Table 6-14: Mean (±SD) Tacrolimus Pharmacokinetic Parameters In Adults And Children Following 8 Days Of Treatment With 0.3% Tacrolimus Ointment (Study 94-0-008)

Treatment Group	Treatment Area (Mean % BSA)	Study Day	AUC_{0-24} (ng/hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
Adults A (Trunk/limbs)	0.5	Day 1	3.7 ± 4.3	0.4 ± 0.4	4.8 ± 3.7
		Day 8	2.2 ± 0.8	0.2 ± 0.1	5.7 ± 3.6
Adults B (Face)	0.5	Day 1	15.2 ± 12.2	1.4 ± 0.9	6.0 ± 3.5
		Day 8	14.9 ± 13.6	0.9 ± 0.9	6.9 ± 3.2
Adults C (Trunk/limbs)	2.4	Day 1	2.4 ± 2.0	0.2 ± 0.1	6.0 ± 4.2
		Day 8	3.1 ± 4.4	0.2 ± 0.2	3.5 ± 4.4
Adults D (Trunk/limbs)	5	Day 1	16.1 ± 20.7	1.2 ± 1.4	6.0 ± 3.1
		Day 8	8.8 ± 12.2	0.6 ± 0.6	5.3 ± 3.5
Adults E (Trunk/limbs)	27	Day 1	42.5 ± 37.1	3.5 ± 3.1	6.3 ± 3.2
		Day 8	27.3 ± 34.0	1.4 ± 1.5	4.8 ± 3.9
Children 5-6 yrs† (Trunk/limbs)	0.7	Day 1	17.3 ± 10.7	1.9 ± 1.3	5.0 ± 2.0
		Day 8	3.7 ± 2.5	0.2 ± 0.1	4.3 ± 2.9
Children 7-11 yrs (Trunk/limbs)	0.8	Day 1	0.9 ± 1.0	0.1 ± 0.1	2.5 ± 1.7
		Day 8	1.9 ± 1.2	0.2 ± 0.1	2.5 ± 1.7

BSA: Body surface area.
LOQ: ~~—~~ ng/mL
†Patients aged ~~3-6 years~~ were allowed to enroll in this treatment group according to the protocol; however, no patients <5 years of age enrolled in the study.
Source: Reference [5]

The AUC value for adults in Group E was 42.5 ± 37.1 ng/hr/ml at Day 1 and 27.3 ± 34.0 ng/hr/ml at Day 8. The AUC values for the no effect dose (0.03% tacrolimus ointment) in the mouse dermal carcinogenicity study was ~180 ng·hr/ml after 1 week. The no effect dose was ~6X higher than the maximum AUC value at Day 8 after dosing with 0.3% tacrolimus ointment in humans. A direct comparison of AUC values for the no effect dose level (0.03% tacrolimus ointment) in the mouse dermal carcinogenicity study and 0.03% tacrolimus ointment in humans is not possible. Tacrolimus ointment concentrations of 0.03% and 0.1% were analyzed in human pharmacokinetic studies. However, AUC values were not obtained in these studies because the levels of tacrolimus measurable were too low to make an accurate estimate. Therefore, it is anticipated that the actual safety margin for the no effect dose and human exposure after maximum use would be much greater than 6 fold.

The sponsor estimated human tacrolimus bioavailability by comparison of mean AUC_{0-24hr} after topical administration of the highest dose of tacrolimus ointment relative to historical (data on file) mean AUC_{0-24hr} from normal adult volunteers who were adminstered intravenous and oral tacrolimus. The results of this estimate are provided in the table below taken directly from the electronic NDA. The estimate of absolute bioavailability in humans for tacrolimus ointment is ≤ 0.5%.

Table 6-15: Estimated Absolute and Relative Bioavailability (%) of Topically Administered Tacrolimus (Study 94-0-008)

Method of Administration	Dose (mg)	Mean AUC_{0-24} (ng•hr/mL)	Absolute $\frac{AUC_{Topical}/dose}{AUC_{IV}/dose} \times 100$	Relative $\frac{AUC_{Topical}/dose}{AUC_{Oral}/dose} \times 100$
Intravenous (IV)	2	346 (173)		
Oral (PO)	5	163 (33)		
Topical (day 1)	45†	42.5 (0.9)	0.5	2.7
Topical (day 8)		27.3 (0.6)	0.3	1.8

( ): AUC normalized to 1 mg
†Highest amount applied in the study.
Source: Reference [5] and calculated by the Sponsor; data on file.

Human systemic exposure after topical administration of tacrolimus ointment will be significantly less than what has been observed for the no effect level in the mouse dermal carcinogenicity study. The no effect dose in the mouse dermal carcinogenicity study (0.03%) provided an ~6 fold greater AUC level than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment application. The dose that lymphomas were observed in the mouse dermal carcinogenicity study (0.1%) provided an ~20X fold greater AUC exposure than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment. It is important to note that the increase in lymphomas was noted at a dose in the mouse dermal carcinogenicity study that caused systemic immunosuppression.

Reliable AUC data in humans could be obtained from the 0.3% concentration of tacrolimus ointment. Reliable AUC data in humans could not be obtained from the 0.1% and 0.03% concentrations of tacrolimus ointment due to the inconsistent plasma levels that were obtained during the pharmacokinetic analysis in clinical studies. Therefore, the safety factor for AUC exposure in the mouse dermal carcinogenicity study would probably be much greater than comparison to human AUC levels obtained from the maximum exposure estimate in humans (from 0.3% tacrolimus ointment). Therefore, it is my opinion that human patients would not have a high risk of getting lymphomas under conditions of clinical use for the 0.03% and 0.1% tacrolimus ointment.

However, it is recommended that the tumor findings of this study be included in the label. A comparison of levels of systemic exposure in humans vs systemic exposure in the mouse dermal carcinogenicity study should be included in the label for reference purposes.

- *Recommendations for Further Analysis:*

No recommendations for further analysis at this time.

Addendum/Appendix Listing:

- *Dose-Ranging Study Report:*

No dose range study was performed for this dermal carcinogenicity study.

- *CAC Report:*

An Executive CAC meeting was held on March 14, 2000 to discuss the results of the dermal carcinogencity study. The minutes from this meeting are attached below. The chair for the Executive CAC, Joseph DeGeorge, signed the minutes from this meeting on March 16, 2000.

Executive CAC
March 14, 2000

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Nakissa Sadrieh, Ph.D., HFD-160, Alternate Member
Ken Hastings, Ph.D., HFD-590, Team Leader
Barbara Hill, Ph.D., HFD-540, Presenting Reviewer

Author of Draft: Barbara Hill

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

NDA # 50-777
Drug Name: Protopic (tacrolimus) ointment
Sponsor: Fujisawa Healthcare, Inc.

Background:

Tacrolimus is a macrolide immunosuppressant to be used in humans for the treatment of moderate to severe Atopic Dermatitis. Atopic Dermatitis is primarily a pediatric indication and the duration of treatment is chronic. Tacrolimus has undergone testing in a full battery of genotoxicity tests and showed no genotoxic potential. Two oral (feed) carcinogenicity studies in mice and rats have been conducted previously for tacrolimus. The results from these two studies were negative but there is some question as to whether the systemic exposure was adequate in these two studies. The sponsor was requested by the division to conduct a 2 year dermal carcinogenicity study in the mouse to support the tacrolimus ointment formulation. The division also requested conduct of a one year photocarcinogenicity study in hairless mice. Although the topic of this Exec CAC meeting was not to discuss the results of the photocarcinogenicity study, it was brought up for completeness sake and to include these results in the final evaluation of the 2 year mouse dermal carcinogenicity study results. The results of the tacrolimus ointment photocarcinogenicity study demonstrated that the vehicle and drug product decreased the time to development of skin tumors. Therefore, both the vehicle and tacrolimus ointment showed a strong signal for increased photocarcinogenic risk.

Mouse Carcinogenicity Study:

No formal dose range study was conducted to support the doses selected in the 2 year mouse dermal carcinogenicity study. The sponsor anticipated that the doses used in this study would include the maximum tolerated dose (MTD). The following dose groups were tested in the study: sham control, vehicle control, 0.03%, 0.1%, 0.3%, 1.0% and 3.0% tacrolimus ointment. High levels of mortality were exhibited in the 0.3%, 1.0% and 3.0% tacrolimus ointment dose groups. All animals died by week 26 in the 3.0% dose group and by week 46 in the 1.0% dose group. Approximately 85% of animals died by the end of the study in the 0.3% dose group. Adequate numbers for statistical evaluation of tumor incidence were available in the sham control, vehicle control, 0.03% and 0.1% tacrolimus ointment groups. The MTD was identified as the 0.1% tacrolimus ointment dose based on mortality.

Both the trend and pairwise statistical comparison performed by the agency's biostatistical reviewer demonstrated that the incidence of pleomorphic lymphoma was statistically significant in high dose male (25/50) and female animals (27/50) and that the incidence of undifferentiated lymphoma was statistically significant in high dose female animals only (13/50). No statistically significant elevation in skin tumors was noted in this study. The committee commented that this did not necessarily mean that there was not a risk of skin cancer in humans with tacrolimus ointment use. Humans have papilloma virus that can contribute to the incidence of skin cancer, which would not be present in the mice. The reviewer agreed with this comment.

The committee expressed concern about the strong lymphoma signal demonstrated in the study. The reviewer was questioned whether she was comfortable with the chronic use of tacrolimus ointment in the pediatric population for treatment of atopic dermatitis. The reviewer responded yes because the AUC data for the mouse dermal carcinogenicity study indicated a 20X fold safety factor or greater between the AUC level that exhibited lymphoma in the mouse dermal carcinogenicity study and the AUC level after maximum use clinically in humans. However, the reviewer did state that a meeting would be held with the entire division review team for tacrolimus ointment to receive clinical input on the potential concern for humans from the strong lymphoma signal observed in the mouse dermal carcinogenicity study.

The committee questioned whether perhaps the hepatocarcinoma incidence in low dose males, the stromal cell sacroma incidence in the cervix of low dose females and the leiomyoma incidence in the uterus of low dose females were statistically significant. This concern was raised as the tumors were generally found only in those animals surviving to study termination and there was a significant reduction in survival at the 0.1% dose level, which may have prevented the effect from being observed at the higher dose. It was noted that if these tumor finding are rare and can be attributed to drug treatment, there is no NOEL for tumor response in the study. The reviewer responded that the agency's biostatistical reviewer had performed the analysis for all tumor types and found that no other tumors, except for those listed for lymphoma, were statistically significant. The committee clarified that this request was to determine whether these tumors might be a weak signal of additional potential carcinogenic effect and that a survival adjusted analysis without the HD group may be needed. The committee inquired if the historical background rates for these three tumor types were provided in the submission. The reviewer replied no and that a request will be submitted to the sponsor to supply the historical background rate for the three types of tumors. The committee requested clarification if the lymphomas were of a B-cell or T-cell origin. The reviewer replied that this was unknown but that a request would be sent to the sponsor to determine if they could clarify this point.

The committee discussed the potential concern for skin cancer in humans with tacrolimus ointment use even though a signal was not seen in the dermal mouse carcinogenicity study. The rationale for this is that the human papilloma virus has been shown to be a factor in the development of skin cancer and that immune suppression can alter the ability of the human papilloma virus to be expressed in humans. In addition, the strong signal for photocarcinogenic risk associated with tacrolimus ointment was

discussed in the meeting. The committee recommended that even though tacrolimus ointment does not cause skin cancer directly it may cause skin cancer after exposure to sunlight or in combination with human papilloma virus. Therefore, it was recommended that wording be added to the label to address this concern.

Executive CAC Recommendations and Conclusions:

1. The committee determined that the mouse dermal carcinogenicity study was adequate and that there was a strong signal for lymphoma.
2. The committee requested historical background incidence rates for hepatocarcinoma, stromal cell sacroma in the cervix and leiomyoma in the uterus for the strain of mouse used in the dermal carcinogenicity study. A request will be sent to the sponsor for these historical background incidence rates.
3. The committee requested clarification whether it was known if the lymphomas noted in this study were of a B-cell or T-cell origin. A request will be sent to the sponsor to determine if this information is known.
4. The committee strongly recommended that if tacrolimus ointment is approved, then the division should consider strong label warnings for the potential lymphoma risk and photocarcinogenic risk associated with tacrolimus ointment use. Also, the committee recommended that wording be added in the label to indicate that even though no skin cancer was noted in the mouse dermal carcinogenicity study, that there may still be a risk in humans due to the presence of human papilloma virus in humans that was not present in mice. It was noted that the exposure at the NOEL for lymphoma were significantly closer to those produced in human at the recommended dose and that this should be considered in the risk benefit as well as the presence of tumors (still of questionable significance) in the lowest dose tested.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:

/Division File, HFD 540
/Abby Jacobs, HFD-540
/Kenneth Hastings, HFD-590
/Barbara Hill, HFD-540
/Millie Wright, HFD-540
/ASeifried, HFD-024

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- *Sponsor's Incidence of Neoplastic Histopathology Findings*: Copied directly from electronic NDA submission.

Reviewer's Comments: It is important to note that in order to obtain a total number for each column the number outside of the parentheses and the one inside the parentheses should be added together. For example, the total number of male mice in group 4 is 21 (survived to terminal necropsy) + 29 (died or euthanized prior to terminal sacrifice) = 50 male mice. The total incidence for bronchiolo-alveolar adenoma for group 4 male mice would be 4 + 3 = 7.

Study Number: 3-A20 Page 1156

Pathology Table 1
Incidence of All Neoplastic Microscopic Findings in Males

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Lungs				
-adenoma, bronchiolo-alveolar	7	8	8 (2)	4 (3)
-carcinoma, metastatic	(1)			1
Number Examined	42	41	39	21
Heart				
-hemangiosarcoma	1			
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Liver				
-adenoma	8 (2)	12	7 (2)	6 (6)
-carcinoma	1 (4)	5 (1)	11 (3)	3 (1)
-hemangiosarcoma	2 (1)	2	1 (1)	(4)
Number Examined	42 (8)	41 (9)	39 (10)	21 (28)
Pancreas				
-adenoma, islet cell		1		
-neurofibroma				(1)
Number Examined	42	41	39	21
Salivary Gland				
-hemangioma				1
Number Examined	42	41	39	21
Urinary Bladder				
-hemangiosarcoma			1	
-hemangioma	1			
Number Examined	(6)	(7)	(7)	(23)
Duodenum				
-adenoma, papillary			(1)	
Number Examined	42	41	38	21
Jejunum				
-adenosarcoma	1			

Numbers in parentheses refer to animals which died prior to study termination

Study Number: 3-A20 Page 1157

Pathology Table 1 (Continued)
Incidence of All Neoplastic Microscopic Findings in Males

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	42 (8)	41 (9)	39 (11)	20 (28)
Femur				
-hemangiosarcoma	1	(1)	1	
Number Examined	42	41	39	21
Testes				
-carcinoma, leydig cell	1			
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Epididymides				
-leiomyosarcoma		1(1)		
Number Examined	42	41	39	20
Prostate				
-leiomyosarcoma			1	
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Spleen				
-hemangioma				1
-hemangiosarcoma	1 (1)	1(1)	2	
Number Examined	42 (8)	41 (8)	39 (11)	21 (29)
Thyroid Gland				
-adenoma			1	(1)
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Administration Site				
-carcinoma, squamous cell				1
-hemangiosarcoma		(1)		
Number Examined	42 (8)	41 (9)	39 (11)	19 (29)
Sternum				
-hemangiosarcoma	1	(1)		

Numbers in parentheses refer to animals which died prior to study termination

Study Number: 3-A20 Page 1158

Pathology Table 1 (Concluded)
Incidence of All Neoplastic Microscopic Findings in Males

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	0	0	0	1
Skeletal Muscle				
-hemangiosarcoma				1
Number Examined	1	3	0	0
Harderian Gland				
-adenoma	1	3		
Number Examined	(0)	(2)	(0)	(0)
Body Cavity, Abdomen				
-sarcoma, NOS		(1)		
Number Examined	42	41	39	21
Adrenal Gland				
-spindle cell adenoma			1	
Number Examined	42 (8)	41 (9)	39 (11)	21 (29)
Hemolymphoreticular system				
-histiocytic sarcoma		(1)	(2)	2
-lymphoma, pleomorphic	6 (1)	2	3 (1)	14 (11)
-lymphoma, undifferentiated		1	(2)	(4)
-plasmacytoma		(1)		1 (2)

Numbers in parentheses refer to animals which died prior to study termination

Study Number: 3-A20 Page 1159

Pathology Table 2
Incidence of All Neoplastic Microscopic Findings in Females

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Lungs				
-adenoma, bronchiolo-alveolar	3	2 (1)	5 (2)	2
-hemangiosarcoma	(1)			
-pheochromocytoma, metastatic	1			
Number Examined	41(9)	44 (6)	39 (11)	20 (30)
Liver				
-adenoma	2	6	2 (3)	5(3)
-carcinoma	3	3 (1)	1	2
-hemangioma	1			
-hemangiosarcoma	1(2)	2 (2)		
-hepatoblastoma			(1)	
Number Examined	41	44	39	20
Kidneys				
-adenoma, tubular epithelium		1		
Number Examined	40	43	39	20
Skin, non-administration site				
-sebaceous adenoma		1		
Number Examined	(9)	(6)	(11)	(30)
Mammary Gland				
-liposarcoma	(1)			
Number Examined	(9)	(6)	(11)	(30)
Brain				
-ependymoma	(1)			
Number Examined	40	44	38	17
Thymus				
-thymoma			1	

Numbers in parentheses refer to animals which died prior to study termination

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Study Number: 3-A20 Page 1160

Pathology Table 2 (Continued)
Incidence of All Neoplastic Microscopic Findings in Females

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	(8)	(6)	(11)	(29)
Stomach				
-papilloma				(1)
Number Examined	41 (9)	44 (6)	38 (11)	20 (30)
Adrenal Glands				
-pheochromocytoma	1			1
-spindle cell adenoma	(1)			
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Femur				
-hemangiosarcoma		(1)	1	
Number Examined	40 (9)	42 (6)	39 (9)	20 (30)
Ovaries				
-cystadenoma	2 (1)	1	2	(1)
-granulosa cell tumor			2	
-hemangiosarcoma	(1)	1 (1)		
-hemangioma				(1)
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Uterus				
-adenocarcinoma	2	1 (1)	3	
-adenoma			1	
-fibroepithelial polyp	1	1	1	
-hemangiosarcoma		1	1	
-leiomyoma			2 (1)	(1)
-myxosarcoma			1	

Numbers in parentheses refer to animals which died prior to study termination

Study Number: 3-A20 Page 1161

Pathology Table 2 (Continued)
Incidence of All Neoplastic Microscopic Findings in Females

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Spleen				
-hemangiosarcoma		(1)	1	
Number Examined	41	44	38	20
Thyroid Gland				
-adenoma			1	1
Number Examined	41	44	38	20
Sternum				
-osteosarcoma			1	
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Hemolymphoreticular System				
-histiocytic sarcoma	3 (2)		(1)	1 (3)
-lymphoma, lymphocytic	1	1	(2)	(2)
-lymphoma, pleomorphic	11 (1)	5 (1)	12 (2)	17 (10)
-lymphoma, undifferentiated	2 (1)	1	1 (2)	1 (12)
-plasmacytoma				1
Number Examined	1	2	1	0
Clitoral Glands				
-hemangioma		1		
Number Examined	2	0	1	0
Harderian Gland				
-adenoma	2		1	
Number Examined	41 (9)	44 (6)	39 (11)	20 (30)
Skin, administration site				
-basal cell carcinoma			(1)	
-sarcoma, NOS			1	
-hemangioma				(1)

Numbers in parentheses refer to animals which died prior to study termination

Study Number: 3-A20 Page 1162

Pathology Table 2 (Concluded)
Incidence of All Neoplastic Microscopic Findings in Females

Group Number / Tissue/Lesion	1	2	3	4
Number Examined	40 (9)	42 (5)	36 (11)	19 (26)
Cervix				
-adenocarcinoma	1	(1)	1	
-fibroma				(1)
-granular cell tumor		1		
-hemangiosarcoma			1	
-neurilemoma		1		
-polyp, fibroepithelial	1			
-stromal cell sarcoma			4 (1)	
Number Examined	40	43	39	18
Pituitary Gland				
-adenoma	7	4	4	
-carcinoma				1
Number Examined	41	44	39	20
Pancreas				
-adenoma, islet cell	1			

Numbers in parentheses refer to animals which died prior to study termination

/S/

Barbara Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 50-777 (000; Addendum)
HFD-340
HFD-540/DIV FILES
HFD-540/TOX/JACOBS
HFD-540/PHARM/HILL
HFD-540/MO/LABIB
HFD-540/CHEM/HATHAWAY
HFD-540/PM/WRIGHT
C:/MY DOCUMENTS/WORD/NDAS/NDA50777/50777carc.DOC

/S/

Concurrence Only:
HFD-540/DivDir/JWILKIN /S/ 6/27/00
HFD-540/PharmTL/AJACOBS 6/27/00
INDFS

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REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Immunosuppressant, Atopic Dermatitis

Reviewer Name: Barbara Hill
Division Name: Dermatologic and Dental Drug Products
HFD#: HFD-540
Review Completion Date: 6-22-00

NDA number: 50-777
Serial number/date/type of submission: 000 / 9-14-99 / Original NDA Submission
Information to sponsor: Yes (X) No ()

Sponsor: Fujisawa Healthcare, Inc.
Parkway North Center, Three Parkway North
Deerfield, IL 60015-2548
(847) 317-8800

Manufacturer for drug substance: Fujisawa Healthcare, Inc.
3125 Staley Road
Grand Island, NY 14072

Drug:

Code Name: FR900506 ointment
Generic Name: FK-506 ointment
Trade Name: Protopic (Tacrolimus) ointment
Chemical Name: [3S-[3R*[E(1S*,3S*,4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate
CAS Registry Number: 104987-11-3
Molecular Formula/ Molecular Weight: $C_{44}H_{69}NO_{12} \cdot H_2O$ / 822.05
UV Absorption: λ_{max} (1:1,000 dil in methanol): ~ nm (active only)

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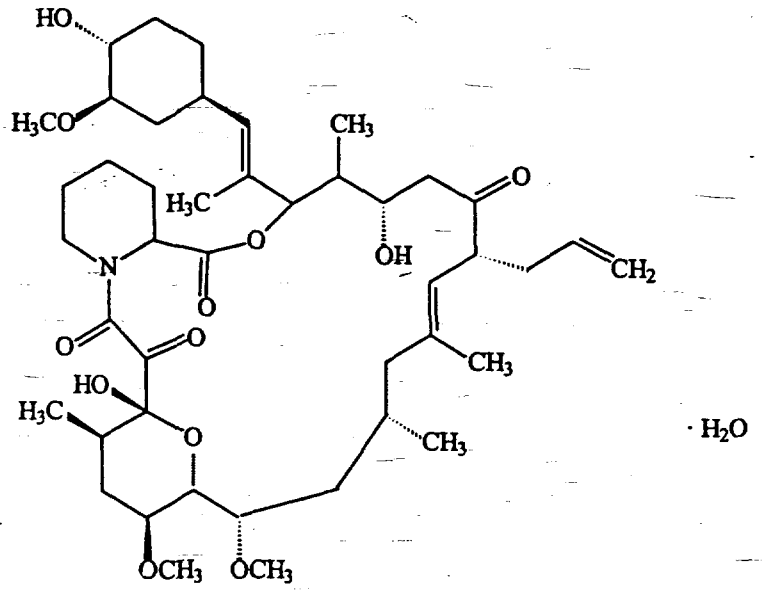
Structure:



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Relevant INDs/NDAs/DMFs:

1) NDA 50-708 (Prograf capsules for prophylaxis of organ {liver} rejection; HFD-590)
2) NDA 50-709 (Prograf injection for prophylaxis of organ {liver} rejection; HFD-590)
3) [redacted]
4) [redacted]
5) [redacted] (Tacrolimus ointment for Atopic Dermatitis; HFD-540)

Drug Class: Macrolide immunosuppressant

Indication: Moderate to severe Atopic Dermatitis

Clinical formulation:

The composition of the test article and vehicle used in Phase 3 clinical studies and nonclinical studies is provided in the following table (the composition of the 0.03% and 0.1% ointment is the same as the to be marketed formulation):

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Substance	Vehicle	0.03%	0.1%	0.3%	1%	3%
FR900506 (Tacrolimus)	[redacted]					
Propylene carbonate						
White wax						
Mineral oil						
Paraffin						
White petrolatum						

Dose:

The proposed dose in adults is 0.1% tacrolimus ointment and in pediatric patients is 0.03% —— tacrolimus ointment applied topically twice daily as a thin layer to affected areas of skin. It is estimated that up to 80% of the body could be treated in a severe case of atopic dermatitis. Approximately 30 g of tacrolimus ointment would be applied per treatment to cover 80% of the body. Therefore, the maximum daily dose of the 0.1% tacrolimus ointment would be 1.2 mg/kg/day (44.4 mg/m^2/day) for a 50 kg person (30,000 mg x .001 x 2/day ÷ 50 kg = 1.2 mg/kg/day).

Route of administration: Topical dermal

Disclaimer: Note some material may be taken directly from sponsor's submission.

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Review Table of Contents

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INTRODUCTION AND DRUG HISTORY:

Tacrolimus (also known as FK506) is a 23 member macrolide immunosupressant produced by *Streptomyuces tsukubaensis*, a soil bacterium found in Mount Tsukba, Japan. Tacrolimus inhibits the early activation of T-lymphocytes. Tacrolimus was originally approved in the United States in April 1994 in Prograf® capsules (NDA 50-708) and injection (NDA 50-709) for the prophylaxis of organ rejection in patients receiving allogenic liver transplants. Supplemental NDAs 50-708 (S-008)/50-709 (S-009) were approved in April 1997 for prophylaxis of rejection after allogenic kidney transplantation.

A topical formulation of tacrolimus, Protopic® (tacrolimus) ointment, has been developed by Fujisawa for dermatologic use. Protopic® ointment is indicated for the treatment of atopic dermatitis. The rationale for this is that atopic dermatitis is considered an immunologic disorder believed to be modified by T-lymphocytes. The sponsor plans to market two strengths of the Protopic® ointment (0.03% and 0.1%).

The nonclinical pharmacology/toxicology of orally or intravenously administered tacrolimus has been established under NDAs 50-708/50-709. A brief summary of the toxicities noted in these studies will be provided below. The sponsor submitted IND ——— to the division in December 1994 for studying the efficacy and safety of Protopic® ointment in the treatment of atopic dermatitis. Additional nonclinical pharmacology/toxicology studies were conducted with the Protopic® ointment under IND ——— to support the safety of topical application of Protopic® ointment.

STUDIES REVIEWED WITHIN THIS SUBMISSION:

Nonclinical Pharmacology Studies:

1) Effect of FK506 ointment on croton oil-induced ear edema and on delayed hypersensitivity in mice (R-94-0180-506-P2-E, CRR940307)

Nonclinical Pharmacokinetic Studies:

1) Metabolism of ^{14}C-tacrolimus by rat skin microsomes (R94-0079-506-P5-E, CRR940353)
2) Pharmacokinetic studies of FK506 ointment after single dermal application to rats (9-0111; CRR970179)
3) Pharmacokinetic study of FR900506 ointment after single dermal application to rabbits (R94-0095-506-P5-E, CRR940383)
4) Blood concentrations of FK506 after intravenous and oral administration and dermal application in Yucatan micropigs (R98-123-506-P5-E, CRD980094)
5) Blood and plasma concentration of FR900506 (FK506) after dermal application of FK506 ointment to micropigs in toxicokinetic study (R96-0087-506-P5-E, CR960501)
6) Absorption, distribution and excretion of ^{14}C- FR900506 ointment after single dermal application to rats (R94-0080-506-P5-E, CRR940364)

7) Absorption, distribution and excretion of ^{14}C- FR900506 ointment after 14 days repeated dermal application to rats (R95-0060-506-PF; CRR950249)
8) 4-week toxicokinetic study of FR900506 (FK506, tacrolimus) ointment administered topically in combination with ultraviolet radiation (UVR) in hairless mice (R98-1066-506-P2-E)

Acute Toxicology Studies:

1) Single dose dermal toxicity of tacrolimus ointment in rat (R94-0061-506-P2-E)

Repeat Dose Toxicology Studies:

1) Twenty-eight day skin irritation study with FR900506 ointment in the New Zealand white rabbit (R94-0098-506-P2-E)
2) 28-day dermal toxicity with FR900506 ointment in the rat (GLR950051)
3) Toxicity to rats by repeated dermal administration for 26 weeks (R96-0107-506-P2-E)
4) 13-week topical toxicity study of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs (R97-0002-506-P2-E)
5) 52-week topical toxicity of FR900506 (FK506, tacrolimus) ointment in Yucatan micropigs (96-0087)
6) 13-week range finding phototoxicity and tolerance of tacrolimus ointment with UV radiation in hairless mice (Study R96-0062-506-P2-E)

Special Toxicology Studies:

1) Local irritation study of FR900506 ointment in rabbits (II) (Primary dermal irritation) (Study R94-0096-506-P2-E; GLR940206)
2) Eye mucosa irritation study of FR900506 ointment in rabbits (Study R94-0099-506-P2-E; GLR940207)
3) Skin sensitization study of FR900506 ointment in guinea pigs (II) (Study R94-0097-506-P2-E; GLR940208)
4) Skin photosensitization study of FR900506 ointment in guinea pigs (II) (Study R94-0059-506-P2-E; GLR940244)
5) Effect of topical FR900506 (FK506, tacrolimus) on cutaneous pigmentation in normal dark Yucatan miniature swine (Study R95-0167-506-P2-E)
6) Comparison of FK506 (Tacrolimus) and glucocorticoids ointment on dermal atrophogenicity in rats (Study R97-0020-506-P2-E)

Degradation Product Toxicology Studies:

1) Single dose toxicity of degradation compound — of FR900506 ointment in mice (R96-0134-506-P2-E; GLR960362)
2) Local irritation study of degradation compound — of tacrolimus ointment in rabbits (Primary dermal irritation) (Study R96-0135-506-12-E; GLR960366)

3) Four-week percutaneous toxicity study of a main degradation compound [redacted] of FR900506 ointment in rats (Study 1998-1011-PJ-1; GLR980300)
4) Mutagenicity study of [redacted] – Reversion test with bacteria
5) Mutagenicity study of [redacted] – Chromosomal aberration test with Chinese hamster lung cells in culture

Carcinogenicity Studies:

1) Twelve-month photocarcinogenesis study of topically administered FR900506 (FK506, tacrolimus) ointment with ultraviolet radiation (UVR) in hairless mice (Study R98-0138-506-P2-E)
2) Topical oncogenicity study of FR900506 (FK506, tacrolimus) ointment in B6C3F1 mice following daily administration for 24 months (Study 95-8005)

Note: The dermal carcinogencitiy study conducted with tacrolimus ointment was reviewed in more detail in an addendum review and will be summarized in this review.

PHARMACOLOGY:

Mechanism of Action (data derived from literature):

Tacrolimus is an immunosuppressant agent. Tacrolimus acts directly on T-lymphocytes to inhibit transcription of genes that encode IL-2, IL-3, IL-4, IL-5, GM-CSF, TNF-α and IFN-γ that may play a role in the pathogenesis of atopic dermatitis. Experimental evidence suggests that tacrolimus diffuses across cell membranes and binds to a class of ubiquitous peptidyl-propyl *cis-trans* isomerases (PPIase), designated FK506 (tacrolimus)-binding proteins (FKBPs). The predominant FKBP in the T-lymphocyte is a cytosolic protein of ~12,000 Daltons, FKBP-12[1,2]. In vitro, tacrolimus binds to FKBP-12 and forms a pentameric complex with Ca^{+2}, calmodulin, and the Ca^{+2}-dependent protein phosphatase, calcineurin[3]. This inhibits the ability of calcineurin to dephosphorylate $NFAT_t/NFAT_p$, which is the cytosolic component of the transcription factor (NFAT) that activates IL-2 gene transcription[4,5]. Consequently, synthesis and release of IL-2 are inhibited and clonal expansion of T-lymphocytes is suppressed[6]. Tacrolimus also inhibits the

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[2] Siekierka JJ, Jung SHY, Poe N, Lin CS, Sigal HN. (1989) A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 341: 755-757.
[3] Tamura K, Fujmura T, Iwaski K, et al. (1994) Interaction of tacrolimus (FK506) and its metabolites with FKBP and calcineurin. Biochem. Biophys. Res. Comm. 202: 437-443.
[4] Shaw KTY, Ho AM, Raghavan A, et al. (1995) Immunosuppressive drugs prevent a rapid dephosphorylation of transcription factor NFAT1 in stimulated immune cells. Proc. Natl. Acad. Sci. USA. 92: 11205-11209.
[5] Ruff VA and Leach KL. (1995) Direct demonstration of NFATp dephosphorylation and nuclear localization in activated HT-2 cells using a specific NFATp polyclonal antibody. J. Biol. Chem. 270: 22602-22607.
[6] Tocci MF, Matkovich DA, Collier KA, et al. (1989) The immunosuppressant FK506 selectively inhibits expression of early T cell activation genes. J. Immunol. 143: 718-726.

transcription and release of other cytokines (IL-3, IL-4, IL-5, IFN-γ, TNF-α, GM-CSF) and protoncogene c-myc, possibly by a similar mechanism[6,7].

The predominant tacrolimus receptor in mast cells and basophils is a membrane-bound protein of ~13,000 Daltons (FKBP-13). FKBP-13 has a 51% nucleotide sequence identity and 43% amino acid sequence identity with FKBP-12. FKBP-13 does not form a pentameric complex with tacrolimus and calcineurin. However, the amino acid residues comprising the tacrolimus binding site and the PPIase activity are identical in the two subtypes[8,9,10,11]. Tacrolimus has been shown to inhibit histamine release in human mast cells and basophils. However, the effect differs between cell type and is dependent on the nature of the stimulus.

Drug Activity Related to Proposed Indication (study submitted to NDA):

Drug Activity Study #1:

Effect of FK506 ointment on croton oil-induced ear edema and on delayed hypersensitivity in mice

Study Title:	Effect of FK506 ointment on croton oil-induced ear edema and on delayed hypersensitivity in mice
Study No:	R-94-0180-506-P2-E, CRR940307
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Study conducted between February and April, 1993
GLP compliance:	No

FK506 ointment was evaluated for anti-inflammatory activity in two mouse models. In the first model, edema was induced on mouse ears using croton oil (3%) and was evaluated based on the thickness of the ear. FK506 ointment (1%) inhibited ear edema up to 44% as measured by ear thickness.

In the second model, delayed hypersensitivity to oxazolone was evaluated by exposure (abdominal skin) and re-exposure (inner side of both ears) 7 days later to 2% oxazolone. The extent of hypersensitivity was evaluated based on the thickness of the ear. FK506 ointment (1%) inhibited ear thickness up to 61%.

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[7] Mori A, Suki M, Kaminuma O, et al. (1997) Enhanced Il-5 gene transcription and allergic inflammation. Japan J. Thoracic Dis. 35 (Suppl): 47-51.

[8] Wiederrecht G, Lam E, Hung S, Martin M and Sigal N. (1993) The mechanism of action of FK-506 and cyclosporine A. Anum. NY Acad. Sci. 696: 9-19.

[9] Futer O, DeCenzo MT, Aldape RA and Livingston DJ. (1995) FK506 binding protein mutational analysis. J. Biol. Chem. 270: 18935-18940.

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[11] Hultsch T, Albers MW, Schreiber SL and Hohman RJ. (1991) Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. Proc. Natl. Acad. Sci. USA. 88: 6229-6233.

PHARMACOKINETICS/TOXICOKINETICS:

Note: Information contained in this section was obtained from either studies conducted with the tacrolimus ointment included in the NDA submission or from previously conducted studies with either the intravenous, oral or topical ointment formulations of tacrolimus. The source of the data provided will be annotated for each section below.

PK Parameters (studies submitted to the NDA):

PK Parameter Study #1:

Pharmacokinetic studies of FK506 ointment after single dermal application to rats

Study Title:	Pharmacokinetic studies of FK506 ointment after single dermal application to rats
Study No:	9-0111; CRR970179
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated December 96
GLP compliance:	No

The objective of this study was to estimate the pharmacokinetic parameters of FK506 ointment after single dermal application to male Sprague-Dawley rats. The effects of application method, strength, skin condition, application area and application amount of FK506 ointment on the absorption were evaluated in this study. Each set of different conditions will be described below followed by a table of the results from each portion of the study. For the dermal portions of this study, blood for pharmacokinetic analysis was obtained at 30 minutes, 1, 2, 4, 6, 8, 24, 30, 48, 72, and 144 hours after dose administration. For the IV portion of this study, blood for pharmacokinetic analysis was obtained at 5, 15 and 30 minutes and 2, 4, 6, 8 and 24 hours after dose administration.

For the first part of the study, 100 mg of 0.5% FK506 ointment was applied to 10 cm^2 of rat intact skin under occlusion and not under occlusion and to rat damaged (tape-stripped) skin under occlusion. In addition, 100 mg of 0.03% and 0.1% FK506 ointment was applied to 10 cm^2 of rat intact skin under occlusion. A group of rats was administered intravenously 1 mg/kg FK506.

The C_{max} values were similar for both application methods but the AUC value for the non-occluded method was higher that the occluded method for the 0.5% FK506 ointment. Occlusion decreased the T_{max} and $T_{1/2}$ values compared to non-occlusion for 0.5% FK506 ointment. C_{max} and AUC levels after a single dermal application of 0.5% FK506 ointment to damaged skin were much higher than when applied to intact skin. The AUC level after application of 0.5% FK506 ointment to damaged skin approached the AUC level seen after 1 mg/kg FK506 intravenous administration. C_{max} and AUC increased in proportion to the strength of FK506 ointment. The results (mean ± SE) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(\infty)}$ (ng·hr/ml)	$T_{1/2}$ (hr)
0.5%, non-occluded, intact skin (n=6)	2.92 ± 1.08	12.0 ± 3.8	73.27 ± 22.90	24.0 ± 5.7
0.5%, occluded, intact skin (n=7)	2.85 ± 1.30	8.3 ± 2.7	49.25 ± 16.55	14.7 ± 1.2
0.5%, occluded, damaged skin (n=7)	43.29 ± 3.22	3.1 ± 0.4	652.1 ± 38.0	20.6 ± 2.4
0.1%, occluded, intact skin (n=5)	0.39 ± 0.05	14.4 ± 3.9	30.44 ± 13.76	66.6 ± 32.9
0.03%, occluded, intact skin (n=6)	0.12 ± 0.07	6.0 ± 0.0	0.65 ± 0.37	--
1 mg/kg IV (n=5)	--	--	678.8 ± 13.1	10.3 ± 0.9

In the next part of the study, 25, 50 and 100 mg of 0.3% FK506 ointment was applied to 2.5, 5 and 10 cm^2 of intact skin under occlusion. C_{max} and AUC values increased in proportion to application area. The results (man ± SE) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(0-144)}$ (ng·hr/ml)
25 mg/2.5 cm^2/0.3%, occluded, intact skin (n=6)	0.50 ± 0.23	14.8 ± 5.1	4.57 ± 2.31
50 mg/5.0 cm^2/0.3%, occluded, intact skin (n=6)	1.04 ± 0.64	17.3 ± 4.9	16.95 ± 7.18
100 mg/10 cm^2/0.3%, occluded, intact skin (n=5)	1.95 ± 1.22	16.4 ± 5.6	42.44 ± 24.69

In the next part of the study, 10, 30, 100 and 300 mg of 0.3% FK506 ointment were applied to 10 cm^2 of intact skin under occlusion. C_{max} and AUC values were almost the same levels in the four different dosing groups. The results (mean ± SE) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(0-144)}$ (ng·hr/ml)
10 mg/10 cm^2/0.3%, occluded, intact skin (n=5)	0.84 ± 0.37	4.4 ± 0.7	8.0 ± 3.4
30 mg/10 cm^2/0.3%, occluded, intact skin (n=5)	0.76 ± 0.35	8.8 ± 3.9	7.93 ± 3.67
100 mg/10 cm^2/0.3%, occluded, intact skin (n=6)	0.91 ± 0.35	6.3 ± 0.8	12.4 ± 5.2
300 mg/10 cm^2/0.3%, occluded, intact skin (n=4)	0.47 ± 0.16	16.5 ± 6.2	7.37 ± 2.13

PK Parameter Study #2:

Pharmacokinetic study of FR900506 ointment after single dermal application to rabbits

Study Title:	Pharmacokinetic study of FR900506 ointment after single dermal application to rabbits
Study No:	R94-0095-506-P5-E, CRR940383
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated July 94
GLP compliance:	No

FK506 was administered to male New Zeland white rabbits intravenously (1 mg/kg) or dermally (250 mg of 0.5% FK506 ointment applied to 25 cm^2 of intact skin under occlusion for 24 hours). For the dermal portion of this study, blood for pharmacokinetic analysis was obtained at 2, 4, 6, 8, 24, 48, 72, and 144 hours after dose administration. For the IV portion of this study, blood for pharmacokinetic analysis was obtained at 5, 15 and 30 minutes and 1, 2, 4, 6, 8, 24, 48 and 72 hours after dose administration. The results (mean ± SE) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(0-\infty)}$ (ng·hr/ml)	$T_{1/2}$ (hr)
250 mg/25 cm^2/0.5%, occluded, intact skin (n=3)	4.57 ± 0.69	7.3 ± 0.7	176.7 ± 32.9	54.8 ± 4.8
1 mg/kg IV (n=3)	--	--	2481 ± 378	18.5 ± 1.5

PK Parameter Study #3:

Blood concentrations of FK506 after intravenous and oral administration and dermal application in Yucatan micropigs

Study Title:	Blood concentrations of FK506 after intravenous and oral administration and dermal application in Yucatan micropigs
Study No:	R98-123-506-P5-E, CRD980094
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated March 98
GLP compliance:	No

FK506 was administered to male Yucatan micropigs intravenously (1 mg/kg), orally (1mg/kg in Peg 400) or dermally (100 mg of 0.1% FK506 ointment applied to 10 cm^2/kg of intact skin under occlusion for 24 hours). Blood samples were obtained at 5 (iv only), 15, 30 and 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hr after dose administration. In addition, blood samples were obtained at 28 and 32 hr after dermal application. The results (mean ± SE) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(0-\infty)}$ (ng·hr/ml)	$T_{1/2}$ (hr)
100 mg/10 cm^2/kg/0.1%, occluded, intact skin (n=3)	0.152 ± 0.051	28.0 ± 0.0	3.66 ± 1.94	0.94 ± 0.50
1 mg/kg oral (n=3)	117 ± 2.9	0.7 ± 0.2	981 ± 99	24.0 ± 1.4
1 mg/kg IV (n=3)	--	--	3903 ± 412	35.4 ± 3.1

Bioavailability (F) was 25.1% after oral administration compared to intravenous administration. Bioavailability (F) was 0.94% after dermal administration compared to intravenous administration. The results from this study indicated that the bioavailability in micropigs is low after dermal administration of FK506 ointment under the conditions of this study.

PK Parameter Study #4:

Blood and plasma concentration of FR900506 (FK506) after dermal application of FK506 ointment to micropigs in toxicokinetic study

Study Title:	Blood and plasma concentration of FR900506 (FK506) after dermal application of FK506 ointment to micropigs in toxicokinetic study
Study No:	R96-0087-506-P5-E, CR960501
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated October 96
GLP compliance:	No

In the first part of this study, a single dose of FK506 was administered to female micropigs intravenously (3.43 mg/kg), orally (3.43 mg/kg) or dermally. Two dermal doses were administered in this study. The first dermal dose was 4 g of 1% FK506 ointment (3.43 mg/kg) and the second dermal dose was 4 g of 3% FK506 ointment (10.11 mg/kg). The conditions for application of the two dermal doses (i.e., under occlusion or not and duration of treatment) were not provided in the study report. For the dermal portion of this study, blood for pharmacokinetic analysis was obtained at 1, 2, 4, 8, 12, 24, 28, 32, 48 and 72 hours after dose administration. For the oral portion of this study, blood for pharmacokinetic analysis was obtained at 1, 2, 4, 8, 12, 24, 48 and 72 hours after dose administration. For the intravenous portion of this study, blood for pharmacokinetic analysis was obtained at 5, 15 and 30 minutes and 1, 2, 4, 8, 12, 24, 48 and 72 hours after dose administration. The results (mean ± SD) are presented in the following table.

Treatment	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{(0-72)}$ (ng·hr/ml)	$T_{1/2}$ (hr)
1% FK506 ointment, 3.43 mg/kg (n=3)	3.13 ± 3.09	24.7 ± 23.0	52.53 ± 8.87	--
3% FK506 ointment, 10.11 mg/kg (n=3)	3.11 ± 1.77	35.3 ± 35.1	69.52 ± 30.61	--
3.43 mg/kg oral FK506 (n=2)	172.4	1.5	1944	19.3
3.43 mg/kg IV FK506 (n=2)	--	--	6777	19.2

No significant differences in blood levels of FK506 were noted after single application of 1% and 3% FK506 ointment. The bioavailabilities after application of 1% and 3% FK506 ointment were 0.83% and 0.36%, respectively. The percutaneous absorption of FK506 after single dermal application of the ointment formulation was very low in the micropig under the conditions of this study.

In the second part of this study, repeat daily dermal doses of FK506 ointment were administered to female micropigs (4 gm of 0.3%, 1% and 3% FK506 ointment, bid) for seven days. The conditions for application of the two dermal doses (i.e., under occlusion or not and duration of treatment) were not provided in the study report. Blood samples for pharmacokinetic analysis were drawn on days 1, 4 and 7 (at 2, 4, 8 and 24 hrs after dose administration). The results (mean ± SD) are presented in the following table.

Treatment	C_{max} (ng/ml)			T_{max} (hr)			$AUC_{(0-24)}$ (ng·hr/ml)		
	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
0.3% FK506 ointment (n=4)	17.4 ± 10.12	18.2 ± 11.39	22.85 ± 15.30	2.0 ± 4.0	12.5 ± 13.3	11.0 ± 8.9	174.5 ± 101.9	243.1 ± 115.5	363.7 ± 226.0
1% FK506 ointment (n=3)	12.42 ± 1.16	9.54 ± 1.40	32.28 ± 18.89	--	3.3 ± 4.2	2.7 ± 4.6	108.3 ± 34.7	172.5 ± 28.0	351.4 ± 118.6
3% FK506 ointment (n=3)	9.54 ± 7.40	43.22 ± 25.67	86.18 ± 84.40	16.0 ± 13.9	24.0 ± 0.0	10.7 ± 12.2	87.9 ± 70.03	584.8 ± 276.4	473.1 ± 189.0

In general, there was an increase in C_{max} and AUC with time for each concentration of FK506 ointment. A dose dependent increase in either C_{max} or AUC was not noted on any of the days analyzed in this study.

Absorption, Distribution and Excretion (studies submitted to NDA):

Absorption, Distribution and Excretion Study #1:

Absorption, distribution and excretion of ^{14}C- FR900506 ointment after single dermal application to rats

Study Title:	Absorption, distribution and excretion of ^{14}C- FR900506 ointment after single dermal application to rats
Study No:	R94-0080-506-P5-E, CRR940364
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated August 94
GLP compliance:	No

Absorption, distribution and excretion of radioactivity were studied in male rats after single dermal application of ^{14}C-FR900506 ointment. ^{14}C-FR900506 (918.4 kBq/kg) was applied to occluded and non-occluded intact skin and occluded tape-stripped damaged rat skin for 24 hours (0.5% FR900506 ointment, 1.6 mg/kg). After single application of ^{14}C-FR900506 ointment on the intact and damaged skin of rats, blood samples were taken at 15 and 30 minutes and 1, 2, 4, 6, 8, 12, 24, 28, 32, 48 and 72 hours after application. After single application of ^{14}C-FR900506 ointment to intact and damaged skin of rats under occlusion, animals were housed in individual metabolism cages. Urine was collected at 0-4, 4-8, 8-24, 24-28, 28-32, 32-48 hr intervals after application and for every 24 hr interval thereafter up to 168 hr. Feces samples were collected for every 24 hr interval up to 168 hr. Rats were euthanized after 168 hr and the level of radioactivity in the remaining ^{14}C-FR900506 ointment, application site and carcass was determined for each rat.

No radioactivity was detected in either blood (limit of detection = [redacted] ng/ml) or plasma (limit of detection = [redacted] mg/ml) in rats administered a single application of ^{14}C-FR900506 ointment to intact skin under occlusion or non-occlusion. The pharmacokinetic parameters calculated after single application of ^{14}C-FR900506 ointment to damaged skin under occlusion is provided in the following table.

Sample	$T_{1/2}$ (hr)	$AUC_{(\infty)}$ (ng·hr/ml)
Blood	9.0 ± 0.7	559 ± 71
Plasma	9.3 ± 1.4	457 ± 35

The cumulative excretion of radioactivity (expressed as percentage of radioactive dose) after single dermal application of ^{14}C-FR900506 ointment to occluded and non-occluded intact skin and occluded tape-stripped damaged rat skin is provided in the following table.

Treatment	Urine (24 hrs)	Urine (168 hrs)	Feces (24 hrs)	Feces (168 hr)	Carcass (168 hr)	Skin (168 hrs)	Ointment (168 hr)
Non-occluded intact skin	0.2 ± 0.1	0.5 ± 0.1	1.9 ± 0.7	5.1 ± 1.4	0.3 ± 0.1	14.6 ± 1.2	77.4 ± 1.5
Occluded intact skin	0.2 ± 0.0	0.4 ± 0.1	1.9 ± 0.4	4.2 ± 0.5	0.5 ± 0.2	22.6 ± 2.7	69.6 ± 3.5
Occluded damaged skin	1.6 ± 0.1	2.4 ± 0.1	41.5 ± 1.7	53.6 ± 0.4	0.4 ± 0.0	4.7 ± 0.4	38.3 ± 1.2

Systemic absorption was approximately the same after administration of ^{14}C-FR900506 ointment to intact skin under occlusion or not occluded. Occlusion of intact skin appeared to increase the amount of radioactivity in the skin sample. Systemic absorption of FR900506 was significantly increased under occlusion in damaged skin.

In the second part of this study, [redacted] was performed on rats killed 30 minutes, 2, 8 or 24 hours after single application of ^{14}C-FR900506 ointment to damaged skin under occlusion. [redacted] indicated that FR900506 was generally equally distributed in the body. An increase in the bile was noted after 2 hours. A 50% decrease at the application site was noted between 8 and 24 hours. An increase in the intestinal contents with up to 4 times the amount found in other organ systems was noted after 8 and 24 hours. The radioactivity levels were reported on a relative level with a scale ranging from 0 to 5.

Absorption, Distribution and Excretion Study #2:

Absorption, distribution and excretion of ^{14}C- FR900506 ointment after 14 days repeated dermal application to rat

Study Title:	Absorption, distribution and excretion of ^{14}C- FR900506 ointment after 14 days repeated dermal application to rats
Study No:	R95-0060-506-PF; CRR950249
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated May 95
GLP compliance:	No

Absorption, distribution and excretion of radioactivity were studied in male rats after 14 days of repeated dermal application of ^{14}C-FR900506 ointment. ^{14}C-FR900506 (941 kBq/kg) was applied to intact skin under occlusion for 24 hours (0.5% FR900506 ointment, 1.6 mg/kg). After single application of ^{14}C-FR900506 ointment on the intact and damaged skin of rats, blood samples were taken at 15 and 30 minutes and 1, 2, 4, 6, 8, 12, 24, 28, 32, 48 and 72 hours after application. After repeat application of ^{14}C-FR900506 ointment to intact skin of rats under occlusion, animals were housed in individual metabolism cages. Urine and feces was collected for 24 hr intervals after each application and up to 336 hr after the 14th application. Rats were euthanized 336 hr after the last dose and the level of radioactivity in the remaining ^{14}C-FR900506 ointment, application site and carcass was determined for each rat. The cumulative excretion of

radioactivity (expressed as percentage of radioactive dose) after repeated dermal application of ^{14}C-FR900506 ointment to occluded intact skin is provided in the following table.

Treatment	Urine (24 hrs)	Urine (Day 14)*	Feces (24 hrs)	Feces (Day 14)*	Carcass (Day 14)*	Skin (Day 14)*	Ointment (Day 14)
Occluded intact skin	0.2 ± 0.1	0.2 ± 0.1	2.5 ± 0.7	1.6 ± 0.4	0.0 ± 0.0	1.1 ± 0.3	92.9 ± 0.5

* - Day 14 values are after 336 hrs of measurement.

Repeat daily administration of ^{14}C-FR900506 ointment to intact skin of rats under occlusion for 14 days did not cause an increase in excretion of FR900506 in urine or feces. A small amount of radioactivity was noted in the skin 336 hours after the last dose administration. The majority of radioactivity was recovered in the ointment.

In the second part of this study, radioactivity levels were determined in rats after single and repeat dose administration of ^{14}C-FR900506 ointment to intact skin under occlusion. Animals were euthanized for analysis at 8 and 24 hours after single application, at 24 hours after 5 and 10 time repeated daily application and at 8, 24, 168, 336 and 672 hours after 14 time daily application of ^{14}C-FR900506 ointment. Blood was collected at all time points for analysis. The following tissues were obtained at all time points for radioactivity measurement: cerebrum, cerebellum, pituitary gland, eyeball, harderian gland, parotid gland, sublingual gland, mandibular gland, thyroid gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, skeletal, muscle (application site), skeletal muscle, fat, brown fat, bone marrow, skin (application site), skin, testis, prostate gland, mesenteric lymph node, stomach, small intestine, large intestine and urinary bladder.

Radioactivity levels of most tissues tended to decrease slightly after repeat dose application. The ratio of radioactive concentration in the kidney and skin of application site after the 14th day application were 76% and 65%, respectively. Application site skin contained the highest levels of radioactivity (40-80 fold greater than the next highest level). Most of the organs contained measurable levels of radioactivity that ranged between 8 – 150 ng equivalents/g tissue that decreased with repeat dose administration. Plasma, cerebrum, cerebellum, pituitary gland, thyroid gland, and testis were tissues that had levels below the level of detection.

<u>Metabolism (data from NDAs 50-708/50-709 {iv/oral pharmacokinetics studies}):</u>

When ^{14}C-tacrolimus was administered to rats either intravenously or orally, total recovery of radioactivity in urine and feces was over 95%[12]. Eight metabolites were isolated and identified following metabolism of tacrolimus in human and/or rat liver microsomes *in vitro*[13,14,15]. The main metabolite produced *in vitro* by human and rat hepatic microsomes was 13-

[12] Iwasaki K. (1988) Absorption and excretion of ^{14}C-labelled FR900506 in rats. Fujisawa Pharmaceutical Co., Ltd.; Company Report CRR880349.

[13] Shiraga T, Matsuda H, Nagase K, et al. (1994) Metabolism of FK506, a potent immunosuppressive agent, by cytochrome P450 3A enzymes in rat, dog and human liver microsomes. Biochem. Pharmacol. 47: 727-735.

demethyl-tacrolimus, which demonstrated approximately less than 10% of the immunosuppressive activity of unchanged tacrolimus. The 31-demethyl-tacrolimus metabolite has an immunosuppressive *in vitro* activity similar to that of tacrolimus. The metabolism of tacrolimus is mediated by cytochrome P450 3A, with only a minor role for other P450 isozymes[16].

Metabolism (study submitted to the NDA):

Metabolism Study #1:

Metabolism of ^{14}C-tacrolimus by rat skin microsomes

Study Title:	Metabolism of ^{14}C-tacrolimus by rat skin microsomes
Study No:	R94-0079-506-P5-E, CRR940353
Conducting laboratory:	Fujisawa Pharmaceutical Co.
Date of study:	Final report dated July 1994
GLP compliance:	No

Skin and liver microsomes were prepared from rats. The microsomal fractions were evaluated for their ability to metabolize FK506, testosterone, and 7-ethoxycoumarin in the presence of NADPH under aerobic conditions. The results of this study are provided in the following table.

Substrate	Reaction	Skin Activity (pmol/min/mg protein)	Liver Activity (pmol/min/mg protein)
FK506	M-I formation*	0.071	147
Testosterone	6β-hydroxylation	1.3	2330
7-Ethoxycoumarin	O-deethylation	0.31	1030

* - M-I structure is provided in figure below.

The results from this study indicate that oxidative metabolism of FK506 by the rat skin is very weak when compared to the metabolism of the rat liver. The metabolic pathway for FK506 has been previously elucidated and is provided in the figure below that was scanned from the NDA submission.

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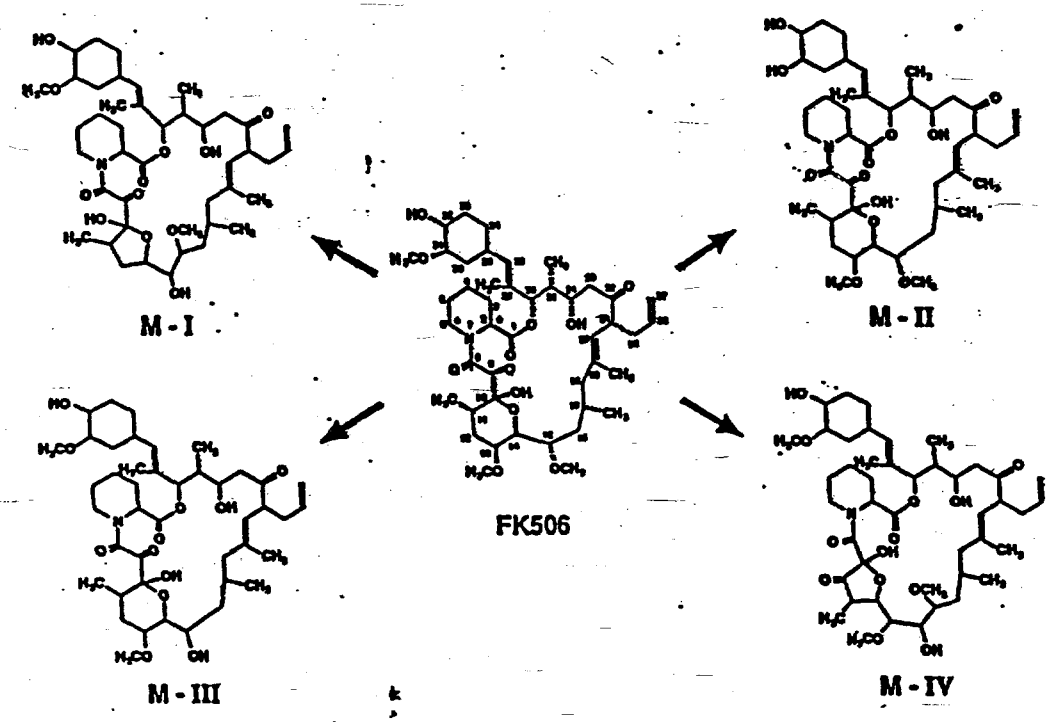
[14] Iwasaki K, Shiraga T, Nagase K, et al. (1993) Isolation, identification, and biological activities of oxidative metabolites of FK506, a potent immunosuppressive macrolide lactone. Drug Metab. Dispos. 21: 971-977.
[15] Iwasaki K, Shiraga T, Matsuda H, et al. (1995) Further metabolism of FK506 (tacrolimus): identification and biological activities of the metabolites oxidized at multiple sites of FK506. Drug Metab. Dispos. 23: 28-34.
[16] Vincent SH, Karanam BR, Painter SK, Chiu SHL. (1992) In vitro metabolism of FK-506 in rat, rabbit and human liver microsomes: identification of a major metabolite of cytochrome P450 3A as the major enzymes responsible for its metabolism. Arch. Biochem. Biophys. 294: 454-460.



Metabolic pathway of FK506

★ : ^{14}C-labelled position

Plasma Protein Binding (data from NDAs 50-708/50-709 {iv/oral pharmacokinetics studies}):

The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of — ng/ml. Tacrolimus is primarily bound to albumin and alpha-1-glycoprotein, and has a high level of association with erythrocytes[17]. Tacrolimus partitions into red blood cells with a blood to plasma ratio of >4[18].

[17] Nagase K, Iwasaki K, Nozaki K and Noda K. (1994) Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. J. Pharm. Pharmacol. 46: 113-117.

[18] Venkataramanan R, Jain A, Warty VS, et al. (1991) Pharmacokinetics of FK506 following oral administration: a comparison of FK506 and cyclosporine. Transplant Proc. 23: 931-933.

Other Study (submitted to the NDA):

Note: This study was conducted per a division request to have toxicokinetic data after the analysis of the 12 month photocarcinogenesis study. This study was conducted as a stand alone study with the results being submitted to the NDA and not submitted to the IND previously.

Other Study #1:

4-week toxicokinetic study of FR900506 (FK506, tacrolimus) ointment administered topically in combination with ultraviolet radiation (UVR) in hairless mice

Study Title:	4-week toxicokinetic study of FR900506 (FK506, tacrolimus) ointment administered topically in combination with ultraviolet radiation (UVR) in hairless mice
Study No:	R98-1066-506-P2-E
Conducting laboratory:	[redacted]
Date of study:	Final report dated June 1998
GLP compliance:	Yes

The purpose of this study was to provide systemic tacrolimus (FK506) blood concentration data for use in evaluating the results obtained from a one-year photocarcinogensis study of tacrolimus ointment in hairless mice. Sixty albino hairless Crl:KSH1-*hr*BR mice/sex were assigned to three dose groups. Tacrolimus ointment concentrations of 0.03%, 0.1% and 0.3% were topically administered to the posterior dorsal skin of mice (~20% of the body surface area) at a volume of 50 µl/mouse. Each dose was administered once daily, five days/week, for four weeks. All mice were exposed to ultraviolet radiation (UVR) five days/week. The UVR source was a 6.5 kilowatt xenon long arc lamp [redacted] with a [redacted] glass optical filter. Mice were exposed to 600 Robinson-Berger Units (RBU) per week. The duration of irradiation of 120 RBU/day was ~72 minutes. On Monday, Wednesday and Friday, UVR exposure for each group began ~1 hour after the completion of test article administration. On Tuesday and Thursday, the test article was administered ~1 hour after completion of UVR exposure for each group.

Viability was checked twice daily during the study. Clinical observations, general appearance and body weight measurements were recorded weekly and at sacrifice. Blood samples were collected from 5 mice/sex/group at 0, 2, 4 and 20.5 hours after UVR exposure on days 1, 12 and 26 of the study. Blood concentrations of FK506 were determined by [redacted]

No test article related effects were noted for clinical observations or body weight in this study. No mortality was noted in this study. Pharmacokinetic results (mean) for male mice are presented in the following table.

Treatment	C_{max} (ng/ml)			T_{max} (hr)			$AUC_{(0-20.5)}$ (ng·hr/ml)		
	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26
0.03% FK506 ointment	4.92	10.16	9.63	0*	4	0	39.6	140.2	115.7
0.1% FK506 ointment	32.68	35.81	41.71	2	4	2	287.3	439.2	441.0
0.3% FK506 ointment	109.8	48.89	56.34	0	2	0	1153	572.8	687.5

* - The T_{max} value of 0 is not the conventional 0 hr which refers to prior to drug application. This 0 refers to 132 minutes after dose administration.

Pharmacokinetic results (mean) for female mice are presented in the following table.

Treatment	C_{max} (ng/ml)			T_{max} (hr)			$AUC_{(0-20.5)}$ (ng·hr/ml)		
	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26
0.03% FK506 ointment	6.68	13.88	14.18	0*	2	0	47.25	137.3	156.5
0.1% FK506 ointment	36.24	64.45	75.95	0	2	2	351.6	706.7	848.0
0.3% FK506 ointment	102.0	63.2	122.8	0	0	2	1340	737.1	1136

* - The T_{max} value of 0 is not the conventional 0 hr which refers to prior to drug application. This 0 refers to 132 minutes after dose administration.

Blood levels tended to increase with increased strength of FK506 ointment. Female mice C_{max} and AUC levels were generally slightly higher than male mice C_{max} and AUC levels. C_{max} and AUC levels generally increased from Day 1 to Day 12 and reached a plateau from Day 12 to Day 26. A more detailed discussion of this pharmacokinetic data as it relates to the photocarcinogenicity study results will be provided during the discussion of this study later in this review.

TOXICOLOGY:

Acute Toxicology Studies (data from NDAs 50-708/50-709):

The acute toxicity of tacrolimus has been determined in adult and immature Sprague-Dawley rats and baboons following oral and intravenous administration. In adult rats, the oral LD_{50} for tacrolimus was 234 mg/kg for males and 194 mg/kg for females. The corresponding intravenous LD_{50} values for tacrolimus were 57.0 mg/kg and 23.6 mg/kg in male and female rats, respectively[19]. In immature rats, The oral LD_{50} values were 70 and 32-100 mg/kg in males and

[19] Acute toxicity study of FR900506 in rats (intravenous and oral dosing). (1988) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR880181 (88-0002-506-P2-E).

females, respectively[20]. An LD_{50} was not determined in baboons following oral doses of 50-250 mg/kg and IV doses of 2-50 mg/kg[21].

Acute Toxicology Studies (submitted to the NDA):

Acute Toxicology Study #1:

Single dose dermal toxicity of tacrolimus ointment in rat

Study Title:	Single dose dermal toxicity of tacrolimus ointment in rat
Study No:	R94-0061-506-P2-E
Amendment #, Vol #:	000, 10
Conducting laboratory:	[redacted]
Date of study initiation:	3/23/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Approximately 24 hours before treatment commenced, the dorso-lumbar region of each rat (~10% of total body surface area) was clipped free of hair using electric clippers. The test substance (8 g/kg) was applied by spreading it evenly over the prepared skin. The treatment area was ~ 50 mm x 50 mm. Abraded application sites were prepared by using the tip of a scalpel blade immediately prior to dosing. The treatment area was covered with gauze held in place with a non-irritating dressing that surrounded the trunk. The treatment period was for 24 hours. At the end of the treatment period, the dressing was removed and the treated area of skin was washed with warm water.

Dosing:

- *species/strain:* Crl:CD Br VAFD Plus Sprague-Dawley rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 7-10 weeks
- *weight:* 255-368 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

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[20] Acute toxicity study of FR900506 in young rats (oral dosing). (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910392 (R91-0020-506-P2-E).

[21] FR900506 acute oral and intravenous toxicity to baboons by single administration. (1989) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R89-0048-506-P2-E).

Dosing Table

Treatment	Skin site	Dose (mg/kg/day)	Number of Main Study Animals	
			Males	Females
Sham Control	Intact	0	5	5
Placebo Control	Intact	0	5	5
0.3% FR900506 ointment	Intact	24	5	5
0.3% FR900506 ointment	Abraded	24	5	5
1.0% FR900506 ointment	Intact	80	5	5
1.0% FR900506 ointment	Abraded	80	5	5

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.3% FR900506 ointment – lot # 66863XK
1.0% FR900506 ointment – lot# 66873XK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily for 14 days
- *Local dermal signs:* daily prior to next dose administration for 14 days
- *Body weights:* days 1, 8 and 15
- *Gross pathology:* at sacrifice

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** No treatment related signs of dermal irritation or other dermal changes were noted at either the intact or abraded treatment sites.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

No overt toxicity was noted in this study. The acute lethal dermal dose of FR900506 ointment is greater that 80 mg/kg in rats.

Repeat Dose Toxicology Studies (data from NDAs 50-708/50-709):

The toxicological profile of orally administered tacrolimus in rats was characterized by such overt signs of toxicity as decreased spontaneous motility, piloerection, hyper-reactivity, decreased body weight gain, and salivation[22,23,24,25,26].

Oral administration of tacrolimus to baboons resulted in decreased body weight and food consumption, incidences of quietness and huddled and/or unnatural posture[27,28,29,30,31,32]. Clinical chemistry showed reduction in hemoglobin, RBC count, and mean corpusclar volume and increase in urinary glucose.

The summary table of the toxicological profile of tacrolimus outlined by end organ toxicity that was provided by the sponsor is reproduced below.

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[22] Preliminary two-week oral toxicity study of FR900506 in rats (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910477 (R91-0112-506-P2-E).
[23] Four-week oral toxicity study of FR900506 in young rats. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910393 (R91-0119-506-P2-E).
[24] FR900506 toxicity to rats by repeated oral administration for 13 weeks. (1988) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R88-0001-506-P2-E).
[25] FR900506 toxicokinetic study in rats by dietary administration for 13 weeks . (1994) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R94-0042-506-P2-E).
[26] FR900506 toxicity to rats by repeated oral administration for 52 weeks. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0074-506-P2-E).
[27] FR900506 toxicity to baboons by repeated intravenous administration for 4 weeks. (1989) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR890444 (R89-0045-506-P2-E).
[28] FR900506 preliminary toxicity study in baboons by repeated oral administration for 28 days. (1988) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R88-0051-506-P2-E).
[29] FR900506 toxicokinetic study in baboons by repeated oral administration for 13 weeks. (1994) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R94-0093-506-P2-E).
[30] FR900506 toxicity to baboons by repeated oral administration for 13 weeks. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0029-506-P2-E).
[31] FR900506 toxicity to baboons by repeated oral administration for 13 weeks. (1989) Fujisawa Pharmaceutical Co., Ltd.; Company Repon GLR890443 (R89-0046-506-P2-E).
[32] FR900506 toxicity to baboons by repeated oral administration for 52 weeks. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0064-506-P2-E).

Organs	Species					
	Rat			Baboon		
	4-wk (IV)	13-wk (PO)	52-wk (PO)	4-wk (IV)	13-wk (PO)	52-wk (PO)
Kidney	++	+	++	--	--	--
Pancreas	++	+M	++	+	++	+
Neural (Peripheral)	--	--	+	--	--	--
Thymus	++	+	+	+	--	--
Eye	--	+M	+	--	--	--
Prostate/Seminal Vesicles	++	+	+	--	--	--
Uterus/Ovaries	+	--	+	--	--	--
Lymph Nodes	++	--	--	+	++	++
Spleen	++	--	--	+	++	+
Intestine	--	--	--	--	+	--

-- = No, low or sporadic incidences of histopathological changes
+ = Moderate incidence of histopathological changes, dose-unrelated
++ = High incidence of histopathological changes, dose-related
M = Changes found in males only

Overall, a similar toxicologic profile was noted in rats and baboons following oral or intravenous administration of tacrolimus. Toxicity following intravenous administration was evident at lower doses than with oral administration for both rats and baboons. Toxicity was seen at lower doses in rats than in baboons. The primary end organs of tacrolimus toxicity in rats and baboons were the pancreas, thymus, lymph nodes and spleen (the kidneys were affected in rats only).

Repeat Dose Toxicology Studies (submitted to the NDA):

Repeat Dose Toxicology Study #1:

Twenty-eight day skin irritation study with FR900506 ointment in the New Zealand white rabbit

Study Title:	Twenty-eight day skin irritation study with FR900506 ointment in the New Zealand white rabbit
Study No:	R94-0098-506-P2-E
Amendment #, Vol #:	000, 15
Conducting laboratory:	
Date of study initiation:	3/2/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Approximately 24 hours before treatment commenced, the dorso-lumbar region of each rabbit (~5 cm x 5 cm area) was clipped free of hair using electric clippers. Subsequent shavings were performed as needed. The test substance (0.5 g) was applied by spreading it evenly over the prepared skin. The test article was applied once daily to intact skin for 28 days. The treatment site was unoccluded during treatment. Each animal was fitted with a 'Elizabethan' collar immediately prior to application of the test substance. The test article remained on the back of each rabbit for 6 hours each day. After 6 hours of treatment, the treated skin was washed with warm water and the Elizabethan collars were removed from the animals.

Dosing:

- *species/strain:* New Zealand white rabbits
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 9-13 weeks
- *weight:* 1.9 – 2.3 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (gm/day)	Number of Main Study Animals	
		Males	Females
No treatment Control	0	5	5
Placebo Control	0	5	5
0.1% FR900506 ointment	0.5	5	5
0.3% FR900506 ointment	0.5	5	5
0.5% FR900506 ointment	0.5	5	5

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.1% FR900506 ointment – lot # 66835XK
0.3% FR900506 ointment – lot # 66863XK
0.5% FR900506 ointment – lot# 66953XK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily
- *Body weights:* prior to first dose and then weekly

- *Histopathology:* Animals were euthanized after 28 days of treatment. Samples of skin were removed from the treatment sites of each animal and fixed in 10% buffered formalin for histological analysis by hematoxylin and eosin staining.

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** Dermal irritation (erythema and edema) and skin rash developed in the majority of rabbits in all treated groups (including the placebo control) and persisted to termination. The incidence of the dermal findings was not related to the concentration of FR900506. The least incidence of dermal findings was noted in rabbits in the high dose group. The dermal findings appeared to be related to the vehicle.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Histopathology** Acanthosis, hyperkeratosis and superficial dermal inflammation were detected in all groups when compared to untreated controls. The degree of these changes was comparable in animals treated with placebo and those treated with FR900506 ointment. The effects noted in treated skin were considered to be due to vehicle.

Key Study Findings:

Dermal effects were noted in this study but were related to vehicle since no increase was noted with increased FR900506 in the ointment formulation.

Repeat Dose Toxicology Study #2:

28-day dermal toxicity with FR900506 ointment in the rat

Study Title:	28-day dermal toxicity with FR900506 ointment in the rat
Study No:	GLR950051
Amendment #, Vol #:	000, 10-11
Conducting laboratory:	
Date of study initiation:	6/2/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Approximately 24 hours before treatment commenced, hair was clipped from the dorsal region of each animal. The treatment area was 5 cm x 5 cm. Most of the treatment groups were to intact skin. In the one group that had abraded skin, the skin sites were abraded using the tip of a scalpel blade immediately prior to dosing on day 1 and then on a weekly basis.

Each dose (2 gm/kg/day) was applied to the appropriate dermal test site as a thin uniform layer. The animals were then fitted with a plastic Elizabethan collar for a six hour period. At the end of this period, the collar was removed and the dermal application site was washed with warm water and blotted dry. The treatment site remained unoccluded through out the treatment period. Animals were treated once daily for 28 days.

Dosing:

- *species/strain:* Crl:CD Br VAFD Plus Sprague-Dawley rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 7-8 weeks
- *weight:* 333-386 grams males; 219-252 grams females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Skin site	Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Study Animals	
			Males	Females	Males	Females
Sham Control	Intact	0	10	10	--	--
Placebo Control	Intact	0	10	10	--	--
0.1% FR900506 ointment	Intact	2	10	10	10	10
0.3% FR900506 ointment	Intact	6	10	10	10	10
1.0 % FR900506 ointment	Intact	20	10	10	10	10
1.0% FR900506 ointment	Abraded	20	10	10	10	10

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 66843XK
0.1% FR900506 ointment – lot# 66853XK
0.3% FR900506 ointment – lot# 66863XK
1.0% FR900506 ointment – lot# 66873XK

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily prior to next dose administration
- *Body weights:* weekly
- *Food consumption:* weekly
- *Ophthalmoscopy:* prior to treatment and during week 4

- *Hematology:* during week 4
- *Clinical chemistry:* during week 4
- *Urinalysis:* during week 4
- *Gross pathology:* at sacrifice
- *Organs weighed:* adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes (with epididymides), thyroid and uterus
- *Histopathology:* The following organs were preserved in 10% buffered formalin: adrenals, aorta, brain (medullary, cerebellar and cerebral sections), caecum, colon, duodenum, eyes, esophagus, femur, harderian glands, head, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (cervical and mesenteric), mammary glands, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin (treated and untreated), spinal cord (cervical level), spleen, sternum, stomach, testes (with epididymides), thymus, thyroid (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix), vagina and any macroscopically abnormal tissue.

  All tissues and organs were examined for the sham control, placebo control, 1% FR900506 intact skin and 1% FR900506 abraded skin treatment groups. The kidneys, lungs, pancreas, spleen, thymus and lymph nodes (cervical and mesenteric) were examined for the 0.1%, and 0.3% FR900506 intact skin treatment groups.

- *Toxicokinetics:* Blood samples were obtained from toxicokinetic animals on days 1, 13, 20 and 29. Blood samples obtained on Days 1 and 29 were taken at 2, 4, 6, 8, 12 and 24 hours after dose application (5 rats/timepoint). Blood samples obtained on Days 13 and 20 were taken at 6 and 24 hours after dose application (5 rats/timepoint).

Results:

- **Clinical signs** No treatment related deaths were noted in this study. A general deterioration in the appearance of all animals treated with the 1.0% FR900506 ointment on intact or abraded skin was apparent from Day 13 to termination. The final study report attributed this to a lack of grooming.

- **Local dermal signs** No treatment related dermal effects were noted in this study.

- **Body weights** Toxicologically significant effects on body weight were noted in male animals only. Bodyweight gain over the first week of treatment was significantly lower in male rats that received 1.0% FR900506

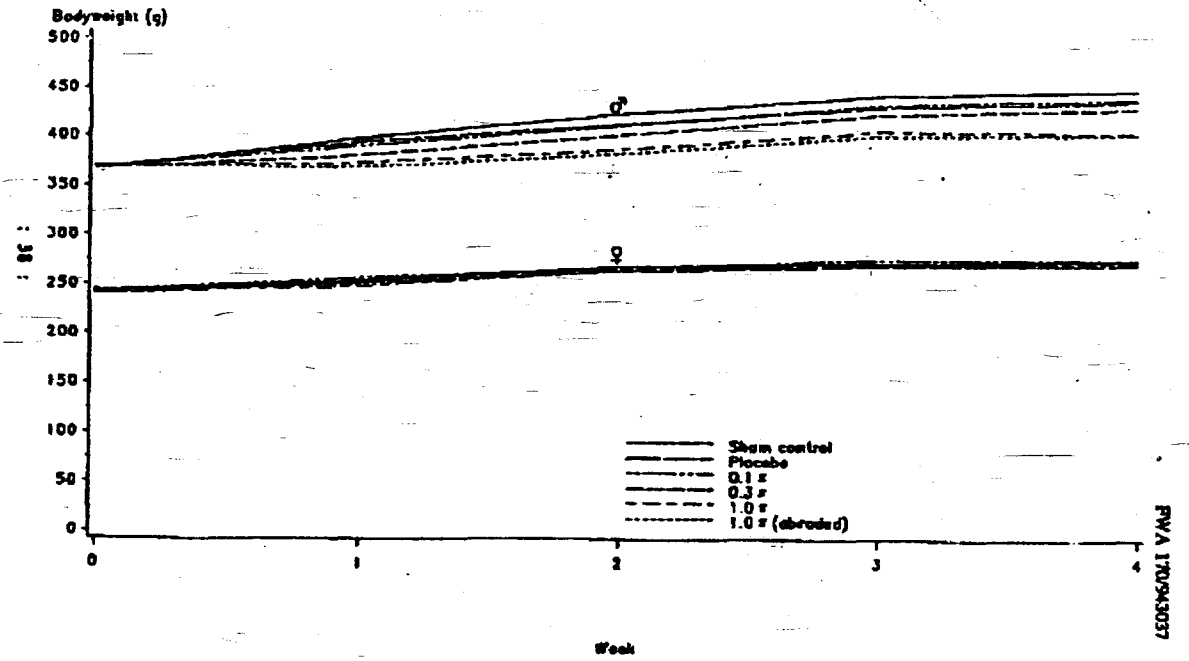
ointment to intact or abraded skin compared to placebo control. Bodyweight gain was decreased to a lesser extent in males treated with the 0.3% FR900506 ointment. Bodyweight gains during week 1 for female rats treated with 1.0% FR900506 to intact or abraded skin were slightly lower than the placebo controls. Refer to figure below copied from the electronic NDA for a graphical representation of the bodyweight data.

The initial significant decrease in bodyweight gain noted in high dose male rats during week 1 was not as significant during the remainder of the study. Bodyweight remained significantly lower than control in the two male high dose groups for the remainder of the study. By the end of the study bodyweight for all other treated male rats (except for the high dose groups) and female rats were comparable to vehicle control.

No significant differences in bodyweight gain was noted between placebo and sham groups.

FIGURE 2

Weekly bodyweights - group mean values



- **Food Consumption** No difference was noted for food consumption in any of the male treatment groups compared to placebo control. Food consumption

was higher in the female high dose groups compared to placebo controls during weeks 2 to 4. Food consumption for rats in the high dose intact skin group was similar to that seen in the high dose abraded skin group.

- **Ophthalmoscopy** No treatment related effects were noted during the ophthalmic examination.

- **Hematology** No significant treatment related hematologic effects were noted in this study. Slightly lower than control mean lymphocyte counts were noted for all treated male and female rat groups compared to placebo control. The slight decrease did not exhibit a dose response relationship.

- **Clinical chemistry** Blood urea nitrogen levels were slightly elevated in male and female rats in both high dose groups (males: ↑25% intact skin and ↑38% abraded skin; females: ↑22% intact skin and ↑33% abraded skin). Cholesterol levels were elevated in male rats only in both high dose groups (↑52% intact skin and ↑54% abraded skin). Slightly lower potassium levels were noted for male and female rats in both high dose groups (males: ↓13% intact skin and ↓18% abraded skin; females: ↓11% intact skin and ↓14% abraded skin).

- **Urinalysis** Higher than control urinary volume was recorded for female rats only in both high dose groups compared to placebo control (↑48% intact skin and ↑24% abraded skin).

- **Organ weights** No treatment related effects were noted for organ weights.

- **Gross pathology** Badly groomed fur was noted for male and female animals in both high dose groups. This finding was noted for occasional animals in the mid and low dose groups.

- **Histopathology**

Treatment related effects were noted in the kidneys. An increased incidence and/or degree of tubular basophilia was seen in males in both high dose groups (8/10 intact and 9/10 abraded) and females in the abraded skin high dose group only (6/10). An increased incidence and degree of foci of mineralization at the corticomedullary junction was seen in females (4/10 intact and 6/10 abraded) in both high dose group and males in the abraded skin high dose group only (4/10).

Treatment related effects were noted in the thymus. Reduced prominence of medulla was noted in low and mid dose male and female rats (males: 8/10 in low and mid dose; females: 9/10 in low and mid dose). Partial absence of medulla was noted in both high dose group male and

female animals (male: 5/10 intact and 8/10 abraded; female: 9/10 intact and 7/10 abraded). Virtual absence of medulla was noted in both high dose group male and female animals (males: 5/10 intact and 1/10 abraded; females: 2/10 intact and 3/10 abraded). A dose related degree of change was noted in the thymus.

Treatment related effects were noted in the cervical lymph nodes. Reduced numbers of germinal centers (males: 6/10 intact and 7/10 abraded; females: 1/10 intact and 2/10 abraded) or reduced prominence of germinal centers (males: 3/10 intact and 3/10 abraded; females 7/10 intact and 8/10 abraded) were seen in both high dose group male and female animals and in one mid dose female animal. No effect was noted in low dose animals.

Treatment related effects were noted in the mesenteric lymph nodes. Reduced numbers of germinal centers (males: 7/10 intact and 10/10 abraded; females: 9/10 intact and 6/10 abraded) were seen in both high dose group male and female animals. Reduced prominence of germinal centers were seen in mid and both high dose male and female animals (males: 1/10 mid, 3/10 intact and 0/10 abraded; females: 1/10 mid, 0/10 intact and 4/10 abraded). No effect was noted in low dose animals.

Treatment related effects were noted in the spleen. Minimal decreased lymphoid cellularity of periateriolar sheath was seen in the majority of both high dose group male animals (6/10 intact and 8/10 abraded). In addition, this effect was noted one female in the intact high dose group and one male and one female in the mid dose group. No effect was noted in low dose animals.

Treatment related effects were noted in the pancreas. Vacolated cells in islets were noted in one mid dose male animal and in both high dose group male and female animals (males: 10/10 intact and abraded; females: 8/10 intact and 10/10 abraded). Apparent reduction in size of islet cells were noted in both high dose group male animals (10/10 intact and abraded). No effect was noted in low dose animals.

Treatment related findings were noted in the lungs. An increased incidence or degree of minor inflammatory lesions (pneumonitis and prominent peribronchiolar and prevascular inflammatory cell infiltration), occasionally with associated prominent goblet cells in bronchiolar epithelium were noted in both high dose group male and female animals. Prominent smooth muscle in alveolar ducts was noted in abraded skin high dose male animals (1/10) and both high dose group female animals (4/10 intact and 3/10 abraded).

Treatment related changes were noted in the skin. An increased incidence of prominent epidermal vacuolation of stratum granulosum was noted in both high dose group male and female animals (males: 5/10 intact and 3/10 abraded; females 2/10 intact and abraded). An increased incidence of acanthosis was noted in both high dose group male and female animals (males: 2/10 intact and 1/10 abraded; females 1/10 intact and abraded). These minor changes were also noted in placebo male and female animals at a lower incidence. These changes were not noted in sham control, low or mid dose group animals.