CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number NDA 50-777

CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW(S)

OCT 2 2000

Clinical Pharmacology/Biopharmaceutics Review

NDA: 50-777 SUBMISSION DATE: 8/10/99, 2/14/00 3/10/00

NDA TYPE: 3S

PRODUCT: PROTOPIC™ (Tacrolimus Ointment, 0.03% and 0.1%)

SPONSOR: Fujisawa Heathcare Inc. REVIEWER: Veneeta Tandon, Ph.D.

NDA Review

I. BACKGROUND

Drug Classification: 3S

Dosage Form: Ointment for topical application (0.03% and 0.1%)

Indication: For the treatment of atopic dermatitis in adults and pediatric patients 2 years of age and older.

Pharmacologic Class: A macrolide immunosuppresant produced by *Streptomyces tsukubaensis* (a soil bacterium found in Mount Tsukuba, Japan). It is also known as FK506. Tacrolimus inhibits the early activation of T-lymphocytes resulting in decreased production of cytokines. Atopic dermatitis is considered to be an immunologic disorder believed to be modified by T-lymphocyte activation.

Dosage and administration: Applied topically twice daily as a thin layer to affected areas of the skin. The use of occlusive dressings is not recommended.

Foreign marketing history: World-wide in both intravenous (Prograf®, NDA 50-708, April 1997) and oral formulations (Prograf®, NDA 50-709, April 1994) for the prevention of organ rejection following allogenic liver or kidney transplantation. 0.1% tacrolimus ointment has been approved for marketing in Japan since June 1999.

Formulation: Tacrolimus Ointment is an oleaginous ointment in which the drug substance is dissolved in droplets of propylene carbonate, which are uniformly dispersed in vehicle. The ointment will be supplied

in 30 and 60 gram tubes. The composition of 0.03% and 0.1% is provided in the following table.

Ingredient	0.03% (%w/w)	0.1% (%w/w)
Tacrolimus	0.03	0.1
White Petrolatum, USP	[redacted]	
Mineral Oil, USP		
Propylene Carbonate, NF		
White Wax, NF		
Paraffin, NF		
Total	100	100

II. RECOMMENDATION

The application is acceptable based on the pharmacokinetic results of the single and multiple dose study in healthy volunteers and adults and pediatric patients with atopic dermatitis. The labeling changes recommended on page 21 of this review along with the comment on analytical validation should be conveyed to the sponsor. Please also refer to the addendum of the review for evaluation of additional studies submitted and the overall conclusions.

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III. ANALYTICAL VALIDATION:

Is the analytical validation adequate for evaluating the concentrations of tacrolimus (FK506) and its metabolites (M-I and M-II)?

Tacrolimus has been analyzed in the blood by two methodologies, both of which have been adequately validated and are summarized in the following section of this review. Blood to plasma partitioning of tacrolimus is saturable and variable, therefore the pharmacokinetics of tacrolimus has been expressed in terms of blood concentration.

Report R94-0150-506-C-E (FK506 in human blood):

[

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In Phase 1 and 2 studies, some subjects that had no exposure to tacrolimus showed measurable concentrations 2-3 times higher than the LOQ, hence ——— was further modified to reduce background noise. The following methodology had a higher limit of detection as compared to the previous report.

Report R97-0070-506-C-E (FK506 in human blood):

[

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Report 1339/1-1010 (FK506 and M-I and M-II in human blood)

[ ]

III. PHARMACOKINETIC STUDIES:

The systemic absorption of tacrolimus ointment (0.03, 0.1, and 0.3%) has been evaluated in healthy volunteers (N=12) as well as adult patients (N=52) and children (N=8) with atopic dermatitis. 0.03 and 0.1% tacrolimus ointments are the to-be marketed strengths with a twice daily treatment regimen. The sponsor has also evaluated the exposure at a higher dose of 0.3%, which would give an understanding to the systemic levels observed at extreme situations. The results of the pharmacokinetic studies are summarized below. The conclusions at the end of each section will answer the questions raised.

In Healthy Volunteers

Is tacrolimus absorbed systemically upon topical application in healthy volunteers as single and multiple doses?

Double-blind, 4-way cross over, randomized and placebo-controlled study with repeated application to investigate the relative bioavailability, tolerance and safety of 3 topical applications of FK506 (0.03%, 0.1%, And 0.3%) to healthy volunteers (Study FG-06-04)

Investigator

Study Design

Tacrolimus ointment or vehicle was applied to a 1000 cm^2 area of back skin of 12 healthy

volunteers (6M & 6F, mean age 31.8 years) once daily for 14 days. Approximately 5g of ointment was applied. To ensure that the volunteers took up the ointment equally, they were asked to remain undressed and abstain from lying down for approximately 0.5 hours after dosing. The subject demographics are attached in the Appendix on page 24. Each treatment period was followed by one week of washout. The randomization was on the basis that each volunteer received the 0.03% tacrolimus ointment before any of the other active treatments.

Serial blood samples were taken on days 1 and 14 of each treatment period for assessment of tacrolimus pharmacokinetics:
Day 1: Predose and 1, 2, 4, 6, 8, 10, 12, and 24 hours postdose
Day 5, 8, 11, 12, 13: Prior to administration (trough concentration)
Day 14: Predose and 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, and 168 hours postdose

Tacrolimus whole blood concentrations were measured by ——— assay (LOQ: — ng/mL).

Observations

The individual subject blood concentration of tacrolimus following administration of 0.03, 0.1 and 0.3% tacrolimus ointment is attached in the Appendix on pages 25-27.

- Following 0.03% tacrolimus ointment administration, whole blood concentrations of tacrolimus were detectable in only one subject starting Day 11 (range: ——— ng/mL). Blood concentrations were detectable up to 120 hours after the final application.

- Measurable blood tacrolimus concentrations (range: ——— ng/mL) were obtained at sporadic timepoints of the sampling schedule in 4 subjects at 0.1%. The maximum concentration of — ng/mL was observed on predose on Day 12 of the study. No pharmacokinetic evaluations were possible for the 0.03% and 0.1% administration periods since a complete concentration-time profile was not obtained for any subject.

- With the 0.3% tacrolimus ointment, only a few measurable concentrations were obtained over the sampling schedule for 6 subjects (range ——— ng/mL); in one subject, a concentration profile was obtained thus providing data for further analysis.
  In this subject, the terminal half-life estimated from blood tacrolimus concentration data obtained 24-120 hours after the last dose was 68.6 hours; the maximum whole blood concentration (C_{max}) was 0.127 ng/mL; the T_{max} was 2 hours; estimated area under the concentration-time curve estimated to infinity (AUC_{0-inf}) was 11.5 ng·h/mL.

- Tacrolimus metabolites (MI and MII) concentrations were also evaluated (LOQ: — ng/mL). The concentrations of both metabolites were below the limit of detection at all time points, at all concentrations of tacrolimus.

Reviewer's Comment

The LOQ used in this study was ~ pg/mL. Tacrolimus blood concentrations as reported in Tables (Pages 22-24) show several samples as — pg/mL. However there are concentrations within brackets as low as 1 pg/mL. When the calibration range for the assay was —— pg/mL, it was unclear how the sponsor could quantitate low levels as 1 pg/mL. Upon additional request the sponsor stated that the numbers within the brackets are at the limit of detection. However, the standard curve or the calibration range does not justify the ability to detect quantities as low as 1 pg/mL. Any values reported below the limit of quantitation have no regulatory significance.

Conclusions

- The results indicate that systemic availability of tacrolimus in healthy volunteers following topical administration of ointment at 0.03%-0.3% strengths was sporadic (blood levels in 1/12 volunteers using 0.03%, 4/12 using 0.1% and 6/12 using 0.3% ointment). The highest concentration seen was — ng/mL (LOQ: - pg/mL).
- The pharmacokinetic parameters could be estimated from only one subject applying 0.3% tacrolimus ointment.
- The metabolites M-I and M-II were not detected in any volunteers (LOQ: — pg/mL)

<u>In adult patients and children with atopic dermatitis</u>

- **Is tacrolimus absorbed systemically upon topical application of single and multiple doses in adult patients and children with atopic dermatitis?**
- **Is there any accumulation after multiple dosing?**
- **Is the systemic aborption similar in children and adults?**
- **Is the systemic absorption similar when the ointment is applied to different parts of the body?**
- **Is the systemic absorption more in patients as compared to healthy volunteers?**

The sponsor has conducted two studies in patients with atopic dermatitis with single as well as multiple applications of the ointment using the 0.1% and 0.3% strengths. The two studies are summarized here, followed by the conclusions obtained. The conclusions at the end of this section will answer the questions raised.

Pharmacokinetics of Tacrolimus 0.3% in adult and pediatric patients with atopic dermatitis following the topical administration of tacrolimus ointment for 8 days. (Protocol 94-0-008)

Investigator

[redacted]

The pharmacokinetics of tacrolimus in patients was assessed in an open label, single and repeat application study. The population was adult (N =31: 16M & 15F, ≥ 13 years of age) and pediatric (N = 8, <13 years of age). The treatment period was 8 days followed by a 3-day washout phase. 0.3% Tacrolimus ointment was applied once daily on Days 1 and 8 and twice daily on Days 2 through 7. The demographics are attached in the Appendix on page 28.

Treatment assignments were made on the basis of patient age, disease location and area of involvement.

Treatment Group	Age Range (Years)	N	Location of Disease	Amount 0.3% Ointment per Application (g)	Area of Application (cm^2)	Tacrolimus Exposure per Application (mg)
Adult A	14-39	6	Trunk/limbs	0.5	100	1.5
B	24-61	7	Face	0.5	100	1.5
C	18-64	6	Trunk/limbs	2.5	500	7.5
D	25-75	6	Trunk/limbs	5.0	1000	15.0
E	14-32	6	Trunk/limbs	15	5000	45.0
Pediatric	5-6†	4	Trunk/limbs	0.25	50‡	0.75
	7-11	4	Trunk/limbs	0.5	100	1.5

†Patients 3-6 years of age were allowed by the protocol, but no one <5 years of age enrolled.
‡For the first application, ointment was inadvertently applied over 100 cm^2 in two patients.

In the adult treatment groups, blood samples for pharmacokinetic analysis were collected just before application and at 1, 2, 4, 6, 8, 12, and 24 hours after application on days 1 and 8; just prior to application (i.e., trough levels) on days 2 through 7; and at 48 and 72 hours after the last application on day 8. In pediatric patients, blood samples were obtained prior to application and at 1, 4, 8, and 24 hours after application on days 1 and 8; just prior to application on days 3 and 6; and 72 hours after the last study application on day 8. Blood tacrolimus concentrations were determined by [redacted] (LOQ: [redacted] ng/mL).

The pharmacokinetic parameters (Mean ± SD) in the adult and children are shown in the following table along with the concentration time profile for the 5 adult patient groups. The individual subject blood levels and pharmacokinetic parameters are attached in the Appendix on pages 29-40.

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.

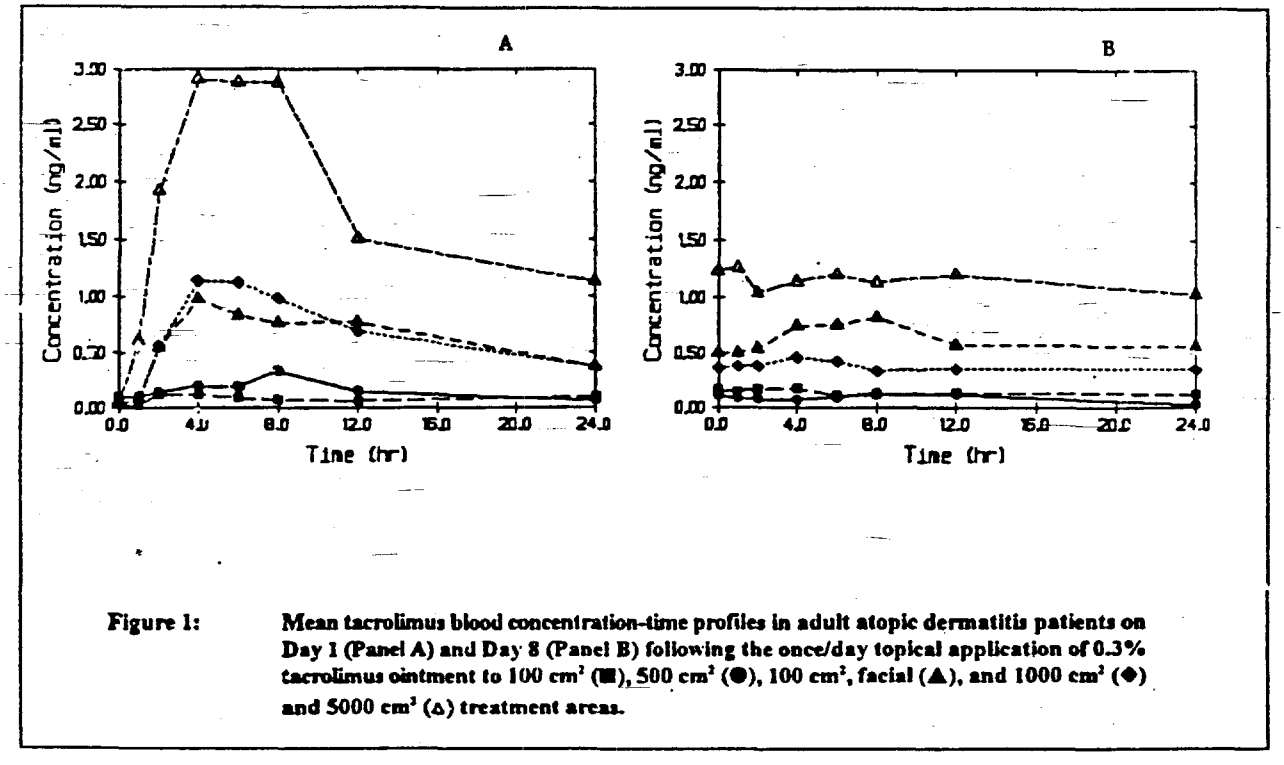


Figure 1: **Mean tacrolimus blood concentration-time profiles in adult atopic dermatitis patients on Day 1 (Panel A) and Day 8 (Panel B) following the once/day topical application of 0.3% tacrolimus ointment to 100 cm² (■), 500 cm² (●), 100 cm², facial (▲), and 1000 cm² (◆) and 5000 cm² (△) treatment areas.**

The mean tacrolimus blood concentration-time profile in pediatric patients on Day 1 and 8 are shown in the following figures.

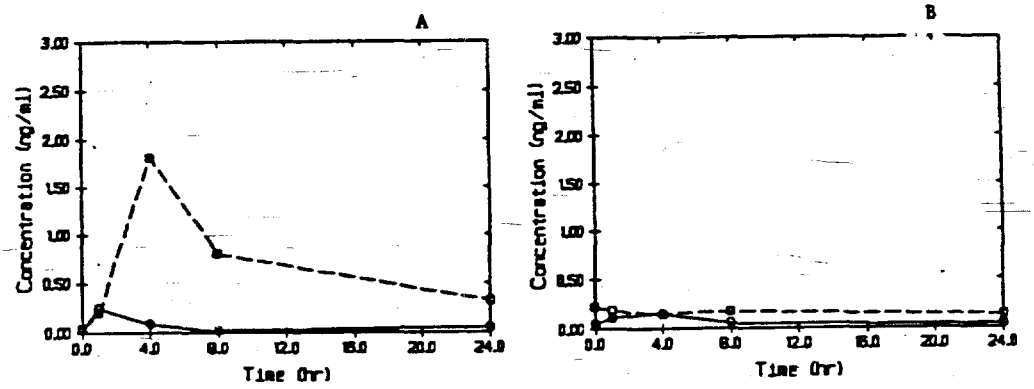


Figure 2: **Mean tacrolimus blood concentration-time profiles in pediatric atopic dermatitis patients on Day 1 (Panel A) and Day 8 (Panel B) following the once/day topical application of 0.3 tacrolimus ointment to 50 cm² (□) and 100 cm² (○) treatment areas.**

Observations

- Although variable, systemic exposure on Day 1 and 8, as measured by the AUC_{0-24}, tended to increase as the treatment area increased when applied to trunks and limbs in both adults and children.

- Day 1 and 8 area under the concentration-time curve (AUC_{0-24}) values were disproportionately higher in adults with facial lesions compared to adults with the same area treated on the trunk/limbs, suggesting site specific permeability.

- On Day 1 AUC_{0-24} values were higher in the four children aged 5-6 years compared to patients in the other groups

Reviewer's Comment

- *For the first application, ointment was inadvertently applied to over 100 cm² in two of the four patients, instead of 50 cm². However, these concentrations were lower than another patient who received the correct dose (see page 30). Hence, the effect of treatment area error on absorption is difficult to ascertain. With only four subjects it is difficult to derive the overall conclusion.*
- *The sponsor has not conducted the study in pediatric patients lower than 5 years of age, however, some information in children between the ages 2-5 is available from the Phase III clinical studies where random blood samples have been analyzed and are discussed on page 15 of the review.*

- AUC_{0-24} values tended to be lower on Day 8 relative to corresponding day 1 values, with the exception of adults treated on the trunk/limbs (area of application 500 cm^2) and children (7-11 years, area of application 100 cm^2).

- The highest concentration observed was — ng/mL (5000 cm^2 exposure area) in one patient (see Appendix page 40).

- More adverse events were seen (100%) when tacrolimus was applied on the facial and neck parts of the body. These group of patients also showed the maximum systemic absorption of tacrolimus.

Tacrolimus bioavailability was estimated by comparison of mean AUC_{0-24} after topical administration relative to historical mean AUC_{0-24} from normal adult volunteers who were administered intravenous and oral tacrolimus.

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.

The relationship of mean Day 1 and 8 AUC_{0-24} to dose of tacrolimus applied on a mg/sq.m basis is shown in the following figure.

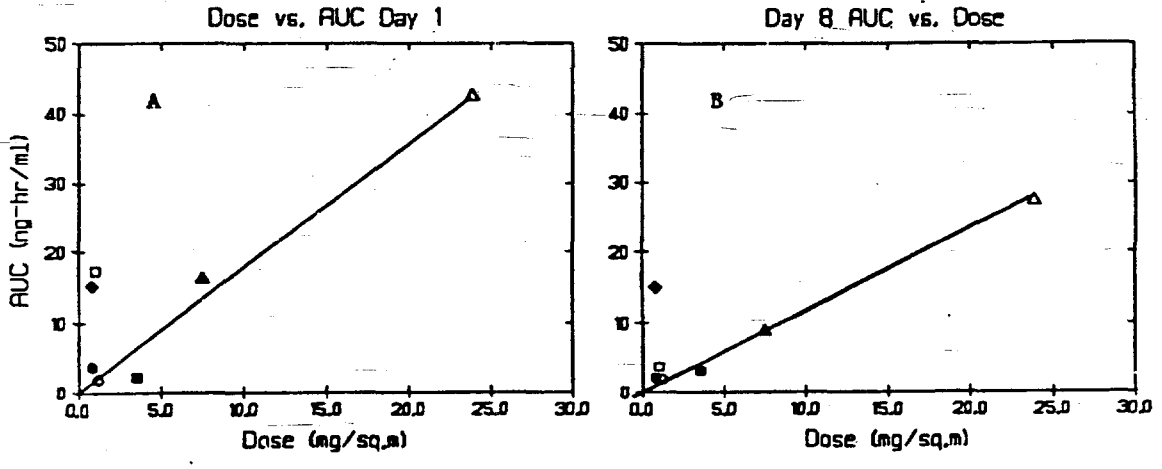
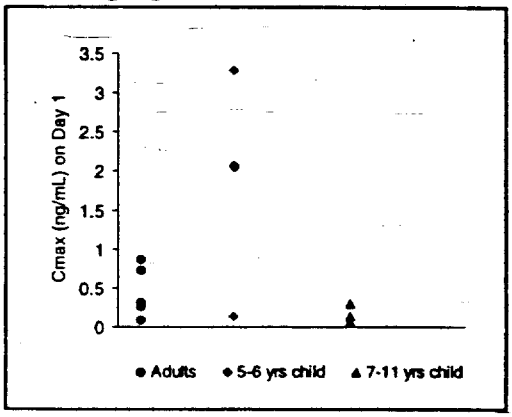


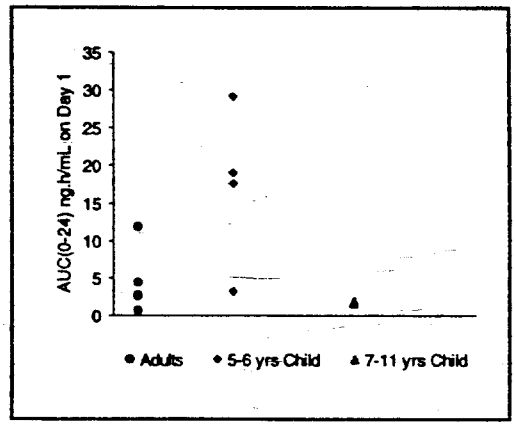
Figure 3: **Mean Day 1 (Panel A) and Day 8 (Panel B) AUC_{0-24} plotted as function of tacrolimus applied. Group 1, 100 cm^2 (●), Group 2, 500 cm^2 (■), Group 3, 100 cm^2 facial (◆), Group 4, 1000 cm^2 (▲), Group 5a, 100 cm^2 (○), Group 5b, 50 cm^2 (□), Group 6, 5000 cm^2(△).**

This shows that facial application area group did not fall in the linear range on both the days.

Comparison between adult and children receiving comparable doses:

From the given adult groups, Group A was chosen for comparison because the older children (7-11 yrs) and the adults had the same area of application (100 cm^2 to the trunks and limbs) and same exposure of tacrolimus per application (1.5 mg). The younger children had both the area of application and the amount of tacrolimus per application that were half of the previous groups (ie. 50 cm^2 and 0.75 mg, respectively). The Cmax and AUC(0-24) hour have been compared graphically for the three groups in the following figures.





From the figure it is clear that the older children (7-11 years) receiving the same dose as that of the adults have similar AUCs and Cmax's, with a trend towards are lower Cmax. But the younger children (5-6 years) who have been exposed to half the dose of the adults have Cmax and AUCs, which are 2-3 times higher than adults and older children.

Conclusions

- Tacrolimus ointment 0.3% is systemically absorbed in adult patients and children. The $AUC_{0\text{-}24}$ values were highly variable (0.9-42.5 ng/hr/mL).

- It appears that atopic dermatitis lesions on the trunk and limbs in older children (7-11 years) and adults have comparable permeability characteristics of tacrolimus. Atopic dermatitis lesions on trunk and limbs of younger children (5-6 years) appear to be more permeable than those of older children and adults, leading to higher exposure and Cmax values.

- Face and neck lesions in adults are more permeable to those on the trunks and limb lesions of adults. Face and neck regions in children have not been evaluated.

- Trough blood concentrations on Days 2-7 (pages 26-31) and lower AUCs on Day 8 indicate that no appreciable accumulation of systemic tacrolimus occurred over the 8 days of the study. However, in children (7-11 years) and in adults with an area of application of 500 cm^2, the Day 8 concentrations were higher than that of Day 1. In adults where tacrolimus ointment was applied on the face, the Day and 8 concentrations were similar (15.2 and 14.9 ng.h/mL, respectively)

- Decreased absorption on Day 8 may be a consequence of improvement of skin condition over the treatment period.

- Comparison of historical systemic data indicated that the bioavailability of topical tacrolimus relative to oral administration is less than 5% and the absolute bioavailability is less than 0.5%.

- The systemic absorption of tacrolimus is more in patients as compared to healthy volunteers. The maximum concentration observed in a healthy volunteers applying 0.3% tacrolimus ointment was [redacted] ng/mL, where as the maximum concentration seen in patients receiving the same dose was [redacted] ng/mL (5 g of 0.3% in 1000 cm^2 area of exposure- Adult Group D).

Phase 2 Pharmacokinetic and safety confirmation study of Tacrolimus ointment (0.1 and 0.3%) in patients with atopic dermatitis (Study FJ-106)

The systemic absorption and safety of tacrolimus ointment after single and multiple applications to extensive area of skin (precise area for subjects not mentioned) was evaluated in this study in 21 adult atopic dermatitis patients. The list of investigators and centers for this study is attached in the Appendix on page 41. The study was divided into 6 stages in a dose-escalation design as shown in the following table.

Study method	Stage	Concentration of FK506 ointment	Amount applied	Amount of FK506 applied
Single application (for 12 hours)	1	0.1%	1.25g/time	1.25mg
	2	0.3%	1.25g/time	3.75mg
	3	0.1%	5g/time	5mg
	4	0.1%	10g/time	10mg
Multiple application (for 7 days twice daily)	5	0.1%	5g/time	5mg×14times
	6	0.1%	10g/time	10mg×14times

For patients who received a single application, blood samples were drawn for tacrolimus concentration measurement at 3, 6, 9, 12, 24, 36, 48, and 72 hours post application. For patients who received twice daily application for 7 days, blood samples were drawn just before the next application on days 1, 3, 6, 7, and 10 (day of first dose=day 0).

Blood levels after single application of tacrolimus ointment

Stage	FK506 ointment: Concentration / amount applied	Amount of FK506 applied (mg)	Case No.	Blood concentration (ng/mL): 3 hrs	6 hrs	9 hrs	12 hrs	24 hrs	36 hrs	48 hrs	72 hrs	Adverse drug reactions / abnormal changes in clinical laboratory test values
1	0.1% / 1.25g	1.25	1-1	[redacted]								—
			1-2									Burning sensation (Mild)
			1-3									Burning sensation (Mild)
2	0.3% / 1.25g	3.75	2-1									Burning sensation (Moderate) /γGTPT
			2-2									—
			2-3									—
			2-4									—
3	0.1% / 5g	5	3-1									Flush (Moderate)
			3-2									—
			3-3									Flush (Moderate)
4	0.1% / 10g	10	4-1									Flush / itching (Moderate)
			4-2									Sensation of irritation (Mild)
			4-3									Flush (Mild)

N.D.: Below detection limit [redacted] ng/mL)

Maximum tacrolimus blood concentrations after single application ranged between [redacted] ng/mL, with the exception of one subject with a 10 g dose showing a maximum concentration of [redacted] ng/mL. This concentration reached to [redacted] ng/mL at the end of 72 hours. Maximum blood concentrations were reached between 3-6 hours.

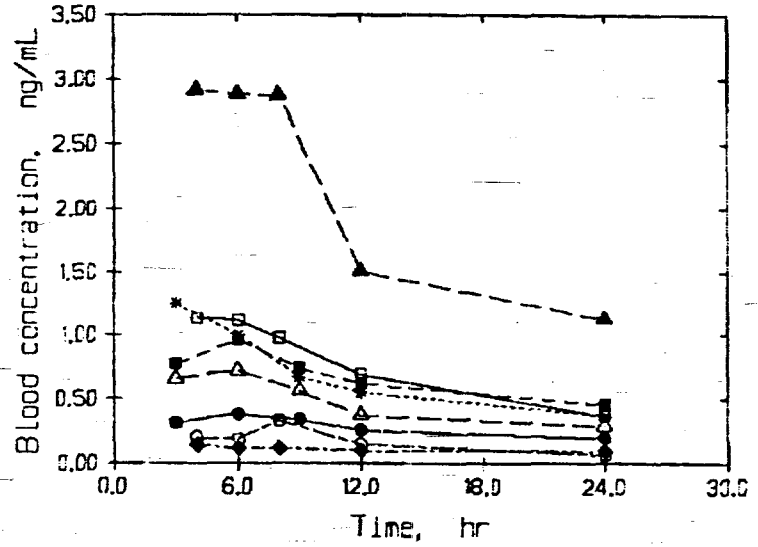
Blood levels after multiple applications (twice daily for 7 days) of tacrolimus ointment:

Stage	FK506 ointment: Concentration / amount applied / application period	Amount of FK506 applied (mg×times)	Case no	Blood concentration (ng/mL): 1 day after	3 days after	6 days after	7 days after (1 day after completion)	10 days after (4 days after completion)	Adverse drug reactions / abnormal changes in clinical laboratory test values / infections
5	0.1 % 5g/time (Twice daily) 7 days	5×14	5-1*	[redacted]					Flush (Mild)
			5-2**						Burning sensation (Moderate)
			5-3						Heat (Mild)
			5-4						Flush / itching (Mild)
									Folliculitis (Mild)
			5-5						Flush (Mild)
6	0.1 % 10g/time (Twice daily) 7 days	10×14	6-1						Heat / hypererythema (Moderate)
			6-2						Flush (Moderate)
			6-3						Burning sensation (Mild) Itching (Moderate) Folliculitis (Mild)

N.D.: Below detection limit [redacted] ng/mL)
*: Discontinued after 4-day application due to patient desire based on symptom improvement
**: Discontinued after 2-day application due to the occurrence of hepatic disorder attributable to antibiotics

Maximum blood concentrations after multiple applications ranged between [redacted] ng/mL with the exception of one subject showing a level of [redacted] ng/mL after one day of application (10 g, twice daily) of tacrolimus ointment. His level decreased t[redacted] ng/mL 7 days after application of the medication. No causal relationship was observed between the blood concentration of tacrolimus observed at various time points and the degree of side effects observed.

A comparison of the concentration-time profiles of tacrolimus in atopic dermatitis patients in U.S. Study 94-0-008 and the Japanese FJ-106 study is illustrated in the following Figure. As shown in the figure, the shapes of the concentration-time curves are in accordance to the doses applied in the two populations. The highest concentration was the U.S. group that received the equivalent of 45 mg of tacrolimus (i.e., 4.5 times the highest amount applied in the Japanese population).



Study	Symbol	Amount of Ointment Applied (g)	Amount of Tacrolimus Applied (mg)
FJ-106	●	1.25	1.25
	△	1.25	3.75
	■	5	5
	*	10	10
94-0-008	○	0.5	1.5
	◆	2.5	7.5
	□	5	15
	▲	15	45

APPEARS THIS WAY
ON ORIGINAL

Mean tacrolimus blood concentrations after the initial topical application of the following amounts of ointment to adult Japanese atopic dermatitis patients (Study FJ-106) or adult Caucasian atopic dermatitis patients (Study 94-0-008). N=3/group in the FJ-106 study; N=6/group in the 94-0-008 study.

Conclusions

- Once again, the blood concentration of tacrolimus was higher in patients as compared to healthy volunteers. Healthy volunteers receiving the same dose showed a maximum concentration of [redacted] ng/mL, whereas one of the patients showed a maximum concentration of [redacted] ng/mL (5 g of 0.1% ointment over extensive area)

- By using the two studies in patients combined, the blood concentration profiles for tacrolimus were in accordance to the doses received.

Comparison of Tacrolimus concentrations in blood during Japanese, European and United States clinical trials

Mean blood concentrations were measured during the Phase 2 and 3 clinical trials after 1 week, 3 or 4 weeks, 3 months, 6 months and 1 year of tacrolimus ointment application. Various clinical trials were conducted with 0.03%, 0.1% or 0.3% tacrolimus ointment.

Adult Patients

The maximum concentration in 7 clinical trials (# 97-0-035, # 97-0-036, #FG-06-12, #FJ-111, #95-0-013, #FG-06-01 and # FJ-103) in adults patients ranged from ——— ng/mL . The distribution of patients with maximum blood concentration is shown in the following table.

Study	Ointment Conc.	N*	Number of Patients With the Indicated Highest Individual Concentration (ng/mL)			Maximum Concentration (ng/mL)
			≤0.5	0.5 – <5	≥5	
97-0-035†#	0.03%	98	74	23	1	
	0.1%	91	55	36	0	
97-0-036†	0.03%	97	63	33	1	
	0.1%	102	59	42	1	
97-0-037† (pediatric)	0.03%	25	22	3	0	
	0.1%	30	24	6	0	
FG-06-12‡	0.1%	311	155	155	1	
FJ-111‡	0.1%	562	217	324	21	
95-0-003‡ (pediatric)	0.03%	42	40	2	0	
	0.1%	45	39	6	0	
	0.3%	42	32	10	0	
95-0-009‡ (pediatric)	0.03%	11	6	5	0	
	0.1%	12	3	8	1	
95-0-013‡	0.03%	7	4	3	0	
	0.1%	6	1	5	0	
	0.3%	7	2	4	1	
FG-06-01§	0.03%	44	38	6	0	
	0.1%	46	27	19	0	
	0.3%	42	23	19	0	
FJ-103‡ (acute AD)	0.03%	11	11	0	0	
	0.1%	9	8	1	0	
	0.3%	7	5	2	0	
(chronic AD)	0.03%	5	4	1	0	
	0.1%	4	4	0	0	
	0.3%	4	4	0	0	
TOTAL		1660	920	713	27	
			55%	43%	1.6%	

Pretreatment values (baseline, screening) are excluded.

*N: Patients who were treated and had tacrolimus blood concentrations measured. AD: Atopic dermatitis. LOQ: limit of quantitation. †LOQ — ng/mL; ‡LOQ — ng/mL; §LOQ — ng/mL

#One 15-year-old was enrolled in this adult study.

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Pediatric Patients

The maximum concentration seen in 3 clinical trials (#97-0-37, #95-0-009, #95-0-003) conducted in pediatric population showed maximum concentrations of tacrolimus between ——— ng/mL.

In the following table tacrolimus blood concentration ranges for the younger children (2-6 years) on the various study days/weeks from two clinical trials have been summarized. This was done because the PK study was not conducted in children lower than 5 years of age and the younger group (2-6 years) showed higher tacrolimus concentration that the older group and adults.

Study 95-0-009 (Limit of detection of detection of blood samples — ng/mL)			
Age	Study Day	0.03% ointment	0.1% ointment
		Tacrolimus blood concentration ranges	
2		-	-
3	1 4/5 8 15/22/29	- (N=0) - - -	[redacted] (N=6)
4	1 4/6 8 22/27/29	[redacted] (N=4)	(N=4)
5	1 4 8 22/29	(N=3)	(N=2)
6	1 4/6 8 22/29	(N=4)	(N=1)

% BSA varied from 6% to 100%. The subject with 100% BSA had a level of — ng/ml on Day 1 and — ng/mL on Day 5 evaluation.

Study 97-0-0379 (Limit of detection of detection of blood samples — ng/mL)			
Age	Study Day	0.03% ointment	0.1% ointment
		Tacrolimus blood concentration ranges	
2	1 8 22/26 83/85/96	[redacted] (N=4)	[redacted] (N=4)
3	1 8 22/26 83/85/96	(N=6)	(N=4)
4	1 8 22/26 83/85/96	(N=1)	(N=7)
5	1 8 22/26 83/85/96	(N=2)	(N=2)
6	1 8 22/26 83/85/96	(N=3)	(N=1)

This study had used ——— method with a higher limit of detection — ng/mL), hence most samples were below the limit of detection and are reported as "0.00"

Th·· the overall distribution of the number of pediatric patients in each category of the younger group is as follows that had been treated with the to-be-marketed 0.03% and 0.1% tacrolimus ointment. In addition to these 58 pediatric patients in the younger pediatric age group there were 4 more in the PK study 94-0-008 with 0.3% tacrolimus ointment:

2 years old,	N=8
3 years old,	N=16
4 years old,	N=16
5 years old,	N=9
6 years old,	N=9

In the 120-day safety update of the submission, study report of study#FG-06-19 (Phase 3) was submitted. This study was conducted in 560 pediatric patients between the ages 2 to 15 years. Few pediatric patients (N=31) study showed extremely high concentrations, with the highest value of — ng/mL. Few other extremely large concentrations observed were ——— ng/mL. These concentrations are much higher than that obtained after oral or intravenous — ng/mL) administration of tacrolimus. Hence, the sponsor explored the reasons of these implausible concentrations of tacrolimus seen in these children.

Evaluation of data revealed that 88% (1737/1972) of samples assayed in this study were "normal volume" blood samples that could be used for the assay and 12% (235/1972) were "low volume" blood samples available for analysis. All these samples showed concentrations >5ng/mL. The sponsor proposes that these samples are quite likely the result of blood collection technique. Obtaining blood samples with venipuncture was not always possible in children. Alternatively, a "finger tick" technique was used, in which a lancet was used to prick the tip of the finger, then the distal end of the bleeding finger was massaged to extract several drops of blood. This resulted in only a small volume for assay (≤0.4 mL) for assay. The finger tips were used to apply the ointment and the patients could have also scratched the affected parts of their body with their finger tips, which may result in potential contamination of blood samples collected from this area.

Based on the pharmacokinetics of tacrolimus and the parameters observed after intravenous and oral doses (0.05 mg/kg and 0.3 mg/kg/day, respectively), these high values seem unrealistic.

Another explanation supporting these values being unrealistic is the absence of metabolites (M-I and M-II) in these samples. If these high concentrations were due to systemic levels then the metabolites would be measurable in these high concentration tacrolimus samples (i.e. low volume samples). The evaluation of normal volume blood samples (N=28, tacrolimus concentrations ≥ 1 ng/mL, maximum — ng/mL) indicated a clearly measurable presence of one or both metabolite in most samples. In contrast only

trace levels of M-I were present in low volume samples, with no evidence of M-II. If the high tacrolimus concentrations were real and indicative of systemic exposure, much higher levels of metabolites would have been observed. A histogram showing the metabolite to tacrolimus ratio is attached in the Appendix on page 46.

V. IN VITRO STUDIES

In Vitro percutaneous absorption of Tacrolimus through human cadaver skin:

The tacrolimus ointment clinical supplies in the US and European Phase I and II studies were manufactured at [redacted] Clinical supply for the Phase III clinical studies were manufactured at FHI, Inc. Grand Island, NY. The equivalence of the tacrolimus ointment, following a manufacturing site change was evaluated using in vitro methodology of assessing percutaneous absorption through human cadaver skin in [redacted] diffusion cells.

Human cadaver skin from 6 donors dermatomed to approximately 0.25 mm was used. Tacrolimus concentrations were measured by [redacted]. Overall mean total penetration (±SE) of tacrolimus over 48 hours was 16.9±3.90% and 17.2±3.71% of the amount applied with the 0.03% ointment and 8.1±0.76% and 8.2±0.85% with the 0.1% ointment for the [redacted] and Grand Island ointments, respectively. The overall total penetration and flux for each lot of 0.03% and 0.1% ointment is attached in the Appendix on pages 42-43.

The overall mean flux of tacrolimus after a single application of the two ointments (0.03% and 0.1%) are shown in then following figures.

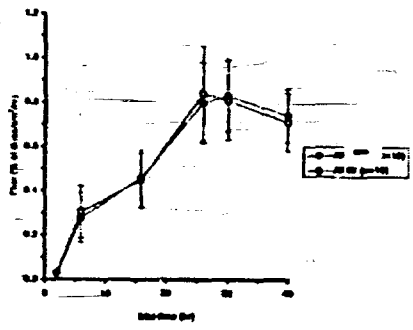

Figure 2 [illegible]

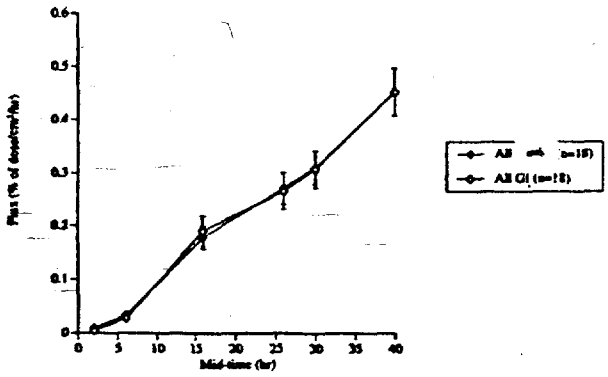

Figure 2 Overall mean flux of FK506 after a single application in 0.1% [redacted] and GI ointment. Data represent mean values ± standard error.

Conclusions

The in vitro percutaneous absorption appears to be similar for tacrolimus ointment manufactured by two facilities.

In Vitro Metabolism Of ^{14}C-Tacrolimus By Human Skin Homogenates

Two major metabolites of tacrolimus have been found in earlier studies, the two being M-I (13 O-demethylated product) and M-II (31-O-demethylated product). M-II has same activity as that of tacrolimus. The metabolism is proposed to be CYP 3A mediated from earlier studies. The sponsor has conducted an in vitro experiment to assess the metabolism of tacrolimus in human skin.

^{14}C-tacrolimus was incubated at 37°C in the presence of NADPH with freshly prepared skin homogenates obtained from the skin of two female donors for 60 minutes. Determination of marker enzyme activities was done with the confirmation of the presence of phenylacetate esterase and cytochrome c reductase activities. Incubates were then extracted with ethyl acetate and a mass balance for the system was determined. The extracts were assayed by [redacted] for total ^{14}C and by [redacted] for tacrolimus and its metabolites M-I and M-II.

One peak, attributed to tacrolimus was present in the extract and the recovery of radioactivity in the incubates was complete. M-I and M-II metabolites were not detected. The total recovery of radioactivity from human skin homogenates in each incubation has been attached in the Appendix on pages 44-45.

Conclusion

In vitro studies suggest that Tacrolimus is not metabolized in human skin.

VI. OVERALL CONCLUSIONS

Systemic absorption of topical tacrolimus, comparison in adult and pediatric patients:

- Tacrolimus is systemically absorbed upon topical application of 0.03, 0.1 and 0.3% ointment in adult patients as well as children (5-11 years) with atopic dermatitis.

- The sponsor has evaluated the systemic absorption of tacrolimus upon topical application in extreme situations using 3 times the to-be marketed dose over a range of body surface areas (0.5, 2.4 and 27% BSA in adult and 0.7 and 0.8% BSA in children). The formulations used in these studies were the to-be-marketed formulation. The lot numbers used in the studies are attached in the Appendix on page 47.

- The $AUC_{0\text{-}24}$ values were highly variable (0.9-42.5 ng.hr/mL in adults and 1.5-29.2 ng.h/mL in children treated with 0.3% tacrolimus ointment).

- The amounts of tacrolimus applied to adults were 21 mg, 105 mg, 210 mg and 630 mg, whereas the amount applied to children were 10.5 and 21 mg (from the 0.3% strength of ointment) and 1.25 to 140 mg (from the 0.1% strength of ointment).

- The C_{max} observed in adult patients was 9.42 ng/ml (from study 94-0-008 with 0.3% tacrolimus) and [redacted] ng/mL (in one patient from study FJ-106 with 0.1% tacrolimus single dose and multiple dose for 7 days) and that observed in pediatric patients was 3.28 ng/mL. The C_{max} observed in adult patients from clinical trials was 14 ng/mL (from study FJ-111) and that in pediatric patients was 9.58 ng/mL (from study 95-0-009)

- The younger children (5-6 years) had higher blood levels of tacrolimus as compared to older children (7-11 years) and adults, when treated on the same body parts (trunk and limbs). The C_{max} observed in a child in the younger group has been 3.28 ng/mL on Day 1 receiving 0.3% ointment on a 50 cm^2 treatment area and the highest AUC_{0-24} was 29.2 ng.h/mL. No obvious differences were seen when the samples from the clinical trials for evaluated closely for the tacrolimus blood levels in the younger children (ages 2-6 years)

- There was no significant accumulation of tacrolimus after twice a day dosing for a week. Drug concentrations on Day 8 were generally lower than that of Day 1. These may be a consequence of improved skin condition during therapy. However, the older children (7-11 years) had twice the C_{max} and AUC_{0-24} on Day 8 as compared to Day 1.

- The pharmacokinetic studies have not been conducted under occlusion.

Systemic absorption in patients with atopic dermatitis vs. healthy subjects:

- The systemic absorption is more (10 times) in patients as compared to healthy volunteers.

Systemic absoprtion; treatment site dependence:

- Tacrolimus ointment was more permeable through atopic dermatitis lesions on the face and neck regions as compared to the trunk and limb regions. The effect of treatment site has not been evaluated in children, only the trunks and limbs have been studied.

- Systemic exposure was the same on Day 1 and Day 8, when tacrolimus ointment was applied on the facial lesions, whereas tacrolimus concentrations were lower on Day 8 when applied to the trunks and limbs.

Systemic absorption and comparison to oral bioavailability:

- The oral absolute bioavailability of tacrolimus is 15-25% (highly variable) and the absolute bioavailability of topical tacrolimus based on historical IV data is 0.5%. The maximum concentration observed with a 0.3 mg/kg/day PO dose was 68.5 ± 30 ng/mL and $AUC_{0-\infty}$ was 519 ± 179 ng.hr/mL in liver transplant adult patients (Data from NDA 50-708). In kidney transplant adult patients receiving an PO dose of 0.2mg/kg/day the $AUC_{0-\infty}$ was 203± 42 ng.hr/mL with a Cmax of 19.2 ±10.03 ng/mL. The maximum concentration in any of the three pharmacokinetic Phase 1 and 2 study

of tacrolimus topical application has been – ng/mL (N=2 subjects, receiving 10 g of 0.1 as a single dose or twice daily for 7 days). The maximum calculable AUC observed has been 42.5 ± 37.1 ng.hr/mL with 0.3% drug exposed to 27% of the body surface area of adult, which is 12-fold lower than that of liver transplant patients and about 5-fold lower than that of kidney transplant patients.

Metabolism of tacrolimus on topical application:

- The metabolites of tacrolimus were not detected in healthy volunteers. The sponsor has not evaluated the presence of metabolite in patients. However, in the clinical study FG-06-19 in pediatrics the levels of metabolites have been evaluated and submitted as an update of 120-day safety update. With the lower concentrations of tacrolimus observed upon topical administration, as compared to oral tacrolimus, this should not be of concern. In vitro studies indicated that tacrolimus is not metabolized in human skin.

VII. LABEL

The following labeling changes in the "pharmacokinetics" section of the label proposed by the sponsor are recommended.

Pharmacokinetics

VIII. COMMENTS (to be sent to the sponsor)

1. The lower limit of the quantitation (LOQ) used for a particular assay is the cutoff point for assessing drug levels. Reporting drug levels below the LOQ and outside analytical validation range are of no regulatory significance. Such practices should be avoided in future.

/S/ 9/25/06

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Pharmacokineticist
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Team Leader: E. Dennis Bashaw, Pharm. D. /S/ 10/2/06

CC: NDA 50-777 (ORIG)
HFD-540/Div File
HFD-540/CSO/Wright
HFD-880(Bashaw/Tandon)
HFD-880(Lazor)
HFD-344(Viswanathan)
CDR ATTN: B.Murphy

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APPENDIX

NDA 50-777

Study FG 06-04 (Healthy Volunteers)

Summary Statistics for Demographic Data

Variable	Sex	N	mean	s.d.	min.	median	max.
Age (years)	Female	7	32.43	5.35	25.0	31.0	41.0
	Male	7	31.14	5.67	22.0	34.0	36.0
	Total	14	31.79	5.34	22.0	32.5	41.0
Height (cm)	Female	7	169.86	4.71	163.0	168.0	176.0
	Male	7	181.00	6.86	171.0	178.0	192.0
	Total	14	175.43	8.08	163.0	176.0	192.0
Weight (kg)	Female	7	66.21	6.18	55.0	66.0	73.0
	Male	7	72.81	6.47	63.0	75.7	79.0
	Total	14	69.51	6.98	55.0	69.5	79.0

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ON ORIGINAL

Concentration of FK506 in whole human blood:
0.03% topical administration

Timepoint	Observed concentration (pg/mL)											
	Subject number(Session number)											
	1(1)	2(1)	3(1)	4(1)	5(1)	6(2)	7(1)	9(1)	10(1)	13(1)	14(1)	15(1)
Day 1 00h												
Day 1 01h												
Day 1 02h												
Day 1 04h												
Day 1 06h												
Day 1 08h												
Day 1 10h												
Day 1 12h												
Day 2 pre												
Day 5 pre												
Day 8 pre												
Day 11 pre												
Day 12 pre												
Day 13 pre												
Day 14 pre												
Day 14 01h												
Day 14 02h												
Day 14 04h												
Day 14 06h												
Day 14 08h												
Day 14 10h												
Day 14 12h												
Day 15 24h												
Day 15 48h												
Day 15 72h												
Day 15 96h												
Day 15 120h												
Day 15 144h												
Day 15 168h												

Comment: The sponsor has incorrectly reported the Days corresponding to the hours. Samples were taken up to 7 days (168 hours) from the 14th Day.

APPEARS THIS WAY
ON ORIGINAL

Concentration of FK506 in whole human blood:
0.1% topical administration

Timepoint	Observed concentration (pg/mL)											
	Subject number(Session number)											
	1(2)	2(2)	3(4)	4(2)	5(3)	6(4)	7(4)	9(4)	10(3)	13(3)	14(4)	15(2)
Day 1 00h												
Day 1 01h												
Day 1 02h												
Day 1 04h												
Day 1 06h												
Day 1 08h												
Day 1 10h												
Day 1 12h												
Day 2 pre												
Day 5 pre												
Day 8 pre												
Day 11 pre												
Day 12 pre												
Day 13 pre												
Day 14 pre												
Day 14 01h												
Day 14 02h												
Day 14 04h												
Day 14 06h												
Day 14 08h												
Day 14 10h												
Day 14 12h												
Day 15 24h												
Day 15 48h												
Day 15 72h												
Day 15 96h												
Day 15 120h												
Day 15 144h												
Day 15 168h												

L.A. = Laboratory Accident

APPEARS THIS WAY
ON ORIGINAL

Concentration of FK506 in whole human blood: 0.3% topical administration

Timepoint	Observed concentration (pg/mL)											
	Subject number(Session number)											
	1(4)	2(4)	3(2)	4(3)	5(4)	6(3)	7(3)	9(3)	10(4)	13(2)	14(2)	15(3)
Day 1 00h												
Day 1 01h												
Day 1 02h												
Day 1 04h												
Day 1 06h												
Day 1 08h												
Day 1 10h												
Day 1 12h												
Day 2 pre												
Day 5 pre												
Day 8 pre												
Day 11 pre												
Day 12 pre												
Day 13 pre												
Day 14 pre												
Day 14 01h												
Day 14 02h												
Day 14 04h												
Day 14 06h												
Day 14 08h												
Day 14 10h												
Day 14 12h												
Day 15 24h												
Day 15 48h												
Day 15 72h												
Day 15 96h												
Day 15 120h												
Day 15 144h												
Day 15 168h												

L.A. = Laboratory Accident, N.S. = No Sample, * Insufficient sample to re-assay

APPEARS THIS WAY
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Study in Patients with Atopic Dermatitis (Study 94-0-008)

Table 2. Patient Demographics

Characteristic	Group 1 N = 6	Group 2 N = 6	Group 3 N = 7	Group 4 N = 6	Group 5A N = 4	Group 5B N = 4	Group 6 N = 6
Sex	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Male	3 (50.0)	3 (50.0)	4 (57.1)	3 (50.0)	1 (25.0)	1 (25.0)	3 (50.0)
Female	3 (50.0)	3 (50.0)	3 (42.8)	3 (50.0)	3 (75.0)	3 (75.0)	3 (50.0)
Race	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Black	1 (16.6)	5 (83.3)	3 (42.8)	4 (66.6)	3 (75.0)	2 (50.0)	3 (50.0)
Caucasian	4 (66.6)	1 (16.6)	3 (42.8)	2 (33.3)	1 (25.0)	2 (50.0)	2 (33.3)
Hispanic	1 (16.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.6)
Oriental	0 (0)	0 (0)	1 (14.2)	0 (0)	0 (0)	0 (0)	0 (0)
Age (years)							
Mean (SD)	27.8 (8.3)	35.3 (16.9)	38.0 (12.7)	43.7 (22.5)	8.8 (1.7)	5.3 (0.5)	25.2 (7.2)
Range	14 - 39	18 - 64	24 - 61	25 - 75	7 - 11	5 - 6	14 - 32
Height (cm)							
Mean (SD)	171 (3.6)	174 (11.5)	172 (8.2)	171 (11.6)	142 (13.9)	109 (3.0)	170(12.1)
Range	168 - 175	163 - 187	159 - 184	153 - 183	123 - 155	107 - 113	159 - 191
Weight (kg)							
Mean (SD)	73 (7.2)	98 (10.3)	74 (8.2)	89 (14.9)	43 (10.7)	19 (2.9)	79 (16.7)
Range	60 - 80	80 - 109	62 - 86	68 - 106	31 - 55	16 - 23	64 - 104

Table 3. Baseline Characteristics and Tacrolimus Dose by Treatment Group

Characteristic	Group 1 N = 6	Group 2 N = 6	Group 3 N = 7	Group 4 N = 6	Group 5A N = 4	Group 5B N = 4	Group 6 N = 6
Disease Severity	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Moderate	6 (100)	4 (66.6)	6 (85.7)	4 (66.6)	4 (100)	4 (100)	4 (66.6)
Severe	0 (0)	2 (33.3)	1 (14.2)	2 (33.3)	0 (0)	0 (0)	2 (33.3)
Duration of Current AD*(mo)							
Median	12.5	5.0	12.0	6.0	4.5	7.5	3.5
Range	1 - 331	1 - 10	2 - 189	1 -35	1 - 43	5 - 9	0 - 13
Total BSA**(M^2)							
Mean (SD)	1.85 (0.09)	2.1 (0.17)	1.9 (0.12)	2.0 (0.17)	1.3 (0.23)	0.75 (0.06)	1.9 (0.24)
Range	1.7 - 1.9	1.9 - 2.3	1.7 - 2.0	1.8 - 2.3	1.0 - 1.5	0.7 - 0.8	1.7 - 2.3
Tacrolimus Dose (mg/M^2)							
Mean (SD)	0.81 (0.04)	3.56 (0.30)	0.80 (0.05)	7.53 (0.65)	1.19 (0.22)	1.01 (0.08)	23.93 (2.77)
Range	0.8 - 0.9	3.3 - 4.0	0.7 - 0.9	6.6 - 8.5	1.0 - 1.5	0.9 - 1.1	19.3 - 27.1
% BSA Treated							
Mean (SD)	0.54 (0.03)	2.4 (0.20)	0.54 (0.04)	5.0 (0.43)	0.79 (0.15)	0.67 (0.05)	26.6 (3.08)
Range	0.5 - 0.6	2.2 - 2.7	0.5 - 0.6	4.4 - 5.6	0.7 - 1.0	0.6 - 0.7	21.5 - 30.1

*: AD - atopic dermatitis;
**: BSA - Body Surface Area

APPEARS THIS WAY
ON ORIGINAL

Blood Concentrations of Tacrolimus in Adult AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 100 cm² Treatment Area (Group 1).

Study Day	Time (hr)	Patient Number						Mean ± SD
		84001	84003	85001	85002	85003	85004	
1	0	[redacted]						0.10±0.08
	1							0.10±0.06
	2							0.14±0.13
	4							0.20±0.25
	6							0.19±0.27
	8							0.33±0.38
	12							0.15±0.20
2	(24)							0.07±0.11
3	0							0.13±0.13
4	0							0.09±0.07
5	0							0.10±0.08
6	0							0.12±0.07
7	0							0.09±0.07
8***	0							0.11±0.05
	1							0.09±0.05
	2							0.08±0.04
	4							0.07±0.05
	6							0.10±0.04
	8							0.13±0.09
	12							0.12±0.05
9	(24)							0.03±0.04
10	(48)							0.03±0.05
11	(72)							0.01±0.03

( ). Time from previous days administration
*. Once per day on Days 1 and 8. 2X/day on Days 2-7
**. Value is less than LOQ — ng/mL.)
***. Last day of application

APPEARS THIS WAY
ON ORIGINAL

Blood Concentrations of Tacrolimus in Adult AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 500 cm² Treatment Area (Group 2).

Study Day	Time (hr)	Patient Number						Mean ± SD
		84002	84005	85006	85007	85008	85005	
1	0	[redacted]						0.01±0.03
	1							0.02±0.03
	2							0.07±0.05
	4							0.14±0.17
	6							0.12±0.16
	8							0.12±0.12
	12							0.10±0.09
2	(24)							0.10±0.09
3	0							0.16±0.17
4	0							0.17±0.15
5	0							0.14±0.17
6	0							0.17±0.20
7	0							0.16±0.19
8	0							0.16±0.17
	1							0.14±0.18
	2							0.16±0.19
	4							0.17±0.23
	6							0.11±0.21
	8							0.11±0.18
	12							0.13±0.18
9	(24)							0.12±0.18
10	(48)							0.07±0.16
11	(72)							0.10±0.11

( ): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: Value is less than LOQ (= ng/mL)

Blood Concentrations of Tacrolimus in Adult AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 100 cm² Treatment Area on Face (Group J).

Study Day	Time (hr)	Patient Number							Mean ± SD
		84019	84021	84022	85009	85013	85014	85016	
1	0	[redacted]							0.03±0.038
	1								0.09±0.09
	2								0.55±0.47
	4								0.98±0.84
	6								0.83±0.78
	8								0.76±0.75
	12								0.77±0.83
2	(24)								0.37±0.26
3	0								0.88±0.82
4	0								0.67±0.61
5	0								0.74±0.68
6	0								0.66±0.64
7	0								0.50±0.37
8	0								0.50±0.35
	1								0.50±0.51
	2								0.54±0.55
	4								0.74±0.84
	6								0.75±0.90
	8								0.82±0.72
	12								0.57±0.50
9	(24)								0.56±0.49
10	(48)								0.29±0.17
11	(72)								0.21±0.16

( ): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: Value is less than LOQ (— ng/mL)

Blood Concentrations of Tacrolimus in Adult AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 1000 cm² Treatment Area (Group 4).

Study Day	Time (hr)	Patient Number						Mean ± SD
		84013	84014	84015	85011	85017	85018	
1	0	[redacted]						0.03±0.052
	1							0.07±0.11
	2							0.56±0.78
	4							1.14±1.37
	6							1.12±1.33
	8							0.98±1.28
	12							0.69±0.93
2	(24)							0.37±0.47
3	0							0.76±1.06
4	0							0.36±0.49
5	0							0.28±0.29
6	0							0.33±0.46
7	0							0.32±0.46
8	0							0.36±0.50
	1							0.38±0.51
	2							0.38±0.52
	4							0.46±0.62
	6							0.42±0.54
	8							0.33±0.50
	12							0.35±0.49
9	(24)							0.35±0.51
10	(48)							0.32±0.51
11	(72)						[redacted]	0.26±0.42

( ): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: Value is less than LOQ (ng/mL)

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Blood Concentrations of Tacrolimus in Pediatric AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 100 cm² Treatment Area (Group

Study Day	Time (hr)	Patient Number				Mean ± SD
		84025	85012	84032	85022	
1	0 1 4 8	[redacted]				0.03±0.03 0.09±0.07 0.09±0.14 0.02±0.03
2	(24)					0.05±0.08
3	0					0.09±0.14
6	0					0.28±0.35
8	0 1 4 8					0.05±0.05 0.11±0.05 0.15±0.09 0.06±0.09
9	(24)					0.06±0.05
11	(72)					0.02±0.04

( ): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: Value is less than LOQ — ng/mL)

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ON ORIGINAL

Blood Concentrations of Tacrolimus in Pediatric AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 50 cm² Treatment Area (Group 5b).

Study Day	Time (hr)	Patient Number				Mean ± SD
		84031	85023**	85024**	85025	
1	0 1 4 8	[redacted]				0.04±0.04 0.20±0.16 1.82±1.29 0.80±0.86
2	(24)					0.32±0.21
3	0					0.46±0.34
6	0					0.12±0.14
8	0 1 4 8					0.22±0.22 0.19±0.09 0.14±0.10 0.17±0.13
9	(24)					0.13±0.09
11	(72)					0.04±0.05

( ): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: An area of 100 cm² (rather than 50 cm²) was inadvertently treated
***: Value is less than LOQ — ng/mL)

Blood Concentrations of Tacrolimus in Adult AD Patients Following the Daily Administration* of 0.3% Tacrolimus Ointment to a 5000 cm² Treatment Area (Group 6).

Study Day	Time (hr)	Patient Number						Mean ± SD
		84027	84033	84036	85019	85020	85021	
1	0	[redacted]						0.05±0.06
	1							0.61±0.43
	2							1.92±1.93
	4							2.91±2.28
	6							2.88±3.40
	8							2.87±3.05
	12							1.51±1.16
2	(24)							1.13±0.96
3	0							2.02±1.93
4	0							1.55±1.58
5	0							1.82±1.95
6	0							1.41±1.61
7	0							1.50±2.09
8	0							1.23±1.47
	1							1.26±1.56
	2							1.04±1.14
	4							1.14±1.18
	6							1.20±1.25
	8							1.13±1.46
	12							1.20±1.58
9	(24)							1.03±1.37
10	(48)							0.72±0.76
11	(72)							0.50±0.51

(): Time from previous administration
*: Once per day on Days 1 and 8, 2x/day on Days 2-7
**: Value is less than LOQ (— ng/mL)

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ON ORIGINAL

Individual Pharmacokinetic Parameters of Tacrolimus On Days 1 and 8 in Adult AD Patients Following Daily Administration of Tacrolimus Ointment to a 100 cm² Treatment Area (Group 1).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-12} (ng-hr/mL)
84001	0.31	8	2.7
84003	0.00	0	0
85001	0.73	8	4.4
85002	0.86	8	11.9
85003	0.25	4	2.6
85004	0.08	1	0.7
Mean (±SD)	0.37±0.35	4.8±3.7	3.7±4.3

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-12} (ng-hr/mL)
84001	0.15	1	2.1
84003	0.14	8	2.6
85001	0.10	1	0.9
85002	0.22	8	3.3
85003	0.10	8	1.6
85004	0.22	8	2.4
Mean (±SD)	0.15±0.05	5.7±3.6	2.2±0.8

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Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Adult AD Patients Following Daily Administration of Tacrolimus Ointment to a 500 cm² Treatment Area (Group 2).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84002	0.14	12	2.2
84005	0.11	8	0.4
85005	0.44	4	5.8
85006	0.13	8	1.5
85007	0.00	0	0
85008	0.24	4	3.7
Mean (±SD)	0.18±0.15	6.0±4.2	2.4±2.0

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84002	0.63	4	11.9
84005	0.11	1	0.6
85005	0.06	0	0.2
85006	0.08	2	1.1
85007	0.16	12	2.1
85008	0.22	2	2.6
Mean (± SD)	0.21±0.21	3.5±4.4	3.1±4.4

Face

Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Adult AD Patients Following Daily Administration of Tacrolimus Ointment to a 100 cm² Facial Treatment Area (Group 3).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0,12}$ (ng·hr/mL)
84019	0.25	4	4.0
84021	1.46	4	12.8
84022	1.01	2	4.6
85009	1.52	8	22.3
85013	2.45	4	21.4
85014	2.43	12	36.4
85016	0.33	8	4.8
Mean (±SD)	1.35±0.89	6.0±3.5	15.2±12.2

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0,12}$ (ng·hr/mL)
84019	0.53	12	11.1
84021	2.74	6	42.8
84022	1.01	8	10.2
85009	0.15	2	2.5
85013	0.71	8	12.4
85014	1.01	8	20.8
85016	0.32	4	4.6
Mean (±SD)	0.92±0.86	6.9±3.2	14.9±13.6

Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Adult AD Patients Following Daily Administration of Tacrolimus Ointment to a 1000 cm² Treatment Area (Group 4).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84013	3.76	4	56.3
84014	0.07	12	0
84015	1.30	4	18.8
85011	0.87	6	9.9
85017	0.19	6	3.1
85018	0.74	4	8.3
Mean (±SD)	1.16±1.35	6.0±3.1	16.1±20.7

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84013	0.73	6	8.8
84014	0.26	4	2.7
84015	1.70	4	33.1
85011	0.11	4	1.4
85017	0.22	12	3.2
85018	0.57	2	3.3
Mean (±SD)	0.60±0.59	5.3±3.5	8.8±12.2

Pediatric (7-11 years)

Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Pediatric AD Patients Following Daily Administration of Tacrolimus Ointment to a 100 cm² Treatment Area (Group 5a).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84025	0.29	4	1.9
85012	0.06	4	0
84032	0.07	1	0
85022	0.14	1	1.6
Mean (± SD)	0.14±0.11	2.5±1.7	0.9±1.0

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84025	0.27	4	3.6
85012	0.12	4	1.5
84032	0.11	1	1.6
85022	0.16	1	0.9
Mean (±SD)	0.17±0.07	2.5±1.7	1.9±1.2

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(5-6 years)

Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Pediatric AD Patients Following Daily Administration of Tacrolimus Ointment to a 50 cm² Treatment Area (Group 5b).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84031	0.14	4	3.2
85023	2.05	8	29.2
84024	2.07	4	17.6
85025	3.28	4	19.1
Mean (±SD)	1.89±1.30	5.0±2.0	17.3±10.7

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
84031	0.32	8	5.8
85023	0.22	4	4.7
84024	0.22	4	4.2
85025	0.08	1	0
Mean (±SD)	0.21±0.10	4.3±2.9	3.7±2.5

Individual Pharmacokinetic Parameters of Tacrolimus on Days 1 and 8 in Adult AD Patients Following Daily Administration of Tacrolimus Ointment to a 5000 cm² Treatment Area (Group 6).

Day 1

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng-hr/mL)
84027	0.52	12	6.3
84033	2.40	4	26.4
84036	1.62	4	13.3
85019	3.08	4	42.5
85020	4.19	8	59.4
85021	9.42	6	107.2
Mean (±SD)	3.54±3.14	6.3±3.2	42.5±37.1

Day 8

Patient No.	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng-hr/mL)
84027	0.34	12	5.7
84033	0.76	6	8.4
84036	0.27	2	5.3
85019	1.25	4	28.0
85020	4.33	1	94.0
85021	1.16	4	22.3
Mean (±SD)	1.35±1.51	4.8±3.9	27.3±34.0

Study in Patients with Atopic Dermatitis (Study FJ-106)

Institution	Department	Physician-in-charge
[redacted]	Dermatology	[redacted]
	Dermatology	
	Dermatology	
	Dermatology	

*: Chairman of Investigators Committee: [redacted]

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In Vitro Studies

Table 1 Overall mean total penetration in each lot of ointment (n=6)

Ointment	Total penetration (% of dose) Mean	S.E.
0.03% GI Prod. Lot. C037-016	18.57	7.54
0.03% — Prod. Lot. 11526095	17.35	7.48
0.03% GI Prod. Lot. C037-017	18.55	6.01
0.03% — Prod. Lot. 11526097	18.44	8.26
0.03% GI Prod. Lot. C037-031	18.52	6.84
0.03% — Prod. Lot. 11527002	14.98	5.49

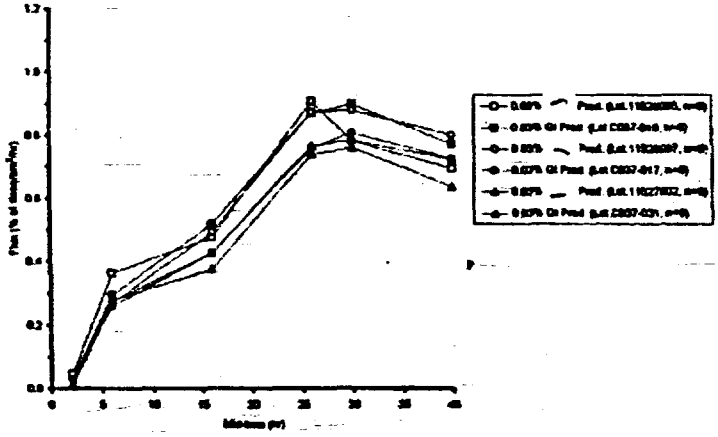

Figure 1 Mean flux of FK506 after a single application in each lot of ointment. Data represent mean values of 6 skins.

Table 1 Overall mean total penetration in each lot of ointment (n=6)

Ointment	Total penetration (% of dose) Mean	S.E.
0.1% — Prod. (Lot 11526079)	8.07	1.31
0.1% GI Prod. (Lot C037-002)	7.91	1.61
0.1% — Prod. (Lot 11526087)	7.74	1.69
0.1% GI Prod. (Lot C037-003)	6.89	1.75
0.1% — Prod. (Lot 11526089)	8.35	1.16
0.1% GI Prod. (Lot C037-025)	7.66	1.38

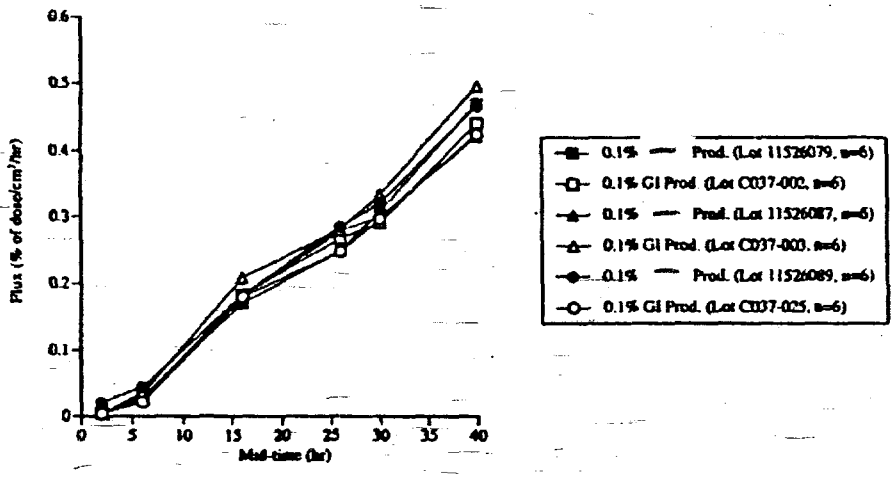

Figure 1 Mean flux of FK506 after a single application in each lot of ointment. Data represent mean values of 6 skins.

In Vitro Skin Metabolism

Extraction efficiencies and recovery of radioactivity in extracts following incubation with human skin homogenates for up to 60 min at 37°C

Sample identity	Replicate	DPM in aqueous (x 1000)	DPM in organic (x 1000)	Total DPM in extract (x 1000)	Extraction efficiency %[1]	Recovery of radioactivity %
1FA	1	15	2585	2600	99	110
1FB	1	18	2050	2068	99	87
	2[2]	21	2285	2306	99	98
1FC	1	25	2402	2427	99	103
	2	27	2725	2753	99	116
1FD	1	30	2316	2346	99	99
	2[2]	28	2241	2269	99	96
1F blank	1	45	2198	2242	98	95
2FA	1	18	2492	2505	99	106
	2	18	2409	2422	99	102
2FB	1	25	2390	2410	99	102
	2	26	2407	2427	99	103
2FC	1	32	2396	2423	99	102
	2	33	2532	2560	99	108
2FD	1	37	2420	2452	98	104
	2	37	2472	2504	99	106
2F blank	1	51	2408	2454	98	104
	2	52	2486	2533	98	107

All values are the result of a duplicate determination

Blank incubations contained no skin homogenate. DPM applied to incubations = 2364300

[1] These values represent the proportion of radioactivity present in the organic extract

[2] Following storage at ca -70°C, it was difficult to distinguish between the labels on the containers for 1FB2 and 1FD2 and it was not possible to confirm the identity of these samples

Concentrations of FK506 and potential metabolites in extracts following incubation with human skin homogenates for incubation periods up to 60 min at 37°C

Sample identity	Replicate no	Concentration [illegible]ncubate (μM)		
		FK506	M-I	M-II
1FA	1	25.10	ND	ND
1FB	1	20.70	ND	ND
	2[1]	27.79	ND	ND
1FC	1	24.50	ND	ND
	2	30.12	ND	ND
1FD	1	24.16	ND	ND
	2[1]	24.38	ND	ND
1F blank	1	24.40	ND	ND
2FA	1	26.07	ND	ND
	2	24.53	ND	ND
2FB	1	24.91	ND	ND
	2	25.25	ND	ND
2FC	1	27.31	ND	ND
	2	27.29	ND	ND
2FD	1	27.27	ND	ND
	2	28.53	ND	ND
2F blank	1	28.33	ND	ND
	2	28.41	ND	ND

ND = Not detected

[1] Following storage at or -70°C, it was difficult to distinguish between the labels on the containers for 1FB2 and 1FD2 and it was not possible to confirm the identity of these samples

Figure 1

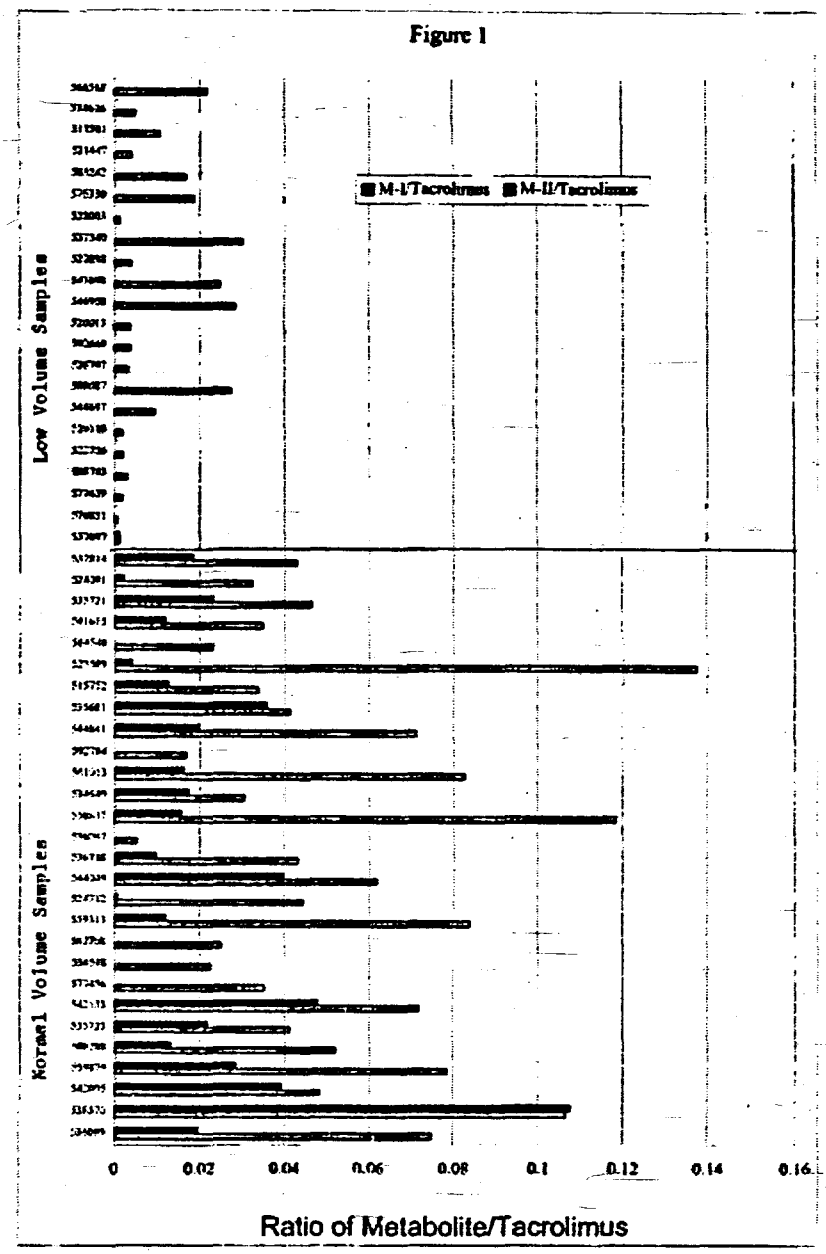

Table 5: Lot Numbers for Fujisawa-Sponsored Studies

Protocol Number	Lot Numbers		
	0.03% Tacrolimus Ointment	0.1% Tacrolimus Ointment	0.3% Tacrolimus Ointment
Clinical Pharmacology Studies			
94-0-004†	11524007	11524009	11524005
94-0-005†	11524007	11524009	11524005
94-0-006†	11524007	11524009	11524005
94-0-007†	11524007	11524009	11524005
95-0-011†	11525025	11525027	11525023
97-0-026†	C037-016	C037-003	N/A
FG-06-04‡	11524007	11524009	11524005
94-0-008†	N/A	N/A	11524005, 11524017
97-0-030†	N/A	C037-002, C238-004A	N/A
FJ-106§	N/A	602541K	602641K
FG-06-17‡	N/A	709467K	709567K
Clinical Studies: Phase 2			
95-0-003†	11525002, 11525025	11525004, 11525027	11524017, 11525023
95-0-009†	11525037	11525035	11525033**
95-0-013†	11525025 11525037	11525035	11525033
FG-06-01‡	11525002	11525004	11524017
FJ-103§	627535K	627635K	627735K
FJ-104§	602441K	602541K	602641K
FJ-105§	N/A	602541K	602641K
FJ-107§	608054K	608154K	N/A

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Clinical Pharmacology/Biopharmaceutics Review

NDA: 50-777 SUBMISSION DATE: 6/2/00, 6/28/00 8/21/00

NDA TYPE: 3S

PRODUCT: PROTOPIC™ (Tacrolimus Ointment, 0.03% and 0.1%)

SPONSOR: Fujisawa Heathcare Inc. REVIEWER: Veneeta Tandon, Ph.D.

NDA Review (ADDENDUM)

I. BACKGROUND

This addendum reviews additional studies and analysis provided by the sponsor upon request. This additional data was called for during the review cycle due to the conclusions provided by the Review Pharmacologist (Dr. Barbara Hill, Ph.D) from the carcinogenicity studies. A strong signal of lymphoma was observed in the mouse dermal carcinogenicity. Hence, further analysis of the exposure levels of tacrolimus were required and compared to that of the levels after systemic use of tacrolimus and the incidence of lymphoma observed in those cases.

The sponsor was requested to conduct a pharmacokinetics study in adult and pediatric population with severe atopic dermatitis using the 0.1% ointment under maximal use conditions, as AUCs after the use of 0.1% ointment was not available in the original submission. The sponsor had conducted a pharmacokinetic study in adults and pediatrics using the 0.3% tacrolimus ointment, which is 3 to 10-times higher than the to-be-marketed strength of tacrolimus ointment. The sponsor was also requested to conduct a population analysis to get rough estimates of AUC while the definitive study was being conducted. The sponsor has submitted results from the definitive pharmacokinetic study and the NONMEM analysis of blood concentrations from clinical studies.

The sponsor has provided data from two studies conducted in Europe in patients with moderate to severe atopic dermatitis to evaluate the pharmacokinetics of tacrolimus following topical application of 0.1% ointment, one which was recently completed in adults (FG-06-22) and one ongoing study in children (6-12 years of age) (FG-06-23). These two studies provide initial and study end AUC data for both adults and children with moderate to severe atopic dermatitis applying the higher of the to-be-marketed concentrations (0.1% tacrolimus ointment) over an extensive portion of their body surface area.

The study results will be summarized in this addendum and conclusions drawn will be highlighted at the end of the study. The contribution of these studies to the overall conclusions will be discussed at the end in this addendum.

II. RECOMMENDATION

The pharmacokinetic studies conducted with single and repeat applications (b.i.d. for 13 days) 0.1% tacroimus ointment in adult and pediatric patients (age 6-12 years) is acceptable from the clinical pharmacokinetics standpoint. The tacrolimus blood concentrations obtained from these studies (*FG-506-06-22 and FG-506-06-23*) were comparable to that obtained from Phase III clinical studies and *Study FJ-106*, with the exception of two samples showing a higher tacrolimus blood concentration of [redacted] ng/mL after single of multiple (twice daily) application of 0.1% tacrolimus ointment of 7 days (from *Study FJ-106*). The 0.03% strength being lower than 0.1% strength of tacrolimus, has not been evaluated in a pharmacokinetic study, though has been studied clinically. The blood concentrations obtained after topical administration of 0.1% tacrolimus ointment was <5 ng/mL in most subjects, hence, that obtained after the application of 0.03% will produce even lower systemic levels of tacrolimus.

TABLE OF CONTENTS

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III. PHARMACOKINETIC STUDIES

Pharmacokinetics in adult patients after single and multiple doses of topical 0.1% tacrolimus ointment

Study Report FG-506-06-22: A pharmacokinetic study of tacrolimus ointment in adult patients with moderate to severe atopic dermatitis following first and repeat application of the tacrolimus ointment.

Study Design: Single center, open label study in adult patients with moderate to severe atopic dermatitis to determine systemic exposure of tacrolimus after single and repeat application of 0.1% tacrolimus ointment for 14 days.

Study population: 37 patients of age 18 and older enrolled, 32 completed (2 withdrew, 3 had incomplete profiles). Diagnosis of severe atopic dermatitis done using Rajka and Langeland criteria.

Treatment Group	Sex	Age (yrs)	Weight (kg)	Height (cm)	Estimated total BSA (m^2)
1	1 M / 10 F	21 ± 5	62 ± 11	167 ± 6	1.69 ± 0.169
2	5 M / 7 F	23 ± 4	67 ± 12	173 ± 10	1.80 ± 0.198
3	2 M / 7 F	25 ± 8	63 ± 10	167 ± 8	1.70 ± 0.160

Dosage: Twice a day dosing for 13 days, single dose on Day 14, three treatment groups stratified by size of application area. The mean (± s.d.) areas treated as an absolute value and as a percentage of total body surface area (BSA) on the days when pharmacokinetic profiles were taken are shown in the tables below. The ointment was applied to the same designated area(s) for 14 days irrespective of any eczema healing.

Treatment Group	Area range (cm^2)	Mean treated area (cm^2)	% of total BSA
1 (n= 11)	≤ 3000	2410 ± 454	16 ± 5
2 (n=12)	>3000 ≤ 6000	4159 ± 892	25 ± 6
3 (n=9)	>6000 ≤ 10000	8553 ± 1308	53 ± 7

Although the treated area remained the same throughout the 14 days the mean amount of ointment required to cover the same area on Days 1, 4 and 14 decreased. This could be related to the improvement of skin condition over a period of time.

Individual information on % BSA involvement and dosing is available in the Appendix on pages 16-18.

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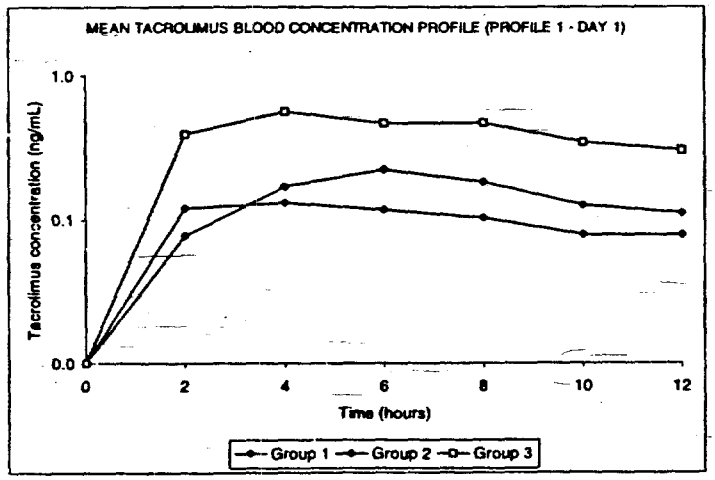
Treatment Group	Profile 1 – DAY 1		Profile 2 – DAY 4		Profile 3 – DAY 14	
	g ointment/ application	mg/kg tacrolimus	g ointment/ application	mg/kg tacrolimus	g ointment/ application	mg/kg tacrolimus
1	3.3 ± 1.4	0.05 ± 0.02	2.9 ± 1.0	0.05 ± 0.01	2.1 ± 0.9	0.03 ± 0.01
2	3.1 ± 1.5	0.05 ± 0.03	3.0 ± 1.3	0.04 ± 0.02	2.3 ± 1.2	0.04 ± 0.02
3	6.3 ± 1.7	0.11 ± 0.04	5.9 ± 2.0	0.10 ± 0.04	4.4 ± 2.5	0.07 ± 0.05

Blood Samples: Collected on Days 1, 4 and 14 following ointment application. Analysed by ———— methodology with a LLOQ of —— ng/mL. The assay validation is acceptable.

Profile 1: 0 (Predose), 2, 4, 6, 8, 10, 12 and 24 hours after first application (Day1).
Profile 2: 0 (Predose), 2, 4, 8 and 12 hours after application on Day 4.
Profile 3: 0 (Predose), 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144 and 168 hours after last application (Day 14).

Pharmacokinetic Results:

The mean plasma concentration time profile for the three groups on Days 1, 4 and 14 is shown in the following figures.



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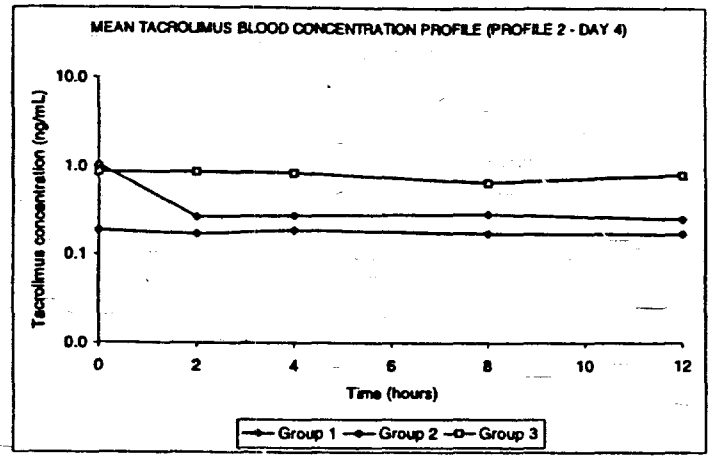

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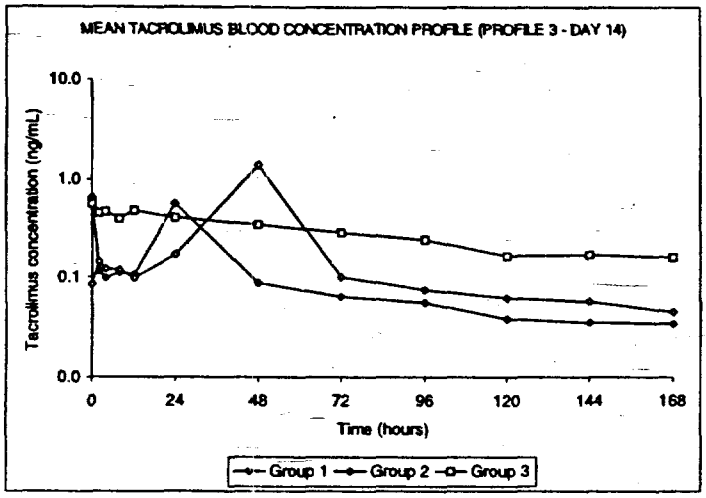


The mean blood concentrations at each time point with the minimum and maximum concentrations are attached in the Appendix on pages 19-21. The summary table showing the highest plasma concentration value and the time point associated with it is given below for all the three groups.

Group I		Absolute Values (Observed)
Day	Time (hr)	Maximum Observed Concentration (ng/mL)
1	4	
4	0	
14	0	
Group II		
Day	Time (hr)	Maximum Observed Concentration
1	4	
4	0	
14	48	
Group III		
Day	Time (hr)	Maximum Observed Concentration
1	4	
4	4	
14	0	

The number of samples showing the various tacrolimus concentrations are shown in the following table.

Tacrolimus conc. (ng/mL)	Number of samples	Cumulative count	Cumulative %
< 0.025	201	201	23.1
0.026 - 0.100	261	462	53.1
0.101 - 0.250	173	635	73.0
0.251 - 0.500	108	743	85.4
0.501 - 1.000	90	833	95.7
1.001 - 2.000	27	860	98.9
2.001 - 3.000	6	866	99.5
>3.000	4	870	100.0

The mean (±SD) pharmacokinetic parameters are tabulated below. The individual subject pharmacokinetic parameter are attached in the Appendix on pages 22-23.

PROFILE 1 – DAY 1

Parameter	GROUP 1(N=11)	GROUP 2(N=12)	GROUP 3 (N=9)
C_{max} (ng/mL)	0.14 ± 0.16	0.25 ± 0.42	0.66 ± 0.85
t_{max} (h)	4 (median)	4 (median)	4 (median)
AUC_{0-12} (ng.h/mL)	1.1 ± 1.4	1.6 ± 2.8	4.8 ± 6.3
$t_{1/2}$ (h)	8.6 ± 2.3	9.4 ± 5.2	8.6 ± 4.5

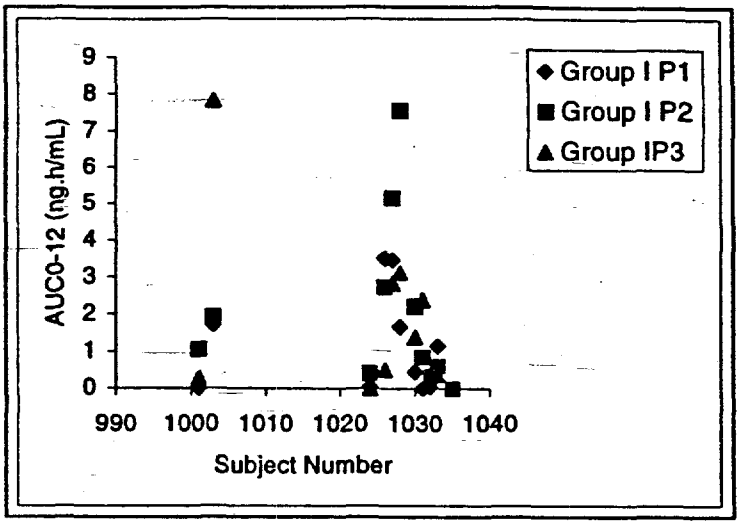
PROFILE 2 – DAY 4

Parameter	GROUP 1	GROUP 2	GROUP 3
C_{max} (ng/mL)	0.21 ± 0.24	1.10 ± 2.78	0.96 ± 0.80
t_{max} (h)	2 (median)	7 (median)	4 (median)
AUC_{0-12} (ng.h/mL)	2.1 ± 2.3	3.9 ± 5.0	10.2 ± 9.2

PROFILE 3 – DAY 14

Parameter	GROUP 1	GROUP 2	GROUP 3
C_{max} (ng/mL)	0.60 ± 1.62	0.19 ± 0.22	0.65 ± 0.38
t_{max} (h)	2 (median)	3 (median)	4 (median)
AUC_{0-12} (ng.h/mL)	1.7 ± 2.3	1.5 ± 1.5	5.4 ± 2.8
$t_{1/2,z}$ (h)	70.3 ± 14.8	78.8 ± 29.8	77.8 ± 20.9

The individual subject AUC_{0-12} values are shown in the following figures for the three groups.



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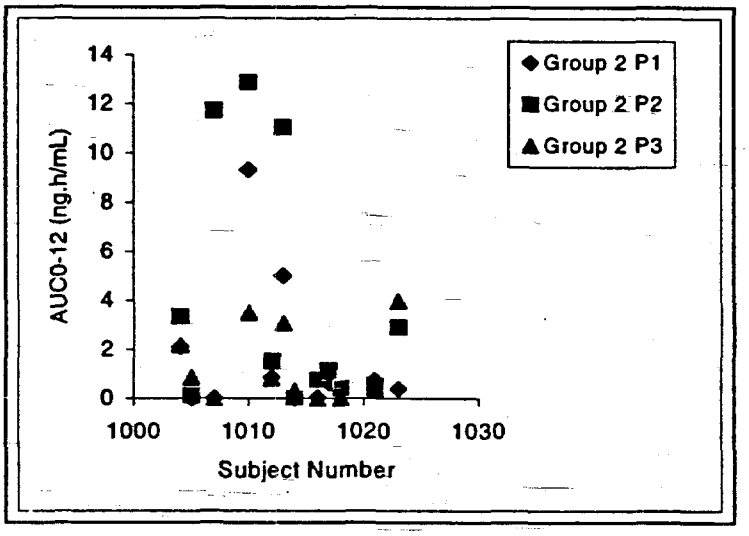

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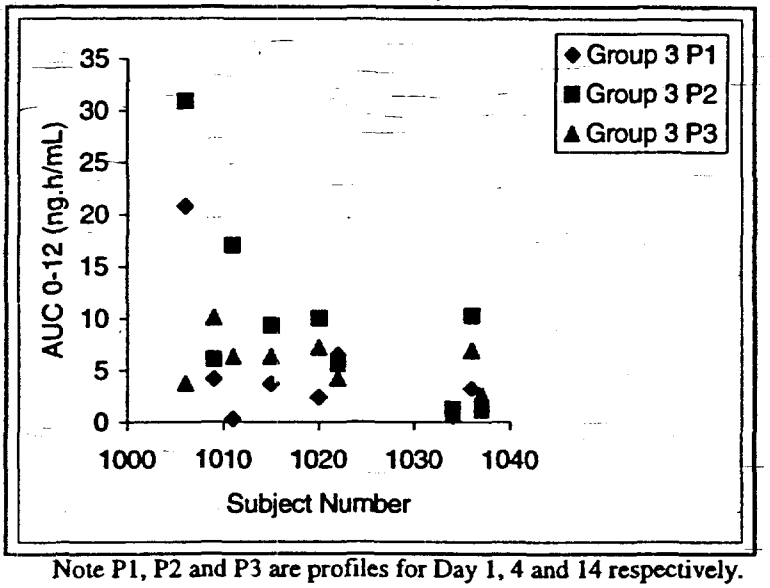


Note P1, P2 and P3 are profiles for Day 1, 4 and 14 respectively.

Observations:

- The highest mean AUC_{0-12} values were obtained on Day 4 for all the groups, with the highest mean AUC_{0-12} being 10.2 ng.h/mL.
- The systemic exposure was low, but highly variable (AUC_{0-12}:0-30.97 ng.h/mL) and increased as the treated body surface area increased. This highest value of 30.97 ng.h/mL was observed with a subject of Group 3 on Day 4. The mean ± SD AUC_{0-inf} following an initial oral dose of Prograf® (dose =0.2 mg/kg/day in two divided doses) for patients receiving a kidney transplant is 203 ± 42 ng.hr/mL (Prograf package insert). If this value is compared with the mean AUC_{0-12} observed in the atopic dermatitis study (10.2 ng•hr/mL), which is 20-fold for atopic dermatitis patients.
- Following repeated application of tacrolimus ointment there was no systemic accumulation observed.
- The Cmax values though not shown graphically were below 5 ng/mL. Two subjects had values higher than 5 ng/ml (— ng/mL for group I subject on Day 14, 9.8 and — ng/mL for Group II subject on Day 4 and 14 respectively). The subject showing a value of - ng/mL had all values on Day 14 below the LOQ, except at 48 hours where a level of — ng/mL was reported. This value was not included in the calculation of the Cmax. The blood levels are comparable to those obtained from study FJ-106 in the original review of the NDA
- On Day 14, following the last application blood samples were collected up to 7 days. The figure for Day 14 profile shows a slow elimination, with a half-life of 77±22 hours (range —— hours). This may be indicative of a slow absorption of the drug from the skin. Slow absorption was also seen from profiles of previous studies.

<u>In pediatric patients after single and multiple doses of topical 0.1% tacrolimus ointment</u>

Study Report FG-506-06-23: A pharmacokinetic study of tacrolimus ointment in pediatric patients with moderate to severe atopic dermatitis following first and repeat application of the tacrolimus ointment.

Study Design:	Multi center, open label study in pediatric patients with moderate to severe atopic dermatitis to determine systemic exposure of tacrolimus after single and repeat application of 0.1% tacrolimus ointment for 14 days.
Study population:	A total of 36 patients of ages between 6 and 12 years of age are planned to be enrolled, and <u>20 have completed</u> so far. This is an interim report submitted by the sponsor. Diagnosis of severe atopic dermatitis done using Rajka and Langeland criteria. Individual subject demographics are attached in the Appendix on pages 25-26.

Treatment Group	Sex	Age (yrs)	Weight (kg)	Height (cm)	Estimated total BSA (m^2)
1	5 M / 6 F	9 ± 2	30 ± 9	132 ± 11	1.041 ± 0.199
2	5 M / 2 F	9 ± 2	31 ± 12	129 ± 17	1.050 ± 0.267
3	2 M / 0 F	12 ± 1	54 ± 4	158 ± 14	1.535 ± 0.109

Dosage: Twice a day dosing for 13 days, single dose on Day 14, three treatment groups stratified by size of application area. The mean (± s.d.) areas treated as an absolute value and as a percentage of total body surface area (BSA) on the days when pharmacokinetic profiles were taken are shown in the tables below. The ointment was applied to the same designated area(s) for 14 days irrespective of any eczema healing.

The mean (± s.d.) areas treated as an absolute value and as a percentage of total Body Surface Area (BSA) on the days when pharmacokinetic profiles were taken are shown below.

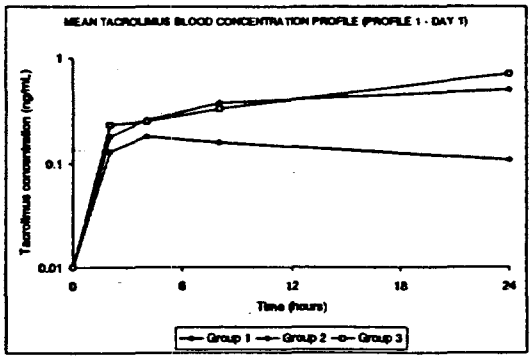
		Profile 1 – DAY 1			Profile 2 - DAY 14		
Treatment Group	Area range (cm^2)	Treatment area (cm^2)	% of total BSA	Dose (g) of ointment	Treatment area (cm^2)	% of total BSA	Dose (g) of ointment
1	≤ 1500	950 ± 329	33 ± 9	3.2	596 ± 315	19 ± 10	1.7
2	>1500 ≤ 3000	2080 ± 583	63 ± 11	3.0	1051 ± 659	32 ± 23	1.9
3	>3000 ≤ 5000	4225 ± 955	61 ± 16	2.5	1325 ± 460	31 ± 8	2.8

Blood Samples: Collected on Days 1 and 14 following ointment application. Analysed by ———— methodology with a LLOQ of — ng/mL. The assay validation is acceptable.

Profile 1: 0 (Predose), 2, 4, 8, and 24 hours after first application (Day1).
Profile 2: 0 (Predose), 2, 4, 8, 24, and 96 hours after last application (Day 14).

Pharmacokinetic Results:

The mean plasma concentration-time profile for the three groups is shown in the following figures for Day 1 and Day 14.



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The mean pharmacokinetic parameters for Day 1 are tabulated below and the individual are in the Appendix on page 29.

PROFILE 1 – DAY 1

Parameter	GROUP 1 (N=11)	GROUP 2 (N=7)	GROUP 3 (N=2)
C_{max} (ng/mL)	0.24 ± 0.26	0.62 ± 0.48	0.70 ± 0.98
t_{max} (h)	4 (median)	8 (median)	12 (median)
AUC_{0-24} (ng.h/mL)	3.2 ± 3.5	8.9 ± 8.8	10.6 ± 15.0

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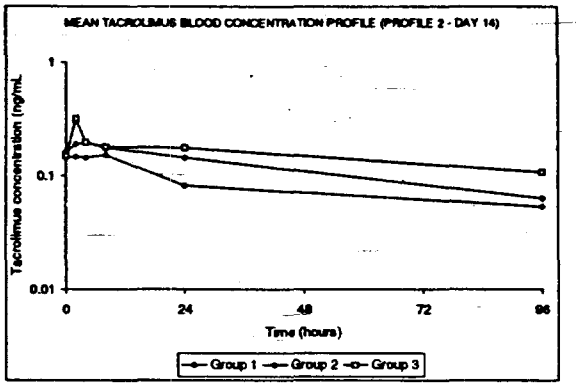

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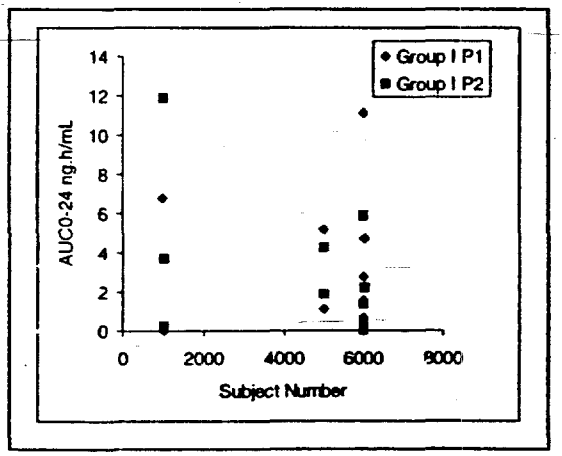
The mean pharmacokinetic parameters for Day 4 and Day 14 are tabulated below.

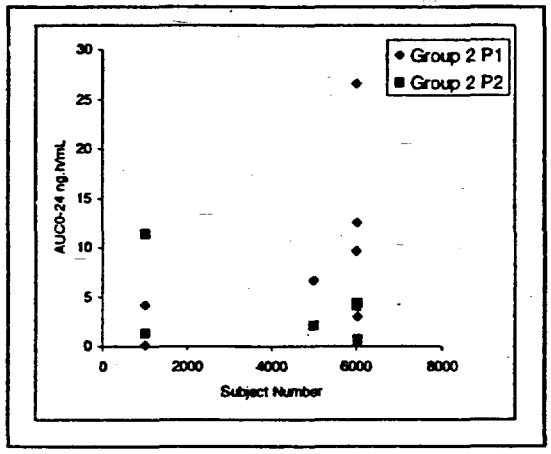
DAY 4 – TROUGH LEVELS

Parameter	GROUP 1	GROUP 2	GROUP 3
C_{min} (ng/mL)	0.12 ± 0.07	0.59 ± 0.38	0.33 ± 0.39

PROFILE 2 - DAY 14

	GROUP 1	GROUP 2	GROUP 3
C_{max} (ng/mL)	0.16 ± 0.22	0.21 ± 0.18	0.33 ± 0.38
t_{max} (h)	2 (median)	4 (median)	1 (median)
AUC_{0-24} (ng.h/mL)	3.0 ± 3.5	4.1 ± 3.6	4.7 ± 5.3





The individual blood concentration at each time point and the trough concentrations are attached in the Appendix on pages 27-28. The summary table showing the highest plasma concentration value and the time point associated with it is given in the following table for all the three groups.

Group I		Absolute Values (Observed)
Day	Time (hr)	Maximum Observed Concentration (ng/mL)
1	8	—
14	8	—
Group II		
Day	Time (hr)	Maximum Observed Concentration
1	8	—
14	4	—
Group III		
Day	Time (hr)	Maximum Observed Concentration
1	24	—
14	2	—

The number of samples showing the various tacrolimus concentrations are shown in the following table.

Tacrolimus conc. (ng/mL)	Number of samples	Cumulative count	Cumulative %
< 0.025	58	58	25.9
0.026 - 0.100	73	131	58.5
0.101 - 0.250	48	179	79.9
0.251 - 0.500	22	201	89.7
0.501 - 1.000	19	220	98.2
1.001 - 1.500	3	223	99.6
1.501 - 2.000	1	224	100

Observations:

- Tne systemic exposure to tacrolimus increased as the treatment area increased.
- These data indicate that following repeated application of tacrolimus ointment, twice daily for 14 days, there is no systemic accumulation of tacrolimus.
- The AUC_{0-24} was highly variable, ranging between 0-27 ng h/mL.
- Tacrolimus blood trough levels decreased with improvement of skin condition.
- The highest blood concentration observed was — ng/mL on Day 1. Typical whole blood trough concentration in pediatric liver transplant patients range from 5-20 ng/mL (from PDR®-Prograf®).
- The highest mean AUC_{0-24} (10.6 ng.h/mL) was observed on Day 1. By Day 14 the mean AUC_{0-24} in the same group of patients decreased by more than 50% to 4.7 ng.h/mL. Pediatric liver transplant patients have a mean AUC of 337 ng.h/mL following oral administration of 0.15 mg/kg/day (PDR®-Prograf®). The AUC after

topical administration is approximately 4% of the oral administration in pediatric patients.

Population Analysis of Cumulative Exposure

The sponsor has also attempted to calculate the average cumulative across 462 subjects from the phase III clinical trials. Blood samples were taken within the first 10 hours of the start of the study and around the end of the study, which was over a treatment period that ranged from 3 weeks to 12 weeks. Whole blood samples were less than 5 ng/mL in most cases. The average cumulative AUC across all 462 subjects was determined to be 332 ng.h/mL and the average steady state concentration was 0.428 ng/mL. This cumulative exposure can only be compared to the theoretical average cumulative exposure obtained from study FG-506-06-22, which would be an overestimation of the exposure based on the average values.

The highest average $AUC_{0\text{-}12\ hours}$ was observed in the adult study (FG-06-22) on Day 4 in the highest %BSA treated group (group 3). For this group, the mean $AUC_{0\text{-}12\ hours}$ for Day 1 was 4.8 ng•hr/mL, for Day 4 it was 10.2 ng•hr/mL and for Day 14 it was 5.4 ng•hr/mL	
1. Convert these mean $AUC_{0\text{-}12}$ values to daily equivalents (i.e., $AUC_{0\text{-}24\ hours}$)	4.8 X 2=9.6 10.2 X 2=20.4 5.4 X 2=10.8
2. Assume that the Day 1 mean daily AUC is maintained for the first 3 days	9.6 X 3=29
3. Assume that the Day 4 mean daily AUC is maintained for the next 10 days of treatment and assume that the Day 14 mean daily AUC is maintained for the next 109 days of treatment, Day 14 through Day 122 (median time to onset of PTLD in the 15 transplant recipients for which a theoretical $AUC_{0\text{-}onset}$ was determined).	20.4 X 10=204 10.8 X 109=1177
4. Determine the total AUC over that 122 day period. In most case the %BSA involved is decreased after a week of treatment	29+ 204 + 1177 =1410 ng.h/mL
5. Average cumulative exposure from Phase III NONMEM analysis of blood levels	332 ng.h/mL

Conclusions:

The average cumulative exposure from the Phase III NONMEM analysis of blood levels is 4-folds lower than the theoretical cumulative exposure obtained. The theoretical exposure is likely to be an overestimation, because the % BSA involved decreases during the treatment duration.

VI. OVERALL CONCLUSIONS

Systemic absorption of tacrolimus in adults patients on 0.1% tacrolimus ointment:

- The systemic exposure was low and highly variable with $AUC_{0\text{-}12}$ ranging between 0-30.97 ng.h/mL or an $AUC_{0\text{-}24}$ ranging between 0-61.94 ng.h/mL and a highest mean $AUC_{0\text{-}12}$ of 10.2 ng.h/mL.

- The Cmax was below 5 ng/mL in most pateints, with the exception of blood levels of [redacted] ng/mL for group I subject on Day 14, 9.8 and [redacted] ng/mL for Group II subject on Day 4 and 14 respectively.
- There was no systemic accumulation of tacrolimus following repeat application.

Systemic absorption of tacrolimus in pediatric patients (ages 6-12) on 0.1% tacrolimus ointment:

- The systemic exposure was low and highly variable with AUC_{0-24} ranging between 0-27 ng.h/mL and a highest mean AUC_{0-24} of 10.6 ng.h/mL.
- The Cmax was below 1.6 ng/mL in all patients.
- There was no systemic accumulation of tacrolimus following repeat application.

Comparison of systemic exposure between adult patients and pediatric patients on 0.1% tacrolimus ointment:

- There are no significant differences in systemic exposure between adult and pediatric patients (6-12 years of age) based on studies FG-506-22 and FG-506-23. The highest AUC_{0-24} observed in a 5 year old child from Study 94-0-008 was 29.2 ng.h/mL. No obvious differences were seen when blood samples from Phase III clinical trials were evaluated closely for blood levels in younger children (ages 2-6 years). The highest Cmax observed from clinical trials was 9.58 ng/ml from Study 95-0-009 in pediatric patients. The pharmacokinetic study (94-0-008) did show a trend towards higher concentration in the younger children (ages 5-6 years), but there were only 4 children in this study and a 3-10 times higher strength (0.3%) than a to-be-marketed strength (0.03 and 0.1%) was used in this study.

Comparison of systemic absorption after oral and topical administration of tacrolimus:
The oral data in the following table has been taken from the PDR® on Prograf®.

Population	N	Route of Tacrolimus administration (Dose)	Highest mean AUC_{0-24} ng.h/mL (range)	Highest Observed C_{max} ng/mL
Healthy	1	Topical (0.3% qd for 14 days)	11.5[b]	0.127
Atopic Dermatitis Adult Patients	37	Topical (0.1% b.i.d for 13 days, 16-53% of total BSA)	20.4 ± 18.4 (0-61.94)	14.975[a]
Atopic Dermatitis Pediatric Patients	20	Topical (0.1% b.i.d for 13 days, 33-61% of total BSA)	10.6± 15.0 (0-27.0)	1.513
Liver Transplant Pediatric Patients	9	Oral (0.15-0.2 mg/kg/day)	337±167[b]	43.4±27.9[c]
Kidney Transplant Adult Patients	26	Oral (0.2 mg/kg/day)	203±42[b]	19.2±10.3[c]
Liver Transplant Adult Patients	17	Oral (0.3 mg/kg/day)	519±179[b]	-

[a]The highest concentration of [redacted] ng/mL was observed in 2 patients after single and multiple application of 0.1% tacrolimus ointment from another study. AUC calculations could not be done for this study. [b]AUC_{0-inf} [c]mean Cmax

- Based on the evaluation of the data it is apparent that the systemic absorption of tacrolimus after topical application of 0.1% tacrolimus ointment is very low relative to the exposure generated from oral dosing. What is left unanswered is the amount of tacrolimus that enters the lymphatic system following topical administration. Drainage of the drug from the skin to the lymph system will be more relevant rather than the skin/blood partitioning.

/S/ 9/25/00

Veneeta Tandon, Ph.D.
Pharmacokineticist
Division of Pharmaceutical Evaluation III

Team Leader: E. Dennis Bashaw, Pharm. D. /S/ 10/2/00

CC: NDA 50-777 (ORIG)
HFD-540/Div File
HFD-540/CSO/Wright
HFD-880(Bashaw/Tandon)
HFD-880(Lazor)
HFD-344(Viswanathan)
CDR ATTN: B.Murphy

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