the number of revertants exceeded the number seen in the solvent control by two multiples.

FR179463 did not increase the mean number of revertant colonies of any strains to twice that of the solvent controls, with or without metabolic activation. Positive and negative controls were satisfactory under the experimental conditions.

FR179463 did not demonstrate the potential to induce gene mutations as assessed using concentrations between 156 to 5000 µg/plate and using *Salmonella typhimurium* TA100, TA98, TA1535 and TA1537 and *Escherischia Coli* WP2 uvrA as tester strains.

14. MUTAGENICITY STUDY OF R179463: CHROMOSOMAL ABERRATION TEST WITH CHINESE HAMSTER LUNG CELLS IN CULTURE.

Key study findings: No evidence of potential mutagenic effects
Study no: GLR9800005
Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Date of study initiation: December 17, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L — purity
Formulation: Dissolved in physiological saline

This study was designed to assess the clastogenicity of FR179463 using the chromosome aberration test with Chinese hamster lung (CHL) cells. CHL cells were exposed to drug for 24 or 48 hours in one study. In another experiment the effect of metabolic activation was assessed after six hour treatments with or without metabolic activation.

FR179463 did not increase the number of cells with structural aberration or polyploidy in either the direct (40-160 µg/ml) or metabolic activation (625-5000 µg/ml) method. As such FR179463 did not show any potential for clastogenicity.

15. MUTAGENICITY STUDY OF R179463: MICRONUCLEUS TEST IN MICE
Key study findings: No evidence of potential clastogenic effects
Study no: GLR970373
Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Date of study initiation: July 29, 1997.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 057AEH - o purity
Formulation: Dissolved in physiological saline

This study was designed to assess the clastogenicity of FR179463. Drug was administered intravenously to ICR mice in a single dose of 25, 50 or 100 mg/kg. Bone marrow cells were taken 24, 48 and 72 hours after dosing and the erythrocytes were examined.

FR179463 did not significantly increase polychromatic erythrocytes with micronuclei in any treated groups at any sampling times. AUC (0-24h) values for the 100 mg/kg dose was 2763 µg*h/ml. This was over seventeen times the exposure seen in humans at the highest recommended human dose. As such, we would not expect a clastogenic effect at clinically relevant doses or up to 17 times that dose.

Study title: Safety Pharmacology Study of FR179463: Action Potential Measurement by Microelectrode Techniques

Key study findings: Micafungin did not affect resting membrane potential, action potential amplitude, or maximum rate of depolarization. No prolongation of action potential duration.

Study no: 4326
Conducting laboratory and location: —

—

Date of study initiation: August 6, 2003
GLP compliance: No
QA report: yes
Drug, lot # GLP-00206IL
Purity: —
Formulation/vehicle: Distilled water

An *in vitro* preparation of Guinea pig papillary muscle was used to determine if micafungin exposure resulted in any effects on the resting membrane potential, action potential amplitude, maximum rate of depolarization and action potential duration. Five preparations were used with micafungin and five were used for the vehicle control.

Control (pre perfusion) values were obtained for each preparation used after which the preparation was superfused with micafungin at $1X10^{-7}$ g/ml then $1X10^{-6}$ g/ml and then $1X10^{-5}$ g/ml. Finally the preparation was exposed to $1X10^{-4}$ g/ml of the positive control sotalol (for a total of four sessions). Vehicle preparations were superfused four times with vehicle. Resting membrane potential, action potential amplitude, maximum rate of depolarization and action potential duration were measured for each superfusion. The preparation was then discarded.

Results:

Micafungin did not show any effects on the resting membrane potential, action potential amplitude or maximum rate of depolarization. Action potential duration was shortened by 10 msec in the presence of $1X10^{-5}$ g/ml micafungin. In contrast, the positive control sotalol prolonged the action potential duration APD_{90} by 43 msec and significantly decreased the action potential amplitude by 2.4 msec.

Summary

Micafungin did not result in a prolongation of the action potential duration and did not affect the resting membrane potential, action potential amplitude or maximum rate of depolarization.

Conclusions: Micafungin did not demonstrate the potential to cause Qt prolongation since it did not result in a prolongation of the action potential duration and did not affect the resting membrane potential, action potential amplitude or maximum rate of depolarization.

Study title: Safety Pharmacology Study of FR179463: hERG Assay

Key study findings: Micafungin did not suppress hERG currents.

Study no: SP-0340
Conducting laboratory and location: —

Date of study initiation: August 18, 2003
GLP compliance: No
QA report: yes
Drug, lot # GLP-002061L
Purity: —
Vehicle: Water for injection

Methods

The whole cell patch clamp technique was used to assess the effects of micafungin on the hERG channel current in HEK293 cells transfected with the human ether-a go-go-related gene. The suppressive effects were determined as the percentage decrease in the peak amplitude of tail currents 10 minutes after application of a test substance using five separate cell preparations in each experimental group. Micafungin was dissolved in water for injection and superfused at $1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$mg/ml. The positive control was E-4031.

Results

Micafungin did not demonstrate a suppressive effect on hERG current compared to vehicle control (see Table 11 below)

Table 11: Suppression of hERG currents by various treatments.

	Supression rates (%)
Water	10
Micafungin ($1x10^{-7}$)	7
Micafungin ($1x10^{-6}$)	11
Micafungin ($1x10^{-5}$)	14
E-4031 (positive control)	86

Summary of individual study findings: Micafungin did not demonstrate a suppressive effect on hERG current compared to vehicle control.

Overall Conclusion

There are no safety issues that would preclude the approval of micafungin for injection for the treatment of [redacted] at the proposed doses of 50 or 150 mg. No additional preclinical studies are being recommended at this time. The label should include [redacted]

Owen McMaster, PhD
Pharmacology/Toxicology Reviewer
DSPIDP

Pharmacology/Toxicology Reviewer, DSPIDP

Concurrences:

HFD-590/DivisionDirector
HFD-590/Pharm/ToxTL

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Owen McMaster
3/14/05 11:50:45 AM
PHARMACOLOGIST

Robert Osterberg
3/14/05 12:03:00 PM
PHARMACOLOGIST

Steven Gitterman
3/14/05 12:46:36 PM
MEDICAL OFFICER

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21506
Review number: 1
NDA submission # 000
Submission date: April 29,2002
Information to sponsor: Yes (√) No ( )
Sponsor: Fujisawa Healthcare, Three Parkway North, Deerfield, Illinois, 60015-2548
Manufacturer of drug substance : Fujisawa Pharmaceutical Company Ltd. 30, Ttoedesakae-machi, Tokaoka, Toyama 939-1118, Japan
Reviewer name: Owen McMaster, Ph.D.
Division of Special Pathogen and Immunologic Drug Products
HFD-590
Review completion date: January 17, 2003
Drug:
Trade name: Mycamine
Generic name: micafungin
Code name: FK 463, FR 179463
Chemical name: *IUPAC:* Sodium 5-[(1*S*, 2*S*)-2-[(3*S*,6*S* ,9*S* ,11*R*, 15 *S* ,18 *S* ,20*R* ,21*R*, 24 *S* ,25*S*,26*S*)-3-[(R)-2-carbomoyl-1-hydroxyethyl]-11,20,21,25-tetrahydroxy-15-[(R)-1-hydroxylethyl]-26-methyl-2,5,8,14,17,23-hexaoxo-18-[4-[5-(4-pentyloxyphenyl)isoxazol-3-yl]benzoylamino]-1,4,7,13,16,22-hexaazatricyclo[22.3.0.$0^{9,13}$]heptacos-6-yl]-1,2-dihydroxyethyl]-2-hydroxyphenyl sulfate.

CAS registry number: 208538-73-2 (sodium salt)
Molecular formula: C_{56} H_{70} N_9 NaO_{23} S
Molecular weight: 1292.26
Structure:

Relevant INDs/NDAs/DMFs: IND 55,322, DMF — , DMF —

Drug class: Echinocandin

Indications:

(1) Prophylaxis of — in patients undergoing hematopoietic stem cell transplantation

Clinical formulation: Micafungin for injection is a sterile,nonpyrogenic lyophilized product for intravenous injection. The drug product is available in a 50 mg vial. Each vial contains micafungin — 50 mg) lactose monohydrate (200 mg), anhydrous citric acid (for pH adjustment, pH 5-- , sodium hydroxide (for pH adjustment, pH 5· —

Route of administration: Intravenous infusion (over 1 hour)

Proposed use: [redacted]

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability

There are no safety issues that would preclude the approval of micafungin for injection for the — at the proposed doses of 50 —

B. Recommendation for Nonclinical Studies

C. Recommendations on Labeling

The label should include a statement —

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

The toxicology program for micafungin included studies in mice, rats, rabbits and dogs. The highest dose administered was 250 mg/kg as a single dose to rats and the longest studies were for 26 weeks in rats and 39 weeks in dogs. The minimum lethal dose was 12-times the highest recommended human dose (based on a body surface area comparison) using data from the more sensitive species, the rat. Using data from the dog, the minimum lethal dose was more than 65 times the maximum recommended human dose.

In animals, the target organs are the liver and the testes, with effects also being seen in clinical chemistry, spleen, and at the injection site. Liver toxicity included enlarged, discolored livers with centrilobular hypertrophy, single cell necrosis, acidophilic bodies, nuclear hypertrophy, vacuolation, bile duct proliferation and mitosis. In rats and dogs, micafungin administration produced testicular atrophy and decreased sperm count. Plasma AST/GOT, ALT/GPT, alkaline phosphatase and LDH were also increased. In the blood, there were decreased erythrocytes, hemoglobin and hematocrit as well as increased reticulocytes, serum total bilirubin and potassium. Congestion, pigmentation and increased weight were recorded in the spleen along with hypercellularity in the femoral bone marrow. Injection sites showed hemorrhage, and cellular infiltration of the perivascular tissue. Injection site irritation was less severe when the drug was infused over one hour compared to bolus injections.

Patients treated with micafungin at the highest recommended clinical dose (100 mg) show a Cmax of 28 mcg/ml and an $AUC_{0\text{-}24h}$ of 110 mcg*h/ml. In the more sensitive rat model, the No Observed Adverse Effect Level was always at a dose which produced exposures less than the exposure expected in the clinic. As such, some mild adverse

effects would be expected at the clinical exposure. In the less sensitive dog model, the clinical exposure was always at or below the NOAEL and so no adverse effects would be expected based on that model. Very high doses, of micafungin, when administered for prolonged periods, produced irreversible changes to the liver. In a 26-week rat study (dosed to 5-times clinical exposure), with four- or 13-week recovery, colored patches/zones, multinucleated hepatocytes and altered cell foci remained at the end of the recovery period. In a similar 13 week dog study with 4 week recovery (dose to 10 times clinical exposure), liver discoloration, cellular infiltration and hypertrophy remained visible at the end of the 13-week recovery period.

B. Nonclinical Safety Issues Relevant to Clinical Use

The predominant safety concern regarding the use of micafungin is the potential for irreversible liver damage if patients are exposed to the drug at high doses for prolonged periods. Thorough, frequent monitoring of liver function should prevent any adverse effects from endangering the patient. In addition,

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

IV. GENERAL TOXICOLOGY:

Toxicology Studies Summary

1. Single dose intravenous toxicity study of FR179463 in rats
2. Single dose intravenous toxicity study of FR179463 in dogs
3. Four week intravenous toxicity study of FR179463 in rats
4. Thirteen-week intravenous toxicity study of FR179463 in rats
5. A 26-week intravenous toxicity study of FR179463 in rats
6. Four week intravenous toxicity study of FR179463 in dogs
7. Thirteen-week intravenous toxicity study of FR179463 in dogs with a 4-week recovery study.
8. A 39-week intravenous toxicity study of FR179463 in beagle dogs.
9. FR179463: Intravenous study for effects on Pre- and postnatal development including maternal function in rats.
10. Study of Fertility and early embryonic development up to implantation in rats treated intravenously with FR179463
11. Intravenous dosing study of FR179463 on embryo-fetal development in rats.
12. Intravenous dosing study of FR179463 on embryo-fetal development in rabbits.
13. Mutagenicity study of R179463:Reversion test with bacteria.
14. Mutagenicity study of R179463: Chromosomal aberration test with Chinese Hamster Lung cells in culture.
15. Mutagenicity study of R179463: Micronucleus test in mice

1. SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: The minimum lethal dose was 125 mg/kg. This dose is equivalent to a human dose of 20 mg/kg, based on body surface area conversions.
Study no: GLR970113
Conducting laboratory and location: —

Date of study initiation: May 15, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot # 00105YL , ~ purity
Formulation: powder was dissolved inphysiological saline

Three groups of SPF ~/:CD (SD) strain rats, 5 per sex per dose group, were injected in the tail vein with micafungin at 62.5, 125 and 250 mg/kg. Drug was infused over 1 minute. Animals were observed for mortalities for 7 days after dosing. Animals were 6 weeks old and weighed males weighed between 200 and 250 g while females weighed between 162 and 190 g.

All animals in the 250 mg/kg dose group died immediately following dosing. Two males in the 125 mg/kg group died the day after administration.

At 125 mg/kg, animals showed decreased spontaneous movement, dark red coloration of the ear auricle and extremities, swelling of the face, tachypnea and frequent approach to the water dispenser. Abnormal signs resolved two hours after dosing. Dark red spots were seen on the lungs of the males that died the day after administration. Mild congestion and perivascular edema and hemorrhage was noted in one and mild alveolar focal hemorrhage was noted in the other male that died.

Additional signs seen at 250 mg/kg included prone position, clonic convulsions, oligopnea. Almost all these animals died within 5 minutes of dosing.

SUMMARY

The minimum lethal dose of FR179463 was 125 mg/kg, a dose equivalent to a human dose of 20 mg/kg based on body surface area conversions.

2. SINGLE DOSE INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS

Key study findings: The minimum lethal dose was greater than 200 mg/kg a dose equivalent to a human dose of greater than 108 mg/kg based on body surface area conversions.
Study no: GLR970114
Conducting laboratory: ~

Location:

Date of study initiation: May 16, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 00105YL , — , purity
Formulation: Dissolved in physiological saline

Two groups of 8 kg, 5 month-old beagle dogs, one/sex/dose group were injected (bolus) with a micafungin at 100 or 200 mg/kg. Animals were observed twice a day for 14 days.

No animals died. At 100 mg/kg, clinical signs included reddened eyelids, and extremities, increased total bilirubin and increased leukocytes. Additional signs seen at 200 mg/kg, included increased salivation, pale mouth and ear, reddened eyelids, ear, abdominal skin, decreased movements, sluggishness. The higher dose also caused decreased erythrocytes, hemoglobin and hematocrit, increased fibrinogen, segmented neutrophil rate, AST/GOT, LDH and reticulocytes.

SUMMARY

The minimum lethal dose was greater than 200 mg/kg a dose equivalent to a human dose of greater than 108 mg/kg or 6.5 grams for a 60 kg subject. The swollen eyelids and extremities and flushing suggest histamine release and the increased AST , LDH suggest that these high doses of micafungin result in damage to the liver

3. FOUR WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 32 mg/kg to rats for four weeks is associated with liver toxicity, hemolysis, bladder injury and irritation of injection site. Slight changes in the spleen, bone marrow and injection site are seen even at 3.2 mg/kg. AUC values for rats treated at 3.2 mg/kg were around 54 mcg*h/ml. Human subjects treated with micafungin at the highest dose of 100 mg show and AUC value around 110 mcg*h/ml. Thus the toxic effects, seen in this study, begin at exposures half of that seen at the highest recommended dose.

Study no: GLR970115
Conducting laboratory:
Location:
Date of study initiation: May 16, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 00105YL , —, purity
Formulation: Dissolved in physiological saline

Four groups of SPF — CD (SD) strain rats, 10 per sex per dose group, were injected once daily for four weeks, in the tail vein with micafungin at 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Drug was infused over 1 minute. Records were kept of

general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 4 weeks old, with males weighing between 190 and 230 g while females weighed between 142 and 192 g.

Animals treated at 3.2 mg/kg showed slight changes, consistent with the changes observed at the higher doses. These included decreased total protein (females), slight hematopoiesis in the spleen (one male), and mild hypercellularity in the bone marrow (4 of 20 animals).

Additional findings at 10 mg/kg, included increased total bilirubin and congestion of the spleen. At 32 mg/kg, changes included small round epithelial cells in the urinary sediment, decreased erythrocytes and lymphocyte rate. Increases were recorded in MCV, MCH, MCHC, reticulocyte rate, segmented neutrophil rate. There was also a significant shortening of the partial thromboplastin time. Clinical chemistry changes included increased plasma AST, ALT and LDH, total bilirubin, potassium, chloride, BUN, α_2-globulin rate. Decreases were recorded in creatinine, α_1-globulin rate and total protein.

Post mortem findings at 32 mg/kg included increased spleen weights and a slight increase in eosinophilic granules in the duct of the submandibular gland (8 of 10 males). In the liver, increased acidophilic bodies (secondary lysosomes), single cell necrosis, slight nuclear hypertrophy, hepatocellular vacuolation, mild round cell infiltration/accumulation in the sinusoid (probably a reaction to necrosis). In the spleen, there was mild congestion of the red pulp (most animals), extramedullary hematopoesis (2 females) and brown pigment in the red pulp (4 females). In the kidney, there was vacuolation in the pelvic mucosal epithelium (in 8/20 animals) and in the bladder, vacuolation in the mucosal epithelium (all animals). Bone marrow hypercellularity was observed in most high dose males. Slight focal atrophy of the seminiferous tubules was observed in one high-dose animal. Injection sites were characterized by slight or mild perivascular hemorrhage, cellular infiltration and fibrosis. Effects were more pronounced and frequent at the higher doses and so these effects are clearly drug related

Pharmacokinetics: $AUC_{0\text{-}24h}$ values (mcg*h/ml) recorded after repeated micafungin dosing in rats.

FK463 Dose	$AUC_{0\text{-}24h}$ mcg*h/ml		
(mg/kg/day)	Day 1	Day 14	Day 28
3.2	54	53	56
10	180	203	223
32	573	680	853

Mean AUC values were proportional to dose on day 1, but increased over time and the dose proportionality was not maintained

Pharmacokinetics: $T_{½}$ values (h) recorded after repeated micafungin dosing in rats.

FK463 Dose	$T_{½}$ values		
(mg/kg/day)	Day 1	Day 14	Day 28
3.2	4.8	5.3	5.2
10	5.7	6.5	6.2
32	6.8	7.7	7.5

Mean terminal half-life increased with dose.

SUMMARY

FK463 produced adverse effects at all doses beginning at 3.2 mg/kg when administered for 4 weeks. The effects seen at 3.2 mg/kg, though milder than those seen at the higher doses, reflected the effects seen at the higher doses. Therefore the lower total protein values, hematopoeisis, and bone marrow hypercellularity, reflect real toxic effects of FK463. At higher doses, effects on the liver, spleen, kidneys, bladder, testes and injection site became evident. AUC values for rats treated at 3.2 mg/kg were around 54 mcg*h/ml. Human subjects treated with micafungin at the highest dose of 100 mg show and AUC value around 110 mcg*h/ml. Thus the toxic effects seen at the lowest dose in this study occur at exposures half of that seen at the highest recommended dose.

4. THIRTEEN-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 1.25 to 2.5 mg/kg to rats for thirteen weeks is associated with extramedullary hematopoeisis, irritation of injection site, decreased urine specific gravity, and increased total excreted potassium. Additional changes at 5 mg/kg included increased blood potassium levels, increased relative thyroid weight, a hematoma (one male) and a mild hemorrhage in the eyeball (one animal). At 10 mg/kg, animals exhibited increased total excreted sodium, potassium and chloride, increased urine volume, increased reticulocyte rate, blood potassium, total bilirubin, extramedullary hematopoiesis and injection site damage. Cmax values for rats treated at 2.5 mg/kg were around 9 mcg/ml. Human subjects treated with micafungin at the highest dose of 100 mg show and Cmax value around 28 mcg/ml. Thus the toxic effects seen in this study at 10 mg/kg (Cmax values ranging from 22 to 40 mcg/ml over the study) could be expected at the recommended human doses. The NOAEL level, 2.5 mg/kg produced an exposure about a third of that expected at the highest recommended clinical dose.

Study no: GLR970291
Conducting laboratory: —
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Location: —
Date of study initiation: August 26, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lot: 002061L , ~ purity
Formulation: Dissolved in physiological saline

Four groups of SPF CD (SD) strain rats, (10/sex/dose group), were injected once daily for thirteen weeks, in the tail vein with micafungin at 1.25, 2.5, 5 or 10 mg/kg. Control animals received physiological saline. A satellite group of 12 animals per dose was used to measure drug plasma levels. Drug was infused as a bolus. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 6 weeks old and males weighed between 192 and 216 g while females weighed between 150 and 177 g.

Animals treated at 1.25 mg/kg FK463 for thirteen weeks showed slight extramedullary hematopoiesis (2/10 females) as well as injection site changes (perivascular hemorrhage, cellular infiltration, necrosis, fibrosis). The next higher dose, 2.5 mg/kg/day also resulted in decreased urine specific gravity and increased total excreted potassium per day.

At 5 mg/kg/day, animals showed extramedullary hematopoiesis, injection site damage, increased blood potassium, decreased relative thyroid weight, a hematoma (one male) and one animal showed a mild hemorrhage in the eyeball.

At 10 mg/kg, animals exhibited increased total excreted sodium, potassium and chloride, increased urine volume, increased reticulocyte rate, blood potassium, total bilirubin, decreased thyroid weight, extramedullary hematopoiesis and injection site damage. Isolated occurrences of thickening of the cecal wall, periarteritis in the cerebrum, myocarditis, granulomatous inflammation of the stomach and urinary bladder, cellular alteration of the adrenals were thought to be possibly unrelated to drug.

Pharmacokinetics: Mean peak plasma *concentrations of FK463 after repeated intravenous* injections

FK463 Dose	**Cmax (mcg/ml)**		
(mg/kg/day)	**Day 1**	**5 weeks**	**13 weeks**
1.25	2.5	3.2	4.1
2.5	4.9	6.7	8.9
5	11.0	14.8	18.9
10	22.3	31.7	40.0

Mean peak (15 minute) FK463 levels were proportional to dose throughout the study. The mean peak drug levels increased over time. Although the dose proportionality was maintained there was a tendency for the Cmax to be slightly superproportional to the dose.

Pharmacokinetics: Mean trough plasma concentrations of FK463 after repeated intravenous injections

FK463 Dose	FK 463 trough levels (24 hours post dose) (mcg/ml)		
(mg/kg/day)	Day 1	5 weeks	13 weeks
1.25	0.03	0.09	0.20
2.5	0.14	0.25	0.52
5	0.36	0.74	1.39
10	1.02	1.89	3.84

Cmax values for rats treated at 2.5 mg/kg were around 9 mcg/ml. Human subjects treated with micafungin at the highest dose of 100 mg show and Cmax value around 28 mcg/ml. Thus the toxic effects seen in this study at 10 mg/kg (Cmax values ranging from 22 to 40 mcg/ml over the study) could be expected at the recommended human doses. The NOAEL level, 2.5 mg/kg produced an exposure about a third of that expected at the highest recommended clinical dose.

5. A 26-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN RATS

Key study findings: Administration of micafungin at 1mg/kg to rats for twenty six weeks is associated with no drug-related findings. At 3.2 mg/kg, drug related findings included altered reticulocyte ratio, extramedullary hematopoiesis and smallness of testes/epididymis. At higher doses effects involved the liver, spleen, kidneys bladder, lungs, bone arrow and epididymis. Cmax values for rats treated at 10 mg/kg were between 20 and 45 mcg/ml. Human subjects treated with micafungin at the highest dose of 100 mg show and Cmax value around 28 mcg/ml. Thus the toxic effects seen in this study at 10 mg/kg could be expected at the recommended human doses. The NOAEL, 1 mg/kg, produced an exposure (Cmax) about 14% of that expected at the highest recommended clinical dose.

Study no: GLR010153
Conducting laboratory: [redacted]

Location: [redacted]
Date of study initiation: June 16, 1999
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L and 003062L, [redacted] purity
Formulation: Dissolved in physiological saline

Four groups of SPF [redacted].CD (SD) strain rats, (14/sex/dose group), were injected once daily in the tail vein with micafungin at 1.0, 3.2, 10 or 32 mg/kg. An additional group of control animals received physiological saline. A satellite group (4/sex/dose) was used to measure drug plasma levels. Drug was infused as a bolus. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 6

weeks old and males weighed between 192 and 216 g while females weighed between 150 and 177 g.

Administration of micafungin at 1.0 mg/kg to rats for twenty six weeks produced no drug related effects. At 3.2 mg/kg, toxic effects included increased reticulocyte ratio, extramedullary hematopoiesis, and bilateral smallness of the testes and epididymis. Injection site damage at this dose was no more frequent than in control animals.

At 10 mg/kg animals showed significant increases in one-day excretion of sodium, decreased red blood cell count, hemoglobin and hematocrit, a significant shortening of the activated partial prothrombin time and an increase in reticulocyte ratio. Extramedullary hematopoiesis and relative spleen weight was also increased at this dose as was nuclear hypertrophy, vacuolation and round cell infiltration/accumulation in liver sinusoid.

At 32 mg/kg, the bodyweights of the males were about 10% lower than controls. Urinalysis changes included lowered urine pH, increased numbers of animals with small round epithelial cells, increased water intake and urine volume, low urine osmolarity and increases in one day excretion of sodium and chloride. Hematology findings (in addition to those seen at 10 mg/kg) included increases in mean corpuscular hemoglobin concentration (MCHC) and platelet count and a shortening of the activated partial thromboplastin time and prothrombin time. Blood chemistry changes relfected changes in the liver and included increases in GOT, GPT, ALP, total bilirubin, urea nitrogen, potassium and calcium and decreases in total cholesterol, triglycerides, phospholipids, glucose, creatinine and toal proteins, increases A/G ration, albumin fraction and globulins fraction (α_2, β and γ) and decrease in the proportion of alpha-1 globulin.

Necropsy findings associated with micafungin administration at 32 mg/kg included increased relative liver, spleen, kidney, heart and lungs weights. There was also a significant decrease in the absolute weight of the testes at this dose. Histopathology examiniation of the liver revealed that 32 mg/kg micafungin was associated with nuclear hypertrophy, single cell necrosis, cytoplasmic acidophilic body in hepatocytes, mild round cell infiltration/accumulation in the sinusoid, vacuolation of the hepatocytes, mitosis in hepatocytes, multinucleated hepatocytes, swelling of sinusoidal cells and increased number of cells with altered cell foci. Extramedullary hemotopoiesis was increased at this dose. In the kidney, deposition of a hemosiderin-like brown pigment was detected in the proximal tubular epithelium as well as mild dilitation of the collecting duct, swelling of the collecting ductal epithelium and vacuolation of the pelvic epithelium. In the bladder there was mild vacoulation and thickening of mucosal epithelium. Slight hypercellularity in the bone marrow was observed in the sternum and the femur. Osteochondroma was detected in the femur, tibia and rib of one male at 32 mg/kg. Slight atrophy of the epididymis was detected in 2 males and slight cellular infiltration of the haderian gland was found in one female.

Pharmacokinetics: Mean peak plasma concentrations of micafungin after repeated intravenous injections in rats

FK463 Dose	Cmax (mcg/ml)		
(mg/kg/day)	Day 1	13 weeks	26 weeks
1.0	2.4	3.6	4.3
3.2	6.2	11.5	14
10	20	38	45
32	76	118	159

Mean peak (15 minute) FK463 levels were approximately proportional to dose throughout the study. The mean peak drug levels increased over time. Although the dose proportionality was maintained there was a tendency for the Cmax to be slightly superproportional to the dose.

Pharmacokinetics: Mean trough plasma concentrations of micafungin after repeated intravenous injections in rats

FK463 Dose	FK 463 trough levels (24 hours post dose) (mcg/ml)		
(mg/kg/day)	Day 1	13 weeks	26 weeks
1.0	0.06	0.15	0.23
3.2	0.20	0.67	0.99
10	1.0	3.5	5.09
32	5.3	13.7	20.6

Trough levels of micafungin were proportional to the dose administered for the two lower doses but were superproportional to the dose above doses of 3.2 mg/kg. Also, trough levels increased as dosing duration increased.

6. FOUR WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS

Key study findings: The NOAEL for micafungin was determined to be 3.2 mg/kg. At 10 mg/kg, changes are detected in MCHC, calcium, A/G ratio and albumin rate. Exposure at 32 mg/kg results in adverse effects in the liver including centrilobular hypertrophy, increased size and weight and discoloration. Exposure at this dose (591 μg*h/ml) is about five times the exposure that would be experienced at the highest recommended human dose (110 μg*h/ml).

Study no: GLR970118

Conducting laboratory: —

Location: —

Date of study initiation: February 7, 1996

GLP compliance: Japanese GLP

QA report: yes (√) no ( )
Drug lots: 00105YL — purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 3 per sex per dose group, were injected once daily for four weeks with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 6 months old.

Dogs treated with micafungin at 3.2 mg/kg did not show any consistent drug related effects. At 10 mg/kg, dogs showed reduced MCHC, increased calcium, A/G ratio and albumin rate. At the higher dose, additional findings included increased urea nitrogen and glucose, liver enlargement, liver discoloration, increased liver weights, centrilobular hypertrophy, increased smooth endoplasmic reticulum in the centrilobular hepatocytes, and mild myelinosomes in the centrilobular and mid lobular hepatocytes.

Pharmacokinetics: $AUC_{0\text{-}24h}$ values (mcg*h/ml) recorded after repeated micafungin dosing in dogs.

Dose (mg/kg)	$AUC_{0\text{-}24h}$ (Day 1)	$AUC_{0\text{-}24h}$ (Week 4)
3.2	45	52
10	154	162
32	557	591

AUC values for micafungin were dose proportional and values on day One were similar to those after four weeks of dosing. Elimination half life was between 3.6 and 4.2 hours.

SUMMARY

The NOAEL for micafungin was determined to be 3.2 mg/kg. At 10 mg/kg, changes are detected in MCHC, calcium, A/G ratio and albumin rate. Exposure at 32 mg/kg results in adverse effects in the liver including centrilobular hypertrophy, increased size and weight and discoloration. Exposure at this dose (591 mcg*h/ml) is about five times the exposure that would be experienced at the highest recommended human dose (110 mcg*h/ml).

7. THIRTEEN-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN DOGS WITH A 4-WEEK RECOVERY STUDY.

Key study findings: The NOAEL for micafungin was determined to be 10 mg/kg. At 32 mg/kg results in adverse effects in the liver including centrilobular hypertrophy, increased size and weight and discoloration. Exposure (Cmax) at this dose (105 mcg/ml) is about four times the exposure that would be experienced at the highest recommended human dose (Cmax, 28 mcg/ml).

Study no: GLR970292

Conducting laboratory: —

Location: —

Date of study initiation: June 28, 1996
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L, — purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 3/sex/dose group, were injected once daily with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Two additional groups of dogs, (2/sex/dose) were treated with saline or 32 mg/kg micafungin and allowed to remain untreated for four weeks after the end of dosing to assess the reversibility of any toxic effects. Records were kept of general signs, bodyweights, food consumption, ophthalmology, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 6 months old and weighed between 6.5 and 9.6 kg.

There were no drug-related effects at doses below 32 mg/kg. Leukocytes were decreased and cholesterol and GPT levels were increased in the 32 mg/kg dose group. Changes in the liver included increased absolute and relative liver weights, enlarged, discolored livers and centrilobular hypertrophy. Spleen congestion and discoloration was found in one male animal.

In the recovery animals, discoloration of the liver was observed, as well as increased liver weight and centrilobular hypertrophy. As such, treatment with micafungin at 32 mg/kg for 13 weeks produced some changes in the dog which were not reversible after a four week recovery period.

Pharmacokinetics: Mean peak plasma concentrations of micafungin after repeated intravenous injections in dogs

Dose (mg/kg)	Day 1	Week 13
3.2	10	9
10	31	30
32	102	105

Pharmacokinetics: Mean trough plasma concentrations of micafungin after repeated intravenous injections in rats

Dose (mg/kg)	Day 1	Week 13
3.2	0.12	0.15
10	0.40	0.6
32	1.6	2.0

Irreversible changes are associated with micafungin, when administered at 32 mg/kg for 13 weeks in beagle dogs. This dose produces a peak plasma concentration that is 105 mcg/ml,

almost four times the peak plasma concentration recorded in the human subjects receiving the maximum recommended dose of micafungin. As such one would not expect these irreversible changes in human subjects under normal dosing conditions.

8. A 39-WEEK INTRAVENOUS TOXICITY STUDY OF FR179463 IN BEAGLE DOGS.

Key study findings: No NOAEL could be established for this study of intravenous micafungin using doses 3.2, 10 and 32 mg/kg. At the lowest dose administered, micafungin resulted in adverse effects on the liver, spleen and testes. At 32 mg/kg, effects included increased liver weights, swollen, discolored livers, centrilobular hypertrophy, increased total cholesterol,alpha$_2$ globulin ratio, platelet count, fibrinogen and phospholipids, high splenic weight, congestion of spleen and dark red coloration of the spleen. Seminiferous tubules showed bilateral atrophy and spermatogenic cells were decreased. Exposure AUC $_{0\text{-}24h}$ at 3.2 mg/kg, (95 mcg*h/ml) is less that the exposure that would be experienced at the highest recommended human dose AUC $_{0\text{-}24h}$, 110 mcg*h/ml). As such the findings observed at the lowest dose in this study could be reasonably expected at the highest clinical dose after 39 weeks of dosing.

Study no: GLR000510
Conducting laboratory:

Location:

Date of study initiation: December 17, 1998.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L purity
Formulation: Dissolved in physiological saline

Four groups of beagle dogs, 4 per sex per dose group, were injected once daily for 39 weeks with micafungin at 0.0, 3.2, 10 or 32 mg/kg. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, ophthalmology, electrocardiography, blood pressure measurements, urinalysis, chemistry, hematology, gross pathology, organ weights, histopathology, electron microscopy and blood plasma concentrations. Animals were 6 months old and weighed between 7.3 and 10.1 kg.

Dogs treated at 3.2 mg/kg/day showed a number of toxic effects which were mirrorred at the higher doses. These included increased liver weight, liver swelling, swollen spleen, hemorrhage in the spleen, white spots on lungs, atrophy of the seminiferous tubules. At 10 mg/kg, animals also showed prolonged QRS interval, liver swelling, and high liver weight, small thyroid, bilateral atrophy of the seminiferous tubules, dilatation of the seminiferous tubules, stasis of spermatid and decreased sperm count.

At 32 mg/kg, livers were increased in weight, swollen, discolored and showed centrilobular hypertrophy. One dog showed increased total cholesterol, alpha2 globulin ratio, platelet count, fibrinogen and phospholipids, high splenic weight, congestion of spleen and dark red coloration of the spleen. Seminiferous tubules showed bilateral atrophy which at times was

severe and spermatogenic cells were decreased. In some animals, seminiferous tubules contained only Sertoli cells. Slight to mild vacuolation of spermatogenic cells was observed in all animals of the 32 mg/kg group. Severe decreases in sperm count were observed at this dose and cell debris was observed in ¾ males. There was also mild cellular infiltration of the interstitium. These findings were thought to be related to the atrophy of the seminiferous tubules.

Pharmacokinetics: Cmax and AUC_{0-24h} values (mcg*h/ml) on Day One of FK463 administration in dogs.

Dose (mg/kg)	AUC (mcg*h/ml)	Cmax
3.2	95	9.8
10	280	31
32	1028	106

AUC values were essentially the same as in week 39 (AUC values 114, 303 and 1132 mcg*h/ml)

9. FR179463: INTRAVENOUS STUDY FOR EFFECTS ON PRE- AND POSTNATAL DEVELOPMENT INCLUDING MATERNAL FUNCTION IN RATS.

Key study findings: At 10 mg/kg, the only abnormal findings related to drug was in one dam that lost its litter after a difficult delivery. Since this did not occur in any animals at the higher dose it was assumed that this effect was not drug related. At 32 mg/kg, dams showed effects on body weight, food consumption and injection site. Pups weighed slightly less than control pups. Thus the NOAEL for general toxicity of the dams and development of their offspring was 10 mg/kg. The NOAEL for reproductive function was 32 mg/kg.

Study no: GLR000640
Conducting laboratory: —

Date of study initiation: December 17, 1998.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L, — purity
Formulation: Dissolved in physiological saline

Groups of — CD(SD) female rats (20/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg from day 7 of gestation through day 20 postpartum. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, physical development, litter data, internal and external abnormalities, locomotor coordination, emotional behavior (grooming, defecation) motor activity, avoidance test, mating performance, copulation and fertility indices.

Dams treated at 32 mg/kg experienced swollen snout, and black coloration at the injection site followed by tail loss. Body weight and food consumption were also reduced on certain days. Pups born to these mothers had slightly (about 5 %) lower weights than controls. One dam

treated at 10 mg/kg showed paleness on delivery and prolongation of labor and this dam lost its entire litter on day 0 of gestation.

There were no other abnormal changes that could be attributed to micafungin.

SUMMARY

At 10 mg/kg, the only abnormal findings related to changes related to one dam that lost its litter after a difficult delivery. Since this did not occur in any animals at the higher dose it was assumed that this effect was not drug related. At 32 mg/kg, dams showed effects on body weight, food consumption and injection site. Pups weighed slightly less than control pups. Thus the NOAEL for general toxicity of the dams and development of their offspring was 10 mg/kg. The NOAEL for reproductive function was 32 mg/kg.

10. STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT UP TO IMPLANTATION IN RATS TREATED INTRAVENOUSLY WITH FR179463

Key study findings: Females treated with micafungin at 32 mg/kg showed temporary reductions in food consumption. Males showed vacuolation of the ductal epithelium of the caput epididymis at 10 and 32 mg/kg as well as reduced sperm count and increased epididymis weight. NOAEL levels for reproductive toxicity was therefore determined to be 3.2 mg/kg for males. For females the NOAEL was 10 mg/kg for general toxicity and 32 mg/kg for reproductive toxicity.

Study no: GLR970119
Conducting laboratory: —
Location: —
Date of study initiation: February 23 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 00105YL. — o purity
Formulation: Dissolved in physiological saline

Groups of —CD(SD) rats (20/sex/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg. Drug was given to males from 9 weeks before mating through the mating period to the day before necropsy. Females were were treated from 2 weeks before mating, through mating to 7 days after confirmed copulation. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, gross pathology of testes and epididymis, plasma GPT activity, sperm counts, number of estrus cycles before mating, litter data, internal and external abnormalities, mating performance, copulation and fertility indices.

Two males from the 10 mg/kg group died on days 71 and 76 of dosing. Foam was found in the renal vein and inferior vena cava of one animal, but cause of death was not determined in the second animal. There were no other abnormal clinical signs. Plasma GPT was significantly higher in the 32 mg/kg group.

In males in the 32 mg/kg group, epididymis weight was higher than in controls. In addition, the number of sperm cells were significantly lower than control even though there was no difference in sperm motility, viability or occurrence of abnormal sperm. Vacuolation of the ductal epithelial cells was observed in almost all animals at 32 mg/kg and 3 animals from the 10 mg/kg group.

In females, there were temporary reductions in food consumption in the high dose group before mating. Copulation, fertility indices, insemination, number of corpora lutea, implantations, live embryos, implantation index or dead embryo rate were no different between treated and control animals.

SUMMARY

Females treated with micafungin at 32 mg/kg showed temporary reductions in food consumption. Males showed vacuolation of the ductal epithelium of the caput epididymis at 10 and 32 mg/kg as well as reduced sperm count and increased epididymis weight. NOAEL levels for reproductive toxicity was therefore determined to be 3.2 mg/kg for males. For females the NOAEL was 10 mg/kg for general toxicity and 32 mg/kg for reproductive toxicity.

11. STUDY TITLE: INTRAVENOUS DOSING STUDY OF FR179463 ON EMBRYO-FETAL DEVELOPMENT IN RATS.

Key study findings: The NOAEL for maternal effects was 10 mg/kg. The NOAEL for reproductive toxicity was 32 mg/kg since no adverse effects on reproductive function could be detected at this dose.
Study no: GLR970379
Conducting laboratory: —
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Location —
Date of study initiation: October 7, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L — , purity
Formulation: Dissolved in physiological saline

Groups of pregnant female _ CD(SD) rats (20/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg from day 7 to day 17 of gestation. This period corresponds to the period from implantation to the closure of the hard palate. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, plasma GPT activity, gross pathology at cesarean section, litter data, internal and external abnormalities.

Plasma GPT levels were higher than control values in the 32 mg/kg group. There were no abnormal findings on cesarean section and no external visceral or skeletal abnormalities that could be ascribed to the drug.

The NOAEL for maternal effects was 10 mg/kg since GPT was increased at 32 mg/kg. The NOAEL for reproductive toxicity was 32 mg/kg since no adverse effects on reproductive function could be detected at this dose.

12 STUDY TITLE: INTRAVENOUS DOSING STUDY OF FR179463 ON EMBRYO-FETAL DEVELOPMENT IN RABBITS.

Key study findings: The NOAEL for maternal effects was 10 mg/kg since an abortion and numerous visceral abnormalities were observed at 32 mg/kg.
Study no: GLR970380
Conducting laboratory: —
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Location: —
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Date of study initiation: August 1, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 002061L, ~ purity
Formulation: Dissolved in physiological saline

Groups of pregnant female SPF New Zealand White (Kbl:NZW) strain rabbits (16-18/group) were given FR 179463 at a daily dose of 3.2 10 or 32 mg/kg for 13 days from day 6 to day 18 of gestation. Control animals received physiological saline. Records were kept of general signs, bodyweights, food consumption, plasma GPT activity, gross pathology at cesarean section, litter data, internal and external abnormalities.

General signs observed at 32 mg/kg included a reduction in feces and one abortion. This obortion occurred in an animal which, upon gross pathological examination was shown to have a congenital absence of the right uterine horn.

The only effects which appeared to be related to drug were seen at 32 mg/kg and were increases in visceral abnormalities. Whereas only one fetus from one control dam showed visceral abnormalities, 10 fetuses from 5 treated (32mg/kg) dams showed visceral abnornmalities. These included abnormal lobation of the lung in 6 fetuses, levocardia in 3 fetuses, retrocaval ureter in 3 fetuses, anomalous right subclavian artery in 1 fetus and dilatation of the ureter in 1 fetus.

SUMMARY

Dosing rabbits with FR 179463 at 32 mg/kg resulted in visceral abnormalities and abortion. This abortion is consistent with abortions observed in a preliminary study at 60 mg/kg. Although this animal also suffered from congenital absence of the right uterine horn, the relationship to drug cannot be ruled out. Visceral abnormalities included abnormal lobation of the lung, levocardia, retrocaval ureter , anomalous right subclavian artery, and dilatation of the ureter.

13. MUTAGENICITY STUDY OF R179463: REVERSION TEST WITH BACTERIA.

Key study findings: No evidence of potential mutagenic effects
Study no: GLR970122
Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Location: Toxicology Research Laboratories, Fujisawa Pharmaceutical Company Ltd. 1-5, Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan
Date of study initiation: May 17, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 00105YL. — . purity
Formulation: Dissolved in physiological saline

This study was designed to assess the ability of R179463 to produce mutations. In this model the mutagenicity is assessed by the drugs ability to increase the number of revertant colonies using *Salmonella typhimurium* TA100, TA98, TA1535 and TA1537 and *Escherischia Coli* WP2 uvrA as tester strains.

The test was performed after preincubation with or without metabolic activation (rat S9 mix) and drug levels ranged from 156 to 5000 μg/plate of FR179463. A positive result was taken as any condition under which the number of revertants exceeded the number seen in the solvent control by two multiples.

FR179463 did not increase the mean number of revertant colonies of any strains to twice that of the solvent controls, with or without metabolic activation. Positive and negative controls were satisfactory under the experimental conditions.

FR179463 did not demonstrate the potential to induce gene mutations as assessed using concentrations between 156 to 5000 μg/plate and using *Salmonella typhimurium* TA100, TA98, TA1535 and TA1537 and *Escherischia Coli* WP2 uvrA as tester strains.

14. MUTAGENICITY STUDY OF R179463: CHROMOSOMAL ABERRATION TEST WITH CHINESE HAMSTER LUNG CELLS IN CULTURE.

Key study findings: No evidence of potential mutagenic effects
Study no: GLR9800005
Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Date of study initiation: December 17, 1996.
GLP compliance: Japanese GLP
QA report: yes (√) no ( )
Drug lots: 003062L. — purity
Formulation: Dissolved in physiological saline

This study was designed to assess the clastogenicity of FR179463 using the chromosome aberration test with Chinese hamster lung (CHL) cells. CHL cells were exposed to drug for 24 or 48 hours in one study. In another experiment the effect of metabolic activation was assessed after six hour treatments with or without metabolic activation.

FR179463 did not increase the number of cells with structural aberration or polyploidy in either the direct (40-160 μg/ml) or metabolic activation (625-5000 μg/ml) method. As such FR179463 did not show any potentail for clastogenicity.

15. MUTAGENICITY STUDY OF R179463: MICRONUCLEUS TEST IN MICE

Key study findings: No evidence of potential clastogenic effects
Study no: GLR970373
Conducting laboratory: Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan
Date of study initiation: July 29, 1997.
GLP compliance: *Japanese GLP*
QA report: yes (√) no ( )
Drug lots: 057AEH. —, purity
Formulation: Dissolved in physiological saline

This study was designed to assess the clastogenicity of FR179463. Drug was administered intravenously to ICR mice in a single dose of 25, 50 or 100 mg/kg. Bone marrow cells were taken 24, 48 and 72 hours after dosing and the erythrocytes were examined.

FR179463 did not significantly increase polychromatic erythrocytes with micronuclei in any treated groups at any sampling times. AUC (0-24h) values for the 100 mg/kg dose was 2763 μg*h/ml. This was over twenty-five times the exposure seen in humans at the highest recommended human dose. As such, we would not expect a clastogenic effect at clinically relevant doses or up to 25 times that dose.