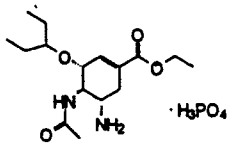
CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-246

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

REVIEWER NAME:	Ita Yuen
DIVISION NAME:	Division of Antiviral Drug Products
HFD#:	530
REVIEW COMPLETION DATE:	
ELECTRONIC FILE NUMBER:	None
NDA NUMBER:	21-246
SERIAL #/DATE/TYPE OF SUBMISSION:	000/June 19, 2000/Original
INFORMATION TO SPONSOR:	Yes ( ) No (X)
SPONSOR (OR AGENT):	Hoffmann-La Roche Inc. 340 Kingsland Street Nutley, NJ 07110-1199
MANUFACTURER OF DRUG SUBSTANCE:	F. Hoffmann-La Roche Ltd. Grenzacherstrasse 124 CH-4070 Basel, Switzerland
DRUG:	
Code Name:	Free base: Ro 64-0796/000; GS-4104 Phosphate salt: Ro 64-0796/002; GS-4104-02
Generic Name:	Oseltamivir phosphate
Trade Name:	Tamiflu®
Chemical Name:	(3R,4R,5S)-4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate (1:1)
CAS Registry Number:	204255-11-8
Molecular Formula/Molecular Weight:	$C_{16}H_{28}N_2O_4$ (free base)/M.W. = 312. $C_{16}H_{28}N_2O_4$ 1:1 H_3PO_4 (phosphate salt)/410.
Structure:	
RELEVANT INDS/NDAS/DMFS:	IND 53,093 DMF Type I #'s DMF Type III #'s DMF Type IV #'s
DRUG CLASS:	Influenza viral neuraminidase inhibitor
INDICATION:	Treatment of influenza infection in pediatric patients
CLINICAL FORMULATION:	The drug product, TAMIFLU™ (oseltamivir phosphate) Powder for Oral Suspension, is a powder, which is reconstituted with water to form a suspension. The powder is reconstituted with water to a concentration of

	of oseltamivir phosphate corresponding to 12 mg/ml of oseltamivir). Each bottle contains of oseltamivir phosphate in of powder for oral suspension. When reconstituted, the active substance is in solution, whereas some of the excipients are suspended. The composition in dry powder contains oseltamivir phosphate, sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium, and Tutti Frutti.
ROUTE OF ADMINISTRATION:	Oral
PROPOSED CLINICAL USE:	Treatment of influenza infection in pediatric patients aged 1-12
DISCLAIMER:	Some material may be taken directly from sponsor's submission

INTRODUCTION AND DRUG HISTORY:

Ro 64-0796 is an oral ethyl ester prodrug of an anti-influenza agent Ro 64-0802, which has poor bioavailability via the oral route of administration. Ro 64-0802 binds specifically to the active site of the neuraminidase enzyme on the surface of the influenza virus. The prodrug, also known as Tamiflu®, was approved for marketing for the treatment of influenza infection for adults on 10/27/99. The approved oral dosage is 75 mg twice daily for 5 days. The present NDA submission contains information for the approval of a new pediatric formulation for the treatment of influenza infection in children aged 1-12 years old. Two types of powder formulations were developed, referred to as types I and II. Formulation type I is a , and was used in clinical trials. Formulation type II, which is proposed for commercial production, is manufactured by a process. The change in formulations was necessary because of Chemistry, Manufacturing, and Control issues.

In the new Type II formulation, temperature- and humidity-dependent degradation is observed. The main degradation products are

Additional degradation products, which are probably Thus, this NDA package contains toxicology studies of oseltamivir plus known amounts of these degradation products.

STUDIES REVIEWED WITHIN THIS SUBMISSION:

NONCLINICAL PHARMACOLOGY STUDIES

Study Summary:

1. The effects of Ro 64-0796 on general hemodynamic and respiratory parameters in the anesthetized dog (Report # W-143176; Study # DHB12201; [redacted] Lot # GPM0229; GLP; With QA statement; Study dates 5/15/99-11/2/99; Vol. 7, pp. 94-191).
2. The tolerability of intravenously administered Ro 64-0796 to the anesthetized ferret (Report # 1001153; Report # 1001153; [redacted] Lot # GPM0229; GLP; With QA report; Study dates 8/30/99-9/1/99; Vol. 7, pp.192-218).

Study Reviews:

1. The effects of Ro 64-0796 on general hemodynamic and respiratory parameters in the anesthetized dog (Report # W-143176; Study # DHB12201; Lot # GPM0229). Four male beagle dogs were used for the study. Two were prepared surgically for the measurement of arterial blood pressure, heart rate, femoral arterial blood flow, and left ventricular pressure. One of these dogs was given 2 and 5 mg/kg and other 2, 5, 10, and 20 mg/kg intravenous doses of Ro 64-0796 as consecutive 30 minute infusions at a dose volume of 4 ml/kg. Hemodynamic and respiratory parameters were recorded continuously. Two conscious dogs received intravenous doses of Ro 64-0796 of 5 or 20 mg/kg as an infusion over 30 minutes. Blood samples for pharmacokinetic analysis were collected from these 2 dogs at 0, 5, 15, 30, 35, 40, 50, and 60 minutes and 2, 3, 4, 6, 8, and 12 hours after the start of the infusion. Pharmacokinetic parameters were calculated using non-compartmental methods for Ro 64-0796 and Ro 64-0802. The areas under the curve (AUC) were calculated using the trapezoidal method, assuming an exponential change in concentration between adjacent time points.

Intravenous administration of Ro 64-0796 to pentobarbitone anesthetized dogs did not have marked effects on the cardiovascular, ECG, or respiratory parameters measured. The pharmacokinetic parameters calculated from the 2 dogs dosed with either 5 or 20 mg/kg Ro 64-0796 are shown as follows:

PK parameters	Ro 64-0796		Ro 64-0802	
	5 mg/kg	20 mg/kg	5 mg/kg	20 mg/kg
T_{max} (hr)	0.5	0.5	2.0	3.0
T_{min} (hr)	12.0	12.0	0.25	0.08
C_{max} (µg/ml)		[redacted]		
$AUC_{0\rightarrow\infty}$ (µg/ml)	3.81	12.5	7.67	29.6
$T_{½}$ (hr)	1.60	1.44	2.32	2.34
Cl (l/hr/kg)	1.31	1.60	0.65	0.67
V_{ss} (l/kg)	1.82	2.50	-	-
F (%)	-	-	38	37

The pharmacokinetic profiles were smooth and the 2 doses provided parallel curves. The exposure to the active drug, Ro 64-0802, was 2-2.4 times greater than to Ro 64-0796. The bioavailability of Ro 64-0802 was about 38%.

2. The tolerability of intravenously administered Ro 64-0796 to the anesthetized ferret (Report # 1001153; Report # 1001153; Lot # GPM0229). Three female anesthetized ferrets each received a single intravenous dose of 2, 5, or 10 mg/kg Ro 64-0796. Blood samples were collected for pharmacokinetic analysis. However, the plasma samples were lost during transfer between [redacted] and the analytical laboratory. In addition, the ferret that was dosed at 2 mg/kg and anesthetized by intravenous infusion of Saffan (19-57 mg/kg/h) died approximately 10.5 hours after the onset of Saffan-induced anesthesia and about 9 hours after administration of the drug. It was determined that the death was related to prolonged anesthesia with the steroid, Saffan, but not with the administration of Ro 64-0796 since the other 2 ferrets dosed with higher doses of Ro 64-0796 (5 and 10 mg/kg) but anesthetized by inhalation administration of 1-2% isoflurane in oxygen/nitrous oxide survived.

NONCLINICAL TOXICOLOGY STUDIES

Study Summary:

1. A 14-day oral (gavage) toxicity study in the rat to investigate degradation products (Report # W-143076; Study # SAR703; Roche Discovery Welwyn, Welwyn Garden City, UK; Lot #'s 80302543 & BS98073444 for Ro 64-0796, 1214-143-20 for [redacted], and 1214-164-20 for [redacted] GLP; With QA report; Study dates 1/6/99-1/20/99; NDA 21-087, Vol. 60, pp. 1-151).
2. A 14-day oral (gavage) toxicity study in the rat to further investigate a degradation product, [redacted] (Report # W-143130; Study # SAR708; Roche Discovery Welwyn, Welwyn Garden City, UK; Lot #'s BS99025246 & BS98124862 for Ro 64-0796 and 1214-124-20 for [redacted], GLP; With QA report; Study dates 4/27/99-5/11/99; Vol. 10, pp. 1-199).
3. A 14-day rat study comparing drug substance spiked with [redacted] impurities [redacted] with Ro 64-0796/002 alone (Report # W-1001810; Study # 276/110; [redacted] Lot #'s 80702944 for Ro 64-0796, 9070012374/M1 for [redacted], RTN113-1-13 for [redacted], GLP; With QA report; Study dates 8/19/99-11/10/99; Vol. 11, pp. 1-160).
4. A 14-day oral toxicity study in rats with a degraded pediatric formulation (Report # W-1001811; Study # 276/114; [redacted], Lot #'s GHM0021 for placebo powder, GHM0022 for undegraded formulation, & GMZ0159/02 for degraded formulation; GLP; With QA report; Study dates 12/22/99-3/7/00; Vol. 12, pp. 1-155).

Study Review:

1. A 14-day oral (gavage) toxicity study in the rat to investigate degradation products (Report # W-143076; Study # SAR703; Lot #'s 80302543 & BS98073444 for Ro 64-0796, 1214-143-20 for [redacted], and 1214-164-20 for [redacted]

Species/Strain: Sprague-Dawley rats	**Route:** Oral (gavage)	**Vehicle:** 0.05 M acetate buffer, pH 4
Weight Range: M = 255-275 g; F = 185-209 g	**Duration of Dosing:** 14 days	**Dose Volume:** 10 ml/kg

Data collected: Intercurrent mortalities, clinical observations, body weights (twice weekly), urinalysis (days 9 & 10), clinical pathology (day 14), necropsy, organ weights, and histopathology (for groups 1, 2, and 4; epididymides from group 3 males also examined).

Important findings

Sex	**Males**				**Females**			
Group	1	2	3	4	1	2	3	4
Number of animal/group	5	5	5	5	5	5	5	5
Daily Dosage of Ro 64-0796 (mg/kg)	0	50	25	50	0	50	25	50
% Degradation product:[#]								
Hematology:								
APTT (sec)	25.1	17.5**	23.0	22.2*	21.8	17.1*	20.9	18.0*
Clinical chemistry:								
Phosphate (mmol/l)	2.0	2.1	2.3**	2.4***	1.8	1.8	1.9	2.0
Glucose (mmol/l)	7.7	8.3	8.8**	9.0**	8.3	8.9*	9.2**	9.3**
Relative organ weight (%):								
Spleen	0.177	0.191	0.19	0.216***	0.216	0.222	0.245	0.225
Epididymides	0.232	0.142	0.153	0.323***	-	-	-	-
Histopathological findings:								
Epididymis – Granulomatous reaction								
# animal affected	0	0	0	2	-	-	-	-
Average grade	0.0	0 0	0.0	2.5	-	-	-	-

#: The amount of degradation product is expressed as the percentage of Ro 64-0796.
* P<0.05 ** P<0.01 *** P<0.001

A No-Adverse Effect Level (NOEL) was not found for this study. Two doses of Ro 64-0796 were studied with a fixed percentage of degradation products of Ro 64-0802 (active metabolite), [redacted] All three compounds are hydrolytic products naturally occurring in solution, especially at higher pH. [redacted] should be converted to [redacted] totally in solution or in body.

There were various statistically significant changes in several parameters measured, even at the low dose. However, most of the changes were small in magnitude and did not correlate with any histological findings. One notable change that may be related to the degradation products was the increased weights of epididymides and a single focus of granulomatous reaction in 2 males receiving 50 mg/kg [redacted] with degradation products. Because of this finding, another 14-day toxicity study was performed with higher amount of degradation. The results of that study are presented below.

2. A 14-day oral (gavage) toxicity study in the rat to further investigate a degradation product, [redacted] (Report # W-143130; Study # SAR708; Lot #'s BS99025246 & BS98124862 for Ro 64-0796 and 1214-124-20 for [redacted]

Species/Strain: Sprague-Dawley rats	**Route**: Oral (gavage)	**Age**: 8 wks old	**Vehicle**: 0.05 M acetate buffer, pH 4	
Weight Range: M = 291-316 g; F = 190-236 g	**Duration of Dosing**: 14 days	**Dose Volume**: 10 ml/kg		
Data collected: Intercurrent mortalities, clinical observations, body weights (twice weekly), urinalysis (days 9 & 10), clinical pathology (day 14), necropsy, organ weights, and histopathology (see addendum 1).				

Important findings

Sex	**Males**				**Females**			
Group	1	2	3	4	1	2	3	4
Number of animal/group	5	5	5	5	5	5	5	5
Daily Dosage of Ro 64-0796 (mg/kg)	0	50	50	50	0	50	50	50
% Degradation product:[#]								
[redacted]								
Histopathological findings:								
Epididymis – Mononuclear cell focus								
# animal affected	2	2	-	3	-	-	-	-
Average grade	1.0	1.0	-	1.0	-	-	-	-

#: The amount of degradation product is expressed as the percentage of Ro 64-0796.
No-Toxic Effect Level (NOEL): 50 mg/kg/day Ro 64-0796 + 0.56% Ro 64-0802 + [redacted]

The design of the present study is slightly different from the previous 14-day toxicity study. The dosage and percentage of Ro 64-0796 and Ro 64-0802 were constant at 50 mg/kg/day and 0.56%, respectively. Only one impurity, [redacted] was studied, albeit at a higher percentage (the amount of [redacted] used for group 4 was [redacted] those for [redacted] combined in the previous study). The rationale for studying [redacted] impurity was presumably because [redacted] is expected to be [redacted] either in solution *in vitro* or *in vivo*. Epididymides were sectioned extensively and all sections examined microscopically because of the histopathological finding in the previous study. Instead of foci containing granulomatous reaction seen previously only in 50 mg/kg/day Ro 64-0796 with degradation products, mononuclear cell foci were seen in all treatment groups examined. There was one more animal with mononuclear cell foci in group 4 as compared to the vehicle and Ro 64-0796 only controls. However, total number of foci at epididymides was the same in all 3 groups examined. Foci containing granulomatous reaction seen in the previous study were absent here. It is believed the high background incidence of mononuclear focus and the absence of granulomatous focus are reassuring that the findings in the previous study may not be representative of toxicity of these degradation products.

3. A 14-day rat study comparing drug substance spiked with two impurities ([redacted], with Ro 64-0796/002 alone (Report # W-1001810; Study # 276/110; Lot #'s 80702944 for Ro 64-0796, 9070012374/M1 for [redacted], RTN113-1-13 for [redacted]. The two impurities, [redacted], are the [redacted] excipients in the pediatric formulation II).

Species/Strain: Sprague-Dawley rats	**Route**: Oral (gavage)	**Vehicle**: Purified water	**Age**: 6 weeks old
Weight Range: M = 173-213 g; F = 153-181 g	**Duration of Dosing**: 14 days		**Dose Volume**: 20 ml/kg

Data collected: Intercurrent mortalities, clinical observations, ophthalmoscopic examination, body weights (twice weekly), food consumption, urinalysis (days 9 & 10), clinical pathology (day 14), necropsy, organ weights, and histopathology (see addendum I).

Important findings

Sex	**Males**				**Females**			
Group	1	2	3	4	1	2	3	4
Number of animals/group	5	5	5	5	5	5	5	5
Dosage of Ro 64-0796 (mg/kg/day)	0	50	50	50	0	50	50	50
Dosage of impurity (mg/kg/day)								

No-Toxic Effect Level (NOEL):

No effects were associated with drug administration. Thus, degradation products at levels up to , respectively, were considered safe.

4. A 14-day oral toxicity study in rats with a degraded pediatric formulation (Report # W-1001811; Study # 276/114; Lot #'s GHM0021 for placebo powder, GHM0022 for undegraded formulation, & GMZ0159/02 for degraded formulation).

Species/Strain: Crl:CD BR rats	**Route**: Oral (gavage)	**Vehicle**: Purified water	**Age**: 6 weeks old
Weight Range: M = 203-233 g; F = 146-185 g	**Duration of Dosing**: 14 days		**Dose Volume**: 10 ml/kg

Data collected: Intercurrent mortalities, clinical observations, ophthalmoscopic examination, body weights (weekly), food consumption (weekly), urinalysis (week 3), clinical pathology (week 2), necropsy, organ weights, and histopathology (see addendum I), and toxicokinetics (blood samples collected from 2 animals/group at 1, 3, 6, and 24 hours postdosing).

Important findings

Sex		**Males**			**Females**		
Group		1	2	3	1	2	3
Number of animals/group		5	5	5	5	5	5
Formulation		Placebo	Undegraded	Degraded	Placebo	Undegraded	Degraded
Dosage of Ro 64-0796 (mg/kg/day)		0	50	50	0	50	50
T_{max} (hr)	**Ro 64-0796**	-	1.0	1.0	-	1.0	1.0
	Ro 64-0802	-	2.0	1.0	-	1.0	2.0
C_{max} (ng/ml)	**Ro 64-0796**	-	510	304	-	674	422
	Ro 64-0802	-	884	1140	-	1940	1150
C_{min} (ng/ml)	**Ro 64-0796**	-	2.21	6.11	-	1.35	2.06
	Ro 64-0802	-	8.41	31.2	-	5.77	5.76
AUC_{0-24h} (µg.hr/ml)	**Ro 64-0796**	-	1.94	1.39	-	2.01	1.73
	Ro 64-0802	-	7.58	6.47	-	10.6	8.28

No-Toxic Effect Level (NOEL): 50 mg/kg/day Ro 64-0796 + degraded pediatric formula II

There was no effect associated with the administration of various pediatric formulations for Tamiflu. As expected, exposures to both Ro 64-0796 and Ro 64-0802 were slightly decreased in the degraded formulation as compared to undegraded formulation.

NONCLINICAL PHARMACOKINETIC STUDIES

Study Summary:

1. *In vitro* experiments of the metabolism of Ro 64-0796 by the marmoset (Report # W-1001666; Study # 99/31/ROC/07; Roche Discovery Welwyn, Welwyn Garden City, England and [redacted] Lot #'s LC571 & LC575 for radiolabeled and 80202143 & GPM0229 for unlabeled Ro 64-0796; non-GLP; Without QA report; Study dates 8/18/99- 2/17/00; Vol. 13, pp. 1-189).
2. A reassessment of the metabolism of Ro 64-0796 by man (Report # W-1001667; Study # DHB11404).
3. Further *in vitro* studies on the metabolism of Ro 64-0796 in animals and man (Report # W-1001812; Study # DHB06803).

Study Review:

1. *In vitro* experiments of the metabolism of Ro 64-0796 by the marmoset (Report # W-1001666; Study # 99/31/ROC/07; Lot #'s LC571 & LC575 for radiolabeled and 80202143 & GPM0229 for unlabeled Ro 64-0796). Experiments in juvenile marmosets indicated that the maximal rate of hydrolysis of Ro 64-0796 to Ro 64-0802 was not achieved until the animals were weaned. The exposure to the more toxic prodrug was 7-fold greater in the 7 day old marmosets than the adults. Because Tamiflu will be administered to infants and children, the difference in the rate of hydrolysis of prodrug to active metabolites needs to be put into perspective. Toxicokinetic information suggested that rat is a poor model for man since most of drug is hydrolyzed in plasma, rather than in the liver as with primates. The marmosets were selected for the study since they handle Ro 64-0796 similar to man so that the comparisons of rates of metabolism of Ro 64-0796 in hepatic preparations from marmosets of various ages may more resemble the degree of expression of esterases in children. To study this, hepatic "S9" fractions were isolated from frozen liver from female marmosets of ages 1 day, 3 and 6 weeks, 3 months, and 4 years. 1 μg/ml of [^{14}C]-Ro 64-0796 was incubated with the "S9" fractions and aliquots removed for quantitation of Ro 64-0796 and Ro 64-0802 and pharmacokinetic analysis at 0, 1, 2, 3, 4, 5, 6, 7, and 24 hours later. The following table depict some pharmacokinetic parameters for the hydrolysis of Ro 64-0796 (1 μg/ml; 2.9 nmole/ml) in "S9" supernatants from female marmosets of various ages:

Age	Initial Rate (pmol/min/mg prot.)	Half-life (hr)	$AUC_{0-\infty}$ (μg.hr/ml)	Intrinsic clearance (μl/min/mg prot.)
1 day old	3.06	36.6	40.5	0.369
3 weeks old	2.78	32.5	36.2	0.414
6 weeks old	6.61	16.0	16.4	0.912
3 months old	16.3	3.54	3.58	4.18
4 years old	18.4	3.07	3.20	4.68

These results indicated that hydrolysis of prodrug Ro 64-0796 to active drug Ro 64-0801 was ~ 5-10-fold slower in juveniles (6 weeks old and younger) than adults (3 months and older). If this is also true in humans, it suggested that neonates and infants may be exposed to the more toxic prodrug which has also been found to be positive in the Syrian Hamster Embryo cell transformation assay.

graded between minimal to slight in severity after 6 months of repeated drug administration at a dose of 1000 mg/kg/day in rats (~300X and 40X human exposure to Ro 64-0796 and Ro 64-0802, respectively). No histopathological changes were associated with Ro 64-0796 administration to marmosets. Increased incidence and severity of mineralization in kidneys may account for the increased relative kidney weight in rats. It is clear that the prolonged and repeated exposure to Ro 64-0796 and its active metabolite causes slight renal dysfunction at fairly high dosages (> 300X and 40X of the expected clinical exposure) in all nonclinical species studied. Since most of the histopathological changes were seen in rats only, the sponsor had postulated that mineralization seen in rats was a species-specific finding. It was argued that at the high dose, the high content of phosphate in the Ro 64-0796/002 (a phosphate salt) would negatively influence the dietary calcium/phosphate ratio in a species known to be sensitive to this kind of change. This led to the precipitation of calcium phosphate and the subsequent mineralization in several renal structures. However, since the sponsor did not offer concrete evidence that the mineral casts/crystals were unequivaocally precipitated calcium phosphate, another possibility also exists. The pharmacokinetic data have indicated that rodents do not hydrolyze Ro 64-0796 to its active metabolite, Ro 64-0802, as efficiently as primates. The urine prodrug/active metabolite ratios for rats and marmosets were 1:3 and 1:15, respectively. Since the free base form of prodrug (the form expected to exist in kidneys) is expected to be 100-1000 times less soluble than the active metabolite, the mineralization in the high dose in rats may be partly due to the precipitation of the prodrug. This scenario may have some clinical implications in severely hepatic impaired patients. All of the renal changes were reversible after a period of drug-free recovery.

GI system: Ro 64-0796/002 was extremely irritating to the GI tract of marmosets. Emesis and salivation were associated with doses greater than 150 mg/kg. Slight to moderately severe gastric mucosal inflammation, atrophy, hemorrhage, erosion, and ulceration were associated with doses of 1000 mg/kg. One animal dosed with 2000 mg/kg had to be sacrificed *in extremis* because of severe gastric irritation. The drug had to be administered as 2 separate daily doses to reduce the drug-induced emesis and gastric irritation. In clinical trials, vomiting and nausea were the two most frequent adverse events during the treatment of naturally acquired influenza with oseltamivir at 75 mg b.i.d.

Liver: The toxicities to this organ were mild and consisted of slightly increased liver weight and plasma levels of glucose, cholesterol, total protein, globulin, and albumin. All of the increases were less than 1-fold greater than the corresponding controls. There were no hepatic histopathological changes associated with drug treatment. All of the

observed changes were reversible upon cessation of drug administration.

Hematology: The toxicities involved, in general, were minor but statistically significant changes in various red and white cell parameters. Since most of the changes occurred in one or the other sex per study without any apparent pattern, it is unclear of their clinical significance.

Bone: There were 3 intercurrent mortalities in the 9 month toxicity study in marmosets related to osteomalacia. These deaths were not dose-related and occurred in the low and mid dose groups only. The sponsor thus attributed the effect to pre-existing osteomalacia and believed that supplementing vitamin D in the diet may have prevented these deaths. Slightly but statistically significant elevation of alkaline phosphatase levels were detected in the high dose (≥ 1000 mg/kg/day) in a few rat toxicity studies. In the Segment II reproductive studies, incomplete or no ossification of various bones was observed in both rats and rabbit fetuses exposed to Ro 64-0796 *in utero*. Thus, the data suggest that Ro 64-0796 and/or its active metabolite may have some effect on the ossification process.

Lung: Pulmonary alveolitis was seen in several toxicity studies in rats, including the 6-month study. The incidence and severity at the high dose (≥ 1000 mg/kg/day) were slightly higher than the control. Since lung is one of the main sites of influenza viral infection, this finding may suggest accumulation of drug at this organ. The inflammation was graded as slightly higher than minimal severity.

Adult vs. juvenile: There were no differences in the toxicity profile between adults and weaned/unweaned juvenile rats. The pharmacokinetic profiles between the weaned (age of 3-7 weeks old) and adult rats were more similar than that of the unweaned juvenile rats. The unweaned juvenile rats (age of 4-21 days) did not hydrolyze the prodrug efficiently at first. The systemic exposure to prodrug after a single dose of Ro 64-0796 was higher than that to the active metabolite and was estimated to be more than 10-fold higher than that in the adult rats. It is possible that the high systemic prodrug exposure was the cause of the 75% mortality rate in the unweaned juvenile rats dosed with 1000 mg/kg/day Ro 64-0796. At this dose, only mild toxicities were detected following 6 months of repeated drug administration in adult rats. The hyrolysis of prodrug to its active metabolite increased following 2 weeks of repeated drug administration to the unweaned rats. However, it is unclear from the data whether the difference was associated with weaning or age. The prodrug to active metabolite ratio was similar in weaned and adults rats.

In conclusion, Ro 64-0796 and its active metabolite, Ro 64-0802, cause mild toxicities at high dosages and systemic exposures. The clinical dosage for the treatment of influenza viral infection is 75 mg b.i.d. for 5 days. The main toxicities caused by long-term (6-9 months)

repeated administration of Ro 64-0796 were those related to kidney and occurred at doses where systemic exposures to prodrug and active metabolites were more than 200- and 30-fold of those seen in humans. These results suggest a large safety margin and high therapeutic index.

The irritation to the GI system will probably be the dose-limiting toxicity in clinical usage, although the study in marmosets suggested that dividing the doses may alleviate some of the GI irritation. The toxicokinetic data suggested that many of the toxicities may be associated with higher prodrug exposure. Human hydrolyzes the prodrug to its active metabolite at 7-fold greater than the rodents, suggesting an added safety margin. However, in severely hepatic impaired and very young pediatric (e.g., newborn) patients, special caution may be needed.

Addendum list:

1. Histopathology inventory.
2. Studies reviewed under IND.

ADDENDUM 1: Histopathology Inventory for NDA # 21087.ori

Study	W-143076	W-143130	W-1001810	# W-1001811	W-143030	W-143066
Species	SD rats	SD rats	SD rats	CDBR rats	SD rats	SD rats
Adrenals	X*	X*	X*	X*	X*	X*
Aorta	X	X		X	X	X
Bone Marrow smear	X	X	X (femur)	X (femur)	X (femur)	X
Bone	X (knee)	X (knee)	X (femur)	X (femur)		
Brain	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix						
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X*†	X*†	X*	X*	X	X
Esophagus	X	X	X	X	X	X
Eye		X	X	X	X	X
Fallopian tube						
Gall bladder						
Gross lesions	X	X	X	X		
Harderian gland			X	X		
Heart	X*	X*	X*	X*	X*	X*
Hyphophysis						
Ileum	X	X	X	X	X	X
Injection site						
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X* (right)	X* (right)
Lachrymal gland			X	X		
Larynx						
Liver	X*	X*	X*	X*	X*	X*
Lungs	X*	X*	X	X	X*	X*
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X		
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves			X	X		
Ovaries	X*	X*	X*	X*	X	X*
Pancreas	X	X	X	X	X	X*
Parathyroid	X	X	X*	X*	X	X
Peripheral nerve						
Pharynx						
Pituitary	X	X	X*	X*	X	X*
Prostate	X	X	X*	X*	X	X*
Rectum			X	X		
Salivary gland, submandibular	X	X	X	X	X	X
Sciatic nerve	X	X	X	X		
Seminal vesicles	X	X	X	X	X	X
Skeletal muscle	X (hindleg)	X (hindleg)	X (quadriceps)	X (quadriceps)		
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	
Spleen	X*	X*	X*	X*	X*	X*
Sternum			X	X		
Stomach	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X	X*
Thymus	X*	X*	X	X	X	X*
Thyroid	X	X	X*	X*	X	X
Tongue	X	X	X	X	X	X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X	X	X	X	X	X*
Vagina			X	X		
Zymbal gland						
Trachea bifurcation			X	X		

* organ weight obtained

† For each epididymis from groups 1, 2, & 4, three pieces were processed in each block and the whole block serially sectioned at 150 μm intervals. The head and tail of the epididymides were identified.

ADDENDUM 1: Studies Reviewed Under IND

Nonclinical Toxicology Review:

1. Ro 64-0796/002: 13 week oral (gavage administration) range-finding toxicity study in the mouse (Report # W-143121; Study # 276/98-D6154; [redacted], Lot # 80202143; GLP; With QA report; Study dates 8/17/98-11/19/98; Vols. 8-9).

Species/Strain: CD-1 mice	**Route:** Oral gavage		**Duration of Dosing:** 13 weeks
Weight Range on Day 1: M = 26-35 g; F = 19-27g		**Age on Day 1:** 6 weeks old	**Dose Volume:** 10 ml/kg
Data collected	**Frequency/Occasion**	**Data collected**	**Frequency/Occasion**
Clinical observation	Daily	Organ weights	Week 14
Body weight	Weekly	Histopathology	Week 14 on control & high dose groups only
Food consumption	Weekly		
Clinical pathology	Week 13	Toxicokinetics	During week 12 at 0.5, 1, 2, 4, 8, 12, 24 hrs postdose
Urinalysis	Week 3		

Important findings

Sex				**Males**					**Females**	
Dosage (mg/kg/day)	0	100	250	600	1000	0	100	250	600	1000
Number of animals:										
Main	12	12	12	12	12	12	12	12	12	12
Toxicokinetic#	6	21	21	21	21	6	21	21	21	21
Pro-drug T_{max} (hr)	-	0.5	0.5	0.5	0.5	-	0.5	0.5	2.0	0.5
Pro-drug C_{max} (µg/ml)	-	16.9	40.3	25.3	41.5	-	8.5	16.1	20.3	37.3
Pro-drug $AUC_{0\rightarrow 24h}$† (µg-h/ml)	-	8.6	36.6	96.6	202	-	10.1	33.0	81.9	191
Multiples human exp.	-	*78*	*333*	*878*	*1836*	-	*92*	*300*	*745*	*1736*
Metabolite T_{max} (hr)	-	0.5	1.0	1.0	4.0	-	0.5	0.5	1.0	1.0
Metabolite C_{max} (µg/ml)	-	11.1	31.5	43.9	54.7	-	26.5	26.5	38.7	47.1
Metabolite $AUC_{0\rightarrow 24h}$† (µg-h/ml)	-	33.9	138	314	474	-	26.5	76.7	182	367
Multiples human exp.	-	*13*	*51*	*116*	*176*	-	*10*	*28*	*67*	*136*
Number of deaths:	0	0	1	2	3	0	0	0	1	3
Cause: Unknown	0	0	1	2	2	0	0	0	1	0
Accident	0	0	0	0	0	0	0	0	0	2
Urogenital lesion	0	0	0	0	1	0	0	0	0	0
Renal lesion	0	0	0	0	0	0	0	0	0	1
Hematology										
Hemoglobin (g/dl)	14.0	13.4	14.1	13.6	14.1	14.7	14.8	15.2	14.4	14.2
PCV (%)	45.6	43.8	46.3	44.5	45.2	47.1	46.1	47.8	45.7	44.8
Clinical chemistry										
ALK PHOS (IU/l)	91	92	139	113	163	137	178	218	192	173
Sodium (mmol/l)	150	146	148	147	146*	146	145	147	146	146
Potassium (mmol/l)	5.1	5.4	5.1	4.7	4.4	5.5	5.1	4.9	5.2	4.9
Chloride (mmol/l)	116	113	114	113	109*	114	113	114	113	114
Creatinine (µmol/l)	35	35	36	36	37	42	41	36	37	34*
Glucose (mmol/l)	5.9	8.2	6.6	8.4*	8.3*	8.4	7.1	6.7	8.9	8.2

Histopathology: *Kidney*										
Inflammatory foci										
Total # affected	4	4	4	5	1	3	2	6	6	5
Average grade	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Papillary mineralization										
Total # affected	3	5	3	6	9	4	6	3	3	3
Average grade	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Focal nephropathy										
Total # affected	3	3	3	5	8	3	3	7	5	4
Average grade	1.0	1.0	1.0	1.0	1.5	1.7	1.0	1.1	1.2	1.0
Tubular necrosis										
Total # affected	0	0	0	0	0	0	0	0	0	2
Average grade	0	0	0	0	0	0	0	0	0	1.5
Papillitis										
Total # affected	0	0	0	0	0	0	0	0	0	1
Average grade	0	0	0	0	0	0	0	0	0	1.0
Papillary necrosis										
Total # affected	0	0	0	0	1	0	0	0	0	1
Average grade	0	0	0	0	1.0	0	0	0	0	2.0

= Blood samples collected from 2-3 mice/sex/group
† = Systemic exposure for the active metabolite Ro 64-0802
* $P<0.05$
For grade used in histopathology, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe. Average grade is calculated as sum of (grade X # of animals with that grade finding)/total # affected.

There were a total of 10 deaths in both the main study and satellite groups. Only the one death caused by renal lesions in the high dose female group was considered to be related to the drug treatment. Body weight for high dose males dropped in week 13 only. Noisy respiration was noted in 3 high dose males at the end of week 1. There were various changes in the hematological and clinical chemistry parameters, some of which reached statistical significance either as compared to the control or by a dose response test. Most of the changes were slight. It is interesting that, this time, plasma glucose levels elevated to statistical significance in the males but not females, as observed in the 1-month study. Treatment-related histopathological findings were limited to kidneys. These findings were slight in nature and non-dose-related. However, a death in the high dose female group was due to renal lesions. Thus, the high dose, 1000 mg/kg/day may be too high for the 2-year carcinogenicity study in this species.

Comment: The results for the toxicokinetic satellite groups were faxed on 5/24/99 to the Division.

OVERALL SUMMARY AND EVALUATION

Introduction:

The toxicity profile of Ro 64-0796 and the active metabolite, Ro 64-0802, have been fully explored as per the ICH guidelines. The acute, subacute, and subchronic toxicities have been studied in rats, mice and marmosets up to 9 months. The studies to assess carcinogenicity are in progress in rats and mice. The toxic potential to many aspects of reproduction and the developing embryos, fetuses, and neonates has also been adequately investigated at dosages that gave systemic exposure >25 fold over human exposures. The genotoxicity of the prodrug, the major and active metabolite, and several of the degradation products in the synthetic process had also been studied. Absorption, distribution, metabolism, and excretion (ADME) studies have also been conducted in various species. Except for a few toxicities noted in the general and reproductive toxicology studies, Ro 64-0796 appeared to be well tolerated in all species studied. The toxicities associated with the GI system, kidneys, and bones and their clinical relevance will be discussed in the following section.

Clinical Relevance of Safety Issues:

The toxicities to the GI system, kidneys, and bone observed in the nonclinical toxicology studies may have some clinical relevance. They will be discussed individually.

GI system Prodrug, Ro 64-0796, was very irritating to the primate GI tract. Emesis and salivation were associated with doses greater than 150 mg/kg. Slightly to moderately severe gastric mucosal inflammation, atrophy, hemorrhage, erosion, and ulceration were associated with doses of 1000 mg/kg. The drug had to be administered as 2 separate daily doses to reduce the drug-induced emesis and gastric irritation. Although it appeared to be less toxic to the rodent GI system, excessive postdosing salivation was observed in some reproductive toxicology studies. In clinical trials, vomiting, nausea, and abdominal pain occurred in a higher number of patients receiving 75 mg b.i.d. Ro 64-0796 as compared to those receiving placebo. Patients who received 150 mg b.i.d. in the phase I and II studies had worse GI-related adverse events. This toxicity will probably be dose-limiting in humans.

Kidneys Slight renal dysfunction manifested as slight plasma and urine electrolyte imbalance (less than 1-fold as compared to the controls) and slight changes of other clinical chemistry parameters (e.g., plasma urea nitrogen and creatinine levels) were evident in mice, rats, and monkeys. These changes did not worsen following long term (up to 9 months) drug administration. Some changes may have improved, suggesting adaptation. Chronic progressive nephropathy, corticomedullary mineralization, tubular mineralization (seen only in the one-month rat study), tubular vacuolation, basophilic tubules, and focal nephropathy were observed in rodents. However, the incidence and severity of these histopathological changes did not worsen and remained minimal to slight in severity after 6 months of repeated drug administration at a dose of 1000 mg/kg/day in rats (~300X and 40X human exposure to Ro 64-0796 and Ro 64-0802, respectively). No histopathological changes were associated with Ro 64-0796 administration in marmosets. It has been suggested by an expert pathologist consulted by the sponsor that these renal changes in rats may be a result of the high content of phosphate in the Ro 64-0796/002 (a phosphate salt) that would

negatively influence the dietary calcium/phosphate ratio in a species known to be sensitive to this kind of change. Thus, the histopathological changes seen in rats, he reasoned, must be species-specific. However, other explanations also exists. The pharmacokinetic data have indicated that rodents do not hydrolyze Ro 64-0796 to its active metabolite, Ro 64-0802, as efficiently as primates. The urine prodrug/active metabolite ratios for rats and marmosets were 1:3 and 1:15, respectively. Since the free base form of prodrug (the form expected to exist in kidneys) is expected to be 100-1000 times less soluble than the active metabolite, the mineralization in the high dose rats may be partly due to the precipitation of the prodrug. This scenario may have some clinical implications in severely hepatic impaired patients who do not convert prodrug to active metabolite as efficiently. However, there is a 5-10 fold of safety margin since humans hydrolyze prodrug 5-10 fold more efficiently as compared to rats. All of the renal changes in animals were reversible after a period of drug-free recovery.

Bone There were 3 intercurrent mortalities in the 9 month toxicity study in marmosets related to osteomalacia. These deaths were not dose-related and occurred in the low and mid dose groups only. They may be attributed the pre-existing osteomalacia. However, slight but statistically significant elevations of alkaline phosphatase levels were detected in high dose animals (≥ 1000 mg/kg/day) in a few rat toxicity studies. In addition, incomplete or no ossification of various bones and various other minor bone abnormalities and variants were observed in both rats and rabbit fetuses exposed to Ro 64-0796 *in utero*. These bone findings in the reproductive toxicity studies will be included in the Label. All of these bone-related findings suggest that Ro 64-0796 and/or its active metabolite may have some effect on the ossification process. The bone effect may have less clinical relevance in the treatment regimen but may be of clinical concern in a prophylaxis regimen.

Juvenile The exposure to prodrug in neonatal (4-21 days old) rats was ~ 6-10-fold higher than in adult and weaned (3-7 weeks old) rats. There were no differences in the toxicity profile between the adults and weaned/unweaned juvenile rats. However, the juvenile rats, especially the unweaned ones, tolerated lower doses of Ro 64-0796. It was suggested that infants and children under 5 (the age range not studied clinically) may have lower tolerance to the drug than adults and older children.

Conclusions:

Ro 64-0796 and its active metabolite, Ro 64-0802, are generally well tolerated and have a good safety profile at fairly high dosages and systemic exposures in all nonclinical animal species studied. The results from the nonclinical pharmacology/toxicology studies do not raise any clinical safety concern for the proposed treatment regimen (75 mg b.i.d. for 5 days) except perhaps during pregnancy and breast-feeding and for very young children. The bone abnormalities associated with rat and rabbit fetuses exposed to the drug *in utero* will be communicated in the drug Label. GI irritation is apparent and detected in clinical trials. This toxicity will probably be dose-limiting. The relationship between bone toxicity and drug exposure and duration of administration is unclear. However, the data do suggest a link between bone effects and Ro 64-0796 administration. Finally, the renal toxicity may be a concern for severely hepatic impaired patients who will take this drug prophylactically. The safety margin for this toxicity is 5-10 fold for healthy patients. There are no issues that would preclude the approval of this drug.

/s/

James Farrelly
12/14/00 01:50:03 PM
PHARMACOLOGIST