

excretion amounted to 70-80% of intake concomitant with reduction of lipid absorption by about 50%. Fractional lipid excretion was only modestly higher at 1000 than at 10 mg/kg/day. The difference in the fractional effect on both lipid parameters was attributed to the increase in food consumption. Triglycerides accounted for more than 80% of fat excretion (glycerides and FFA) at all dose levels. FFA excretion showed a significant increase at the low dose with lesser apparent effects at higher doses. Excretion of DAG was increased at all dose levels - 5 to 10 fold in males and up to 40 fold in females (due to lower values in female controls). All treated groups showed increased daily bile acid excretion in contrast to previous rodent or dog studies in which either no effect or reduced excretion was noted.

The concentration of soluble FFA and BA was reduced at the two higher dose levels with no apparent effect at the lowest dose. According to the sponsor, although diglyceride concentration was increased, the major fraction will likely be dissolved and sequestered in the at least 10-fold larger amounts of triglycerides and thereby reduce colonic epithelial exposure.

Effects on the concentration of total and soluble lipids in the lumen of the rectum showed statistically significant increased DAG at the lowest dose and reduced concentration of FFA at the largest dose. Soluble surfactants, FFA and BA were not changed. Changes in fecal and rectal lipid parameters were qualitatively similar except for the soluble surfactants that were reduced in fecal but not in rectal water.

Antigenicity Study of Ro 18-0647 in Guinea Pigs:

Lot 3034009.

Q.A. - Present

Research Report J-146'466 dtd Oct 95.

An active systemic anaphylaxis (ASA) and a passive cutaneous anaphylaxis (PCA) test were performed to assess an antigenic potential of the drug.

Male Hartley guinea pigs were immunized with 30 mg/animal of Ro 18-0647 plus Freund's adjuvant (FA), or 1 mg/animal of Ro 18-0647-guinea pig serum albumin (GPSA) mixture plus Freund's adjuvant, and subjected to ASA test and PCA test challenged with 30 mg/animal of Ro 18-0647 or 1 mg/animal of Ro 18-0647-mouse serum albumin (MSA) mixture. Immunization was performed three times at 2-week intervals with antigen solutions injected intradermally into the dorsal region. Challenging injection was performed 2-days later.

Positive reactions were seen in 1/10 guinea pigs immunized with Ro 18-0647-GPSA mixture plus FA and challenged with Ro 18-0647-MSA mixture. Positive PCA reactions were also observed in 7/19 guinea pigs immunized with Ro 18-0647-GPSA mixture plus FA and challenged with Ro 18-0647-MSA mixture. The sponsor concluded these reactions to be caused with reactions to carrier protein MSA, rather than positive antigenicity of Ro 18-0647. No positive ASA reaction nor PCA reaction was seen in guinea pigs immunized with Ro 18-0647 plus FA, and in those immunized with Ro 18-0647-GPSA mixture plus FA and challenged with Ro 18-0647 alone.

The sponsor thus concluded that there was no positive reaction indicating an antigenicity to Ro 18-0647 under this experimental condition.

APPEARS THIS WAY ON ORIGINAL

Chemical vs Fermentation Process**Ro 18-0647/081 and Ro 18-0647/090: 2-Week Oral Toxicity Study in Male Wistar Rats**

[Han Ibm: WIST (SPF)]: F. Hoffmann-La Roche Ltd. Study Protocol 185 P 94. Research Report B-159'673 dtd May 1995. Ro 18-064/081 (fermentation process) Batch GQC0063; Ro 18-0647 (synthetic material) Batch PT2157T62. Q.A. - Present

The object of this study was to compare material produced by fermentation (Ro 18-0647/081) to that produced by chemical synthesis (Ro 18-0647/090). [Each test article contained about 50% of active ingredient.] Each form was finely milled and utilized for the preparation of pellets which were then also finely milled.

8M;8F Wistar rats were assigned to low (B) and mid-dose (C) groups and 13M;13F to control (A) and high dose (D) groups and comparison Group E (synthetic). 3M;3F per group were used for toxicokinetic study. Ro 18-0647/81 (fermentation) was administered orally (feed admix) for 15 consecutive days at daily doses of 20 (B), 200 (C), and 2000 mg/kg (D). Group E received Ro 18-0647/090 (synthetic) at a daily dose of 2000 mg/kg. Control (Group A) received powdered diet alone. Recovery period: 14/15 days for 5/sex from groups A, D and E.

There were no deaths and no drug-related clinical signs, body or organ weight changes or effects on hematology (see below), urine analysis, necropsy or histopathological (Groups A, D and E) findings.

Food consumption was increased by 6.1 to 9.4% for males in groups C, D and E and by 4.3 to 9.4% for females in all dose groups.

Hematology: Formation of crystals occurred in blood films from 4 females treated with 2000 mg/kg fermentation product (Group D) and from 5 females treated with 2000 mg/kg synthetic product (Group E). [It is stated that the electronic leukocyte count of blood samples of these rats resulted in erroneously high values.] According to the sponsor, the marked hypertriglyceridemia among Groups D and E females was considered to be the reason for the formation of crystals seen in the blood films. Recovery animals did not show any abnormalities in either blood films or leukocyte counts at the termination of the recovery period.

Serum bilirubin concentrations were elevated in male rats in Group D by 40% and Group E by 37% and among females in all dose groups in a dose-dependent manner by 8 to 176%. Serum triglycerides showed a dose-related increase of 89-1113% for males to 10 to 5254% for females. Serum cholesterol was increased 21 to 86% among males and 5 to 108% among females. There were no statistical differences in these parameters after recovery.

Ophthalmologic examination showed no treatment-related findings.

Various other sporadic findings were not of apparent biological importance.

Based on this study the sponsor considered the toxicological profile of the fermentation and synthetically produced products to be similar. However, in some cases it would appear that although quite variable (n=only 3) mean AUC values for the fermentation product might be slightly larger than that of the synthesis product at the 2000 mg/kg/day level. The systemic exposure to unchanged Ro 18-0647 (and to Ro 42-3988) was in most instances higher (in general 2-3 fold for Ro 18-0647) in female than in male rats.

Vitamin Supplementation**Ro 18-0647/008: 15-Month Oral (dietary admix) Study to Monitor the Efficacy of Vitamin Supplementation in Rats administered 1000 mg/kg/day Ro 18-0647/008.**

06098 dtd. May 1994. Research Report N-130940 dtd. Jun 1995. Project 323965; HLR Study Batch/Lot: 26031, 26033, 26034. Study Termination July 1993. Q.A. - Present

The purpose of this study was to monitor the efficacy of once a week oral supplementation of a lipid soluble vitamin mixture in rats treated with 1000 mg/kg/day of Ro 18-0647/008 for 15 months. This study was conducted in parallel

with the oncogenicity study of Ro 18-0647/008 Study 319623; HLR Study 06087) in rats to monitor the adequacy of the vitamin supplementation; the dose of 1000 mg/kg/day is the same as the high dose used in the rat oncogenicity study.

Diet: Kliba rat 30-343-7 - contains 7.94% [82.4 mg/g (8.4%) by analysis] of crude fat (22% of calories) and 0.98% of calcium.

All rats were given once weekly oral (gavage) doses of 0.4 ml of vitamin supplement independent of weight - thus females received ca 50% more vitamin than males.

Vitamin supplement: (as mg/ml peanut oil) Vitamin A (alcohol) 1,725; dl- α -tocopherol 100; Vitamin D₃ 7; Vitamin K₁ 1,400; β -Carotene 7.

35 rats/sex/group were allotted to two groups 0 and 1000 mg/kg/day of Ro 18-0647/008 in a dietary admix in pelleted food. 5 Rats/sex/group were sacrificed after 1, 2, 3, 4, 5, 9 and 16 months of treatment, for determination of plasma levels of vitamins A, D₃, and E, and liver levels of vitamins A and E. [Study was planned for 15 months, however, actual terminal blood collection and sacrifice were at 16 months.] Animals were discarded after liver tissue removal.

Results:

Plasma levels of vitamins A, D₃, and E showed no consistent adverse effects. Plasma vitamin E levels were approximately halved in males sacrificed after 1 and 2 months as was vitamin D₃ in males sacrificed after 2 months. After this, these values were similar to or on one occasion higher than controls.

For females plasma vitamin E levels were slightly decreased by ca 24% in rats sacrificed after 2 months, but increased ca five-fold for females sacrificed after 5 and 9 months, but were similar to controls at other time periods.

However, male hepatic vitamin A levels were decreased ca 20%; that of females was similar to controls. It is reported that effects on the hepatic concentration of vitamin A in male rats were considered to be minimal and no attempt was made to correct the ca 20% deficit in male rats. [During the treatment period mean vitamin A levels increased continually in both treated and controls, presumably, according to the sponsor, as a result of normal age-related accumulation.]

On the other hand, compared to controls hepatic vitamin E levels were on average ca 30% (17-61%) for males and ca 44% (16-75%) for females. After about 26 weeks of dosing, d- α -tocopherol was substituted for dl- α -tocopherol in the formulation to attempt to restore hepatic levels of vitamin E (The former is considered to be more readily bioavailable than the latter). However, only a negligible effect was obtained.

There were no deaths considered associated with treatment. One treated female was sacrificed in poor condition after about one year of dosing.

Clinical signs were similar to that of controls. Reportedly treatment had no influence on the frequency or group distribution of palpable masses.

Male rats showed a minimal, transient body weight gain deficit (4.6 - 7.1% weeks 5 - 14 but not wk 11). Thereafter weights for these rats were consistently lower (not statistically significant) than controls.

There was increased food intake by male and female rats. Increases were on the average 30% for males and 34% for females. Reportedly, other findings were similar to that of controls.

Drug intake averaged over the 15 month period was 1000.2 mg/kg/day for males and 1009.3 mg/kg/day for females.

From Sponsor's Tables:

Plasma Vitamin Levels (Percent of Control) in
Male and Female Rats Treated with Ro 18-0647/008

Months of Dosing	Vitamin A		Vitamin D ₃		Vitamin E	
	Males	Females	Males	Females	Males	Females
1	82.4	100.0	69.4	79.6	52.2	89.1
2	86.7	100.0	57.4*	86.3	51.8	76.4*
3	106.7	85.7	82.2	98.5	71.3	171.3
4	92.9	100.0	84.2	88.5	84.6	139.6
5	92.9	150.0	103.1	106.9	170.5	548.1
9	100.0	83.3	111.7	125.8	132.7	527.2
15	109.1	66.7	88.5	76.8	64.7	91.6
Mean	95.8	98.0	85.2	94.6	89.7	234.8
±S.D.	±9.9	±26.0	±18.6	±17.3	±45.1	±209.6

Hepatic Vitamin Levels (Percent of Control) in
Male and Female Rats Treated with Ro 18-0647/008

Months of Dosing	Vitamin A		Vitamin E	
	Males	Females	Males	Females
1	86.2	124.9	19.2	23.8
2	68.0	96.3	17.0	16.1
3	82.8	110.3	25.7	50.3
4	94.1	125.0	27.1	74.5
5	90.5	102.0	38.4	52.7
9	76.0	99.5	60.9	42.5
15	68.1	106.0	24.0	45.4
Mean	80.8	109.1	30.3	43.6
±S.D.	±10.4	±11.7	±15.1	±19.3

No clinical manifestations of hypovitaminosis were seen.

Fecal Analysis from Above Study (Research Report N-130940 Vitamin
Supplementation):

Research Report B-162'799. (Day 163-166 of 1000 mg/kg/day by diet.)
Treated rats showed larger food and lipid intake, increased fecal excretion and reduced absorption of lipids.

Ro 18-0647 markedly increased excretion of total lipids. Expressed as percentage of intake, output of total lipid amounted to 75.4% (controls 15.6%) for males and 75.3% (controls 15.7%) for females.

Fractional excretion of dietary lipids was markedly increased from 16% in controls to 72% in treated animals. The reducing effect on lipid absorption, due to a compensatory increase of food and lipid intake, was 58% (0.90 g/day) for males and 55% (0.63 g/day) for females which was less than the increase of excretion 1.47 and 1.14 g/day for males and females, respectively.

The increase in fecal fat was mainly due to triglycerides and to a minor extent diglycerides. However, due to the low fecal concentration of diglycerides in control rats (below the limit of detection), the corresponding concentration of treated rats was increased at least 7-fold. There was no significant effect

on daily excretion of unesterified fatty acids (large bowel lipolytic activity may have almost been abolished.)

Control group males showed higher daily excretion of bile acids (24.4 $\mu\text{mol/day}$) than females (16.0 $\mu\text{mol/day}$). Treated males, but not females, showed a reduction in daily excretion of bile acids.

Additional Studies (Impurities, Degradation Products, etc.)

Acute Studies: (single oral dose, 14 days observation) [Roche Research Reports B-164'579; B-119'090; B-161'219]

THL Intermediates: Ro 18-6934 [N-Formyl-(S)-Leucine]
Ro 47-4520 [Diisopropylazodicarboxylate (DIAD)]
Ro 19-3052/000 [THL-Hydroxy-Beta-Lactone (THL-HBL)]

Rats (M&F): Ro 18-6934 2000 mg/kg: No mortalities; no notable toxicity
Ro 47-4520 500 mg/kg: No mortalities; within a few hours after dosing piloerection and increased salivation were noted in 2/10 animals each. Moderate dyspnea occurred in 1M Day 7 and 1F Day 1. All recovered within 24 hrs.

Ro 1495/001 (diastereoisomer of THL, a contaminant): There were no mortalities following a dose of 1000 mg/kg. Behavior was similar to controls.

Ro 19-3052/000 2000 mg/kg: There were no mortalities and no relevant findings.

Research Report N-138802: Acute Oral Tox. In 2-Week Old Rats + 2-week recovery. The max. Limit dose of 2000 mg/kg Ro 18-0647/008 did not have any significant pharmacological or toxicological effect. Body weight decreases (non sig.) of -5% for males and -4% for females were seen on day 14.

Acute Toxicity of Ro 41-4522 in Mice: intravenous (iv) and oral (p.o.) Dose Range Finding Study: F. Hoffmann-La Roche Ltd Research Report B-167'656 dtd. Sep 1996.

Ro 41-4522 is a breakdown product obtained from Ro 18-0647/002 by thermolysis at 120°C for 3 days. SPF MORO strain albino mice were used. The HTD (highest tolerated dose) following i.v. administration was >250<500 mg/kg and after p.o. administration >4000 mg/kg. No signs of respiratory depression or other autonomic effects were noted. About 3 hrs. after the high doses of 250, 500 and 1000 mg/kg/p.o. transient signs of mild CNS stimulation (increased muscle tone, increased locomotion and hyper reactivity) were noted; at even higher doses of 2000 and 4000 mg/kg these signs were intermingled with and followed by mild signs of CNS depression (decreased locomotion, absence of rearing (vertical exploration movements) and motor incoordination).

Results are reported to show a very low acute toxicity in mice with results of the same order of magnitude as those of the parent compound.

28-Day Oral (gavage) Toxicity Study With THL-HBL in the Rat:

And [redacted] Project 371316 dtd Jan 95. Batch 33005. Diet: standard Kliba no 343. Q.A. - Present
THL-HBL - Ro 19-3052/000 [THL-Hydroxy-Beta-Lactone] - THL intermediate
THL-HBL was administered to 5M;5F/group SPF-bred Wistar rats (HanIbm) at dose levels of 0, 50, 200, 1000 mg/kg for 28 days.

There were no apparent treatment-related deaths (1F died day of necropsy after anesthesia for blood sampling) or clinical signs. Body weights were unaffected. Food consumption was increased at the highest dose (possibly due to lower fat absorption and food efficiency).

Hematology, Clinical Chemistry, and Urinalysis at the end of treatment showed no remarkable findings and the minor changes at the high dose were not toxicologically relevant. The slight statistically significant increase in RBC counts, Hb concentration and HCT in high dose females were within limits of historical data. There was a slightly increased total protein and globulin concentration in high dose males, and a slightly increased urea and uric acid concentration in females; these findings were reported within limits of historical controls. Group 4 males had a slightly increased overnight urine output and a slightly decreased specific gravity and osmolality - possibly attributed to greater fluid intake.

Organ weights for livers of high dose males were slightly increased - correlated with the slight increase in protein and globulin in males. Testes weights of males of the low dose group only were increased.

Macroscopically there were no treatment-related findings at necropsy.

Microscopically the only drug related finding was of an increased incidence and severity of hyaline droplet (alfa-2 μ -globulin - unique to male rats) deposits in the epithelial cells of the renal cortical proximal tubules.

Ophthalmoscopic examination showed no treatment-related abnormal findings.

Based on this study the no-observed adverse effect level (NOEL) THL-HBL was considered by the sponsor to be 200 mg/kg for males and 1000 mg/kg for females.

13-Week Oral (intubation) Toxicity Study in Rats with Ro 18-0647/008 (Orlistat) and its Degradation Products Ro 61-1227/000, Ro 43-2042/000 and Ro 47-2139/000 (06780): Hoffmann La-Roche, Inc., Nutley, N.J. Ro 18-0647/008 Bulk Lot 3084028 (Batch L183525; L1183565) Q.A. - Present

Following production, a new impurity, Ro 61-1227/000, was detected in new lots of Ro 18-0647/008 at concentrations of up to 0.1%. Two degradation products, Ro 43-2042/000 and Ro 47-2139/000, were also detected in finished capsules at concentrations of <0.1% at the time of release, increasing to <0.1% - 0.4%, at the end of one year at 25°C and 60% relative humidity.

This oral study was conducted in Charles River Crl:CD BR rats to characterize the degradation products of Ro 18-0647/008. The synthetic impurity, Ro 61-1227/000, and two degradation products, Ro 43-2042/000 and Ro 47-2139/000 were added to Ro 18-0647/008 at levels of 0.5%, 3.0% and 3.0%, respectively. These levels are greater than their proposed upper limit in orlistat capsules. [w/w = with or without degradation products]

Rats (12/sex/group Charles River Crl:CD BR) were dosed for 13-weeks by oral intubation (vol. 5.0 ml/kg/day) with 0 (canola oil) and 150 mg/kg Ro 18-0647/008 without degradation products and at 15 and 150 mg/kg/day with degradation products.

There were no treatment-related mortalities (1 low dose - apparent intubation accident). Clinical signs consisted of unkempt appearance, primarily of the anogenital region in both sexes of all treated groups.

Body weight gain of males was slightly less (ca 8%) than that of controls for treated rats with or without degradation products. Mean male body weight averaged ca 6% at the end of study. For females of the high dose with

degradation products body weight gain was slightly reduced (11%) for the last two months of dosing. At the end of study the lower mean body weight (7%) was statistically significant.

Food consumption was increased (ca 20% compared to controls) in both sexes of all treated groups after two weeks and remained so throughout treatment.

Hematology and clinical chemistry showed minimal to slight changes (within normal background ranges) in some parameters in rats with or without degradation products. Hematologically these consisted of increased platelet counts (150 mg/kg gpc ca 15%) and decreased platelet volume (ca 7%); increased APTT and PT in males (low dose 16 and 30%, respectively; high dose 47 and 60%, respectively). Clinical chemistry changes consisted of increased BUN [Males 21% in high dose (w/w)] and decreased calcium [M;F low dose ca 1.6% with deg. prod.; M;F high dose (w/w) 3.4%] decreased alkaline phosphatase [ca 32% high dose males (w/w)] and triglycerides [ca 32% in low dose males with degradation product and ca 42% in high dose groups (w/w)] in males.

Ophthalmic observations and neurologic examination showed no treatment-related effects.

Liver weights (absolute and relative) were slightly decreased in males of all treatment groups without any histopathologic correlation. Other organ weight changes were within background.

No treatment-related histopathologic changes were observed.

Treatment with Ro 18-0647/008 with and without degradation products was well tolerated and there did not appear to be any adverse effects of treatment attributable to the degradation products in this study.

Segment II (Teratogenicity) Rat Study of Ro 18-0647 Spiked With Three Impurities: Supplementary Oral Study for Effects on Embryo/Fetal Development in the Rat:

F. Hoffmann-La Roche Ltd., Switzerland. Research Report B-161'863 dtd Nov 1995. Study 193R94. Ro 18-0647/008 Batch 3074024 Q.A. - Present
 [Formulation: Ro 18-0647/114 Placebo (rape seed oil); Ro 18-0647/115 Orlistat 'pure' batch (with a total of 0.3% impurities); Ro 18-0647/116 Orlistat, spiked batch low dose; Ro 18-0647/117 Orlistat, spiked batch, high dose. Impurities in low and high dose = Ro 47-2139 (3%), Ro 43-2042 (3%), and Ro 61-1227 (0.5%).] Diet: Kliba 25-343-4 ad libitum.

Ro 43-2042/000 = N-Formyl-L-leucine (3S, 4R, 6S)-tetrahydro-3-hexyl-2-oxo-6-undecyl-2H-pyran-4-ylester

Ro 61-1227/000 = name not given

Ro 47-2139/000 = (2S, 3R, 5S)-5-[(S)-2-Formylamino-4-methyl-pentanoyloxy]-2-hexyl-3-hydroxy-hexadecanoic acid

The test compound was administered orally by gavage at doses of 0 (vehicle, rape seed oil), 15 mg/kg/day (spiked) or 150 mg/kg/day ('pure' or spiked), on Days 6 through 15 of gestation to 24 mated female rats [Wistar (RORO) SPF] per group (Groups 1-4).

Maternal Findings:

There were no mortalities in treated groups. 1F control died due to a gavage accident.

There were no adverse treatment-related clinical observations.

There was a slight trend for a reduced body weight gain during the treatment period of all orlistat-treated groups compared to concurrent controls. Upon cessation of treatment, the reduction in weight gain was made up.

Compared to concurrent controls, maternal food consumption was statistically significantly increased in all Orlistat treated groups with no difference between the 'pure' and spiked batch.

Necropsy of the dams did not indicate any drug-related adverse effects.

Reproductive Parameters:

All experimental groups showed comparable findings in numbers of corpora lutea, implantations, fetuses per dam, and resorptions. The incidence of females with total fetal death was 0, 2, 1, 1 Groups 1-4. Although not significant, the percent per group and percent per animal was slightly higher for the 150 mg/kg groups, pure and spiked, and the percent of resorptions per group was slightly greater for the spiked groups.

Fetal Parameters:

Fetal bodyweights and sex distribution were comparable among study groups.

Teratology Findings:

Some fetuses with externally visible abnormalities were observed in treated groups (Groups 2-4). The most frequent finding, 'protruding tongue' was seen in 6 fetuses from one litter in Group 2 (150 mg/kg/day, 'pure' batch) and in 4 fetuses from one Group 3 (15 mg/kg/day, spiked batch) litter. This abnormality was not seen in high dose Group 4 and was associated with abnormal head shape in 2 fetuses from Group 3. Skeletal assessment of the head of one of these showed incomplete skull ossification, the other was used for visceral examination. [It is reported that no 'protruding tongue' was seen in any of the fetuses in the preceding Segment II studies at dosages up to 800 mg/kg/day.] Other anomalies noted at external assessment were isolated without indicating drug adverse effects.

Compared to other experimental groups, the total incidence of visceral abnormalities (fetal and litter incidence) was slightly higher at 150 mg/kg (Group 2) - reportedly there was no pattern that indicated any compound effect. With regard to blood vessels the fetal incidence of the absence of brachiocephalic trunk was greatest in the 150 mg/kg 'pure' group.

Incidences of skeletal abnormalities, visceral or skeletal variation and retardations (fetal and litter incidences) were comparable among study groups. Although not reported as significant the incidence of total variations and of total retardations was increased in the low dose spiked Group 3.

Mutagenicity Studies with Impurities:

Ames Test - Mutagenicity Evaluation of Ro 18-0647/008 (Orlistat) spiked with Three Impurities (Ro 61-1227, Ro 47-2139 and Ro 43-2042): F. Hoffmann-La Roche Ltd. Study 190M94. Research Report B-163'242 dtd Nov 1994. Ro 18-0647/008 Batch 3084029. Q.A. - Present

Ro 18-0647/008 and the spiked solution were evaluated in parallel at 25, 100 and 400 µg/plate. The top dose was selected on the basis of an earlier study reporting precipitation of the compound making evaluation at higher doses impossible. No toxic effects were observed up to 400 µg/plate. A standard plate incorporation and a preincubation modification assay were performed in absence and in the presence of an exogenous metabolic activation system (S9).

Ro 18-0647/008 (Orlistat) and Ro 18-0647/008 spiked with three impurities [Ro 61-1227 (0.5%), Ro 47-2139 (3.0%) and Ro 43-2042 (3.0%)] were not mutagenic in the Ames test using six Salmonella typhimurium test strains TA1535, TA1537, TA97, TA98, TA100 and TA102 and Escherichia strain WP2uvrA.

Chromosome Analysis in Human Peripheral Blood Lymphocytes Treated with Ro 18-0647/008 (Orlistat) Alone and Spiked with Three Impurities (Ro 61-1227, Ro 47-2139 and Ro 43-2042): Study 211 M 94. F. Hoffmann-La Roche Ltd Report B-164'901. Batch 3084029 Q.A. - Present

Concentrations of 5000 µg/ml of Ro 18-0647/008 alone and of 2500 and 5000 µg/ml of Ro 18-0647/008 spiked with the three impurities were evaluated in the presence of a metabolic activation system after short-term treatment of 3 hrs. Concentrations of 200 µg/ml of Ro 18-0647/008 alone and 100 and 200 µg/ml of Ro 18-0647/008 spiked with three impurities were analyzed without metabolic activation after long-term treatment of 22 hours. Upper concentration limits were selected on the basis of toxicity (without metabolic activation) or the recommended maximal dose level for non-toxic compounds (with metabolic activation).

Ro 18-0647/008 (Orlistat) and Ro 18-0647/008 spiked with three impurities [Ro 61-1227 (0.5%), Ro 47-2139 (3.0%) and Ro 43-2042 (3.0%)] were not clastogenic in human peripheral blood lymphocytes.

Additional (more recent) Study Findings Include the Following:

Orlistat inhibits lysosomal acid lipase (an enzyme which plays a pivotal role in cellular lipoprotein metabolism) of rat intestinal mucosa in vitro.

Orlistat exhibits its therapeutic anti-obesity properties in the intestinal lumen whereas systemic exposure to intact parent drug is low. It appears highly unlikely, therefore, that inhibition of intracellular hormone-sensitive lipase, located in systemic organs could occur to any significant extent under normal therapeutic use of orlistat.

In vitro Ro 18-0647 was a very potent inhibitor of diacylglycerol lipase but almost inactive on monoacylglycerol lipase, and weakly inhibitory on phosphatidylinositol-phospholipase C.

Ro 18-0647/002 did not significantly inhibit the rat brain acetylcholinesterase up to a concentration of 10 µM. At 100 µM it produced a significant inhibition of the enzyme (36%). From this result, it was concluded that Ro 18-0647 is an extremely weak inhibitor of rat brain acetylcholinesterase with an IC_{50} exceeding 100 µM.

Orlistat is almost completely degraded by components of rat, dog, and human plasma during prolonged incubation at room temperature in vitro. Current data is reported to suggest that orlistat absorbed systemically may be subject to bioinactivation in the plasma compartment. Particularly in the rat, this process is likely to significantly attenuate inhibitory interaction of orlistat with lipolytic enzymes located at the vascular endothelium.

In the rat differences in biliary as well as urinary excretion may be explained by different absorption from different galenic formulations.

In younger rats (3 mos), the pharmacokinetic behavior of orlistat was characterized by a relatively low systemic plasma clearance (11.5 ± 2.2 ml/min/kg) which represents 15% of the liver blood flow. The volume of distribution at steady state was low (0.14 ± 0.05 l/kg) indicating limited extravascular distribution. The terminal elimination half-life could not be determined reliably. Following oral administration, the systemic bioavailability was far below 1%, probably due to low GI absorption and/or extensive first-pass intestinal metabolism. The pharmacokinetics of orlistat in rats were only slightly influenced by age and the systemic clearance was only slightly decreased in 1-year old rats (8.1 ± 1.2 ml/min/kg).

Orlistat assessed in dogs up to 248 hours post dosing showed a relatively low systemic plasma clearance of 13.5 ± 4.6 ml/min/kg; a large volume of distribution at steady-state of 114 ± 84 l/kg, consistent with the high lipophilicity of orlistat, and a relatively long apparent terminal half-life of 130 ± 30 hours. A parallel study in rats also demonstrated a low plasma clearance of 12.9 ± 2.1 ml/min/kg, with a high volume of distribution and slow rate of elimination. Reliable values for the last two parameters could not be calculated due to a non-exponential decline in the plasma concentrations.

Within 72 hours trapped $^{14}\text{CO}_2$ amounted to $\leq 1\%$ of the administered dose to two rats.

There is a very low systemic availability of oral doses of orlistat in both animals and humans. In beagles orlistat was characterized by a relatively low systemic plasma clearance i.e. 5.0-8.5 ml/min/kg, which represents 12-20% of the liver blood flow. The volume of distribution at steady state appeared to be relatively low (0.2-0.4 l/kg) in the range of extracellular space. Terminal elimination half-life could not be determined here (5 hrs. previously reported).

The pharmacokinetics of Ro 18-0647/008 was studied in rats during 10 days of oral administration at 800 mg/kg/day. Absorption was relatively rapid with peak concentrations (mean, n=2) of 432 (day 2) and 813 ng/ml (day 10) within 1 hour of administration, followed by a high clearance from plasma. Systemic exposure was low compared to the dose and inter-individual variations in plasma concentrations were high. Compared to day 2, day 10 showed a 2-fold higher systemic exposure.

Plasma levels were measured in rabbits (Burgundi) during a 13-day repeat oral administration at 800 mg/kg/day (in rape seed oil Protocol 228R93) and following a single oral administration at 800 mg/kg [in SSV (0.5% methylcellulose, 0.4% Tween 80, 0.5% benzylalcohol and 0.9% NaCl) Protocol 063/94-Co]. Conditions were similar to those of the Segment II Rabbit study (GCR B-153'508).

The drug in rape seed oil produced only sporadically extremely low plasma concentrations of unchanged Ro 18-0647 in the range of 9.62 to 28.9 ng/ml, observed in less than 20% of the plasma samples collected. [These data indicated that the systemic exposure of dams to unchanged drug in the Seg II rabbit study was extremely low.]

Attempts were made to increase the systemic exposure of rabbits to Ro 18-0647 by using the SSV formulation which was reported to have given satisfactory results in the rat during segment II trials. Following a single dose oral administration at 800 mg/kg to female rabbits the systemic exposure to Ro 18-0647/008 was again extremely low, with plasma concentrations in the range of 15.5 to 31.3 ng/ml achieved within 15-30 min of dosing (with the exception of one test rabbit for which it is said that misdosing might have occurred: plasma concentration of 340 ng/ml at 15 min.).

Whole body phosphor imaging was carried out in the male rat following single and multiple (over 30 days) oral administration of [^{14}C]-Ro 18-0647/004. Interim results showed that Orlistat related material was largely confined to the GI lumen. Despite doses of 50 mg/kg (0.5 mCi/kg/d) only low or non quantifiable levels of orlistat related materials were present in tissues including intestinal walls (<5% of that of luminal contents). Mild accumulation occurred during the multiple oral dosing. Accumulation factors were estimated at ca 3 for the liver and kidney and ≥ 5 for white fat, based on 24 hour tissue levels. The increase in tissue levels parallels the increase in plasma levels of orlistat during long term toxicology studies in rats. Organs or tissues in order of decreasing

concentration after 24 hours: after a single dose - GI contents >> urinary bladder, GI walls, liver, kidney, white fat; after multiple doses - GI contents >> urinary bladder, GI walls, white fat, liver, adrenal, kidney, brown fat, preputial gland, skin, bone marrow, Harderian gland, pancreas, lung, thyroid, cardiac muscle, submaxillary salivary gland. Concentrations were lower to non-detectable after 72 and 120 hours.

In vitro studies using isolated perfused rat gut have shown that orlistat is metabolized before and/or during its passage across the intestine. Comparing the relative rates for the disappearance of the test compound and the formation of products indicated that orlistat is metabolized within and/or during its passage across the intestine. Due to technical difficulties, quantitation was not possible in this study.

A study was undertaken to elucidate the structure of the compounds formed by incubation of THL (Ro 18-0647) in rat or human plasma for several hours under conditions close to physiological ones (pH 7.9, 37°C). A similar study was performed with Ro 40-1379, a putative metabolite of THL sharing the 4-membered ring structure.

When THL is incubated in rat or human plasma, it is mainly transformed to a more polar compound which was identified as the open ring compound Ro 42-3988 (M1) which seems rather stable in plasma but not in organic solvent or under slightly acid or basic conditions. Another compound was detected whose structure may correspond to the 6-membered ring lactone obtained by recyclization of Ro 42-3988. The formation of Ro 40-1379 was not observed.

During incubation of Ro 40-1379 with rat or human plasma, the formation of the open compound Ro 42-2556 was detected. Besides this compound the corresponding 6-membered ring lactone Ro 41-9252 was observed.

This study shows a qualitative but not quantitative picture of compounds obtained in rat or human plasma from Ro 18-0647 and Ro 40-1379. Both compounds are unstable in human or rat plasma yielding open ring structures or recyclized 6-membered ring lactones. In the opinion of the sponsor due to the hydrolysis of the 4-membered ring lactones, the risk of inhibition of enzymes in the circulation is probably of minor importance.

Research report B 165'960 concerns plasma levels of metabolites M1 (Ro 42-3988), M3 (Ro 42-2556) and M9 (Ro 61-0591) during short- and/or long-term toxicity studies in mouse [Protocol RCC-346860 (2 yr CA study)], rat [Protocols RCC-359550 (2 wk tox study), and RCC-319623 (2 yr CA study)] and dog [Protocol 116P94 (1 yr tolerance study)].

The small fraction of orlistat absorbed (typically 5-10%) following oral administration undergoes (in rat) extensive presystemic (intestinal) metabolism. Thus, the major chemical species which reach the systemic circulation (in rat and dog) are biotransformed products. The major plasma metabolites in both rat and dog are M9 (Ro 61-0591) and the glucuronic acid conjugate of M13 (Ro 61-0593). In human plasma, two major orlistat metabolites were identified, namely M1 (Ro 42-3988) and M3 (Ro 42-2556).

Various species differences are indicated regarding the contribution of orlistat metabolites to the overall systemic exposure of test animals. 97-99% of the systemic exposure in the mouse was related to metabolites, mainly M3. Metabolites in the rat contributed ca 50-60% to systemic exposure and in the dog systemic exposure to orlistat represented 60-80% of the total exposure at 1000 mg/kg. Considering the sum (orlistat + M1 + M3 + M9 + M13 + M13-glucuronide) in plasma, figures of the same order of magnitude were seen in mouse, rat and dog

for similar administered doses, indicating that the extent of gastrointestinal absorption is likely to be similar in all three species.

The sponsor also included these additional findings:

•A dose-proportional systemic exposure, with the exception of female mice in the 750-1500 mg/kg dose range, of rats for doses of 150 to 500 mg/kg and of male dogs for doses of 100 to 1000 mg/kg. They indicate that the disproportionate increase of systemic exposure with dose probably results in the mouse from a dose-dependent increase of the GI absorption, and in rat and dog from both increased absorption and saturation of metabolism. •A moderate accumulation of orlistat (3-5 fold) and metabolites (2-3 fold) in rat plasma over the 2 year treatment, partly due to an age-related decrease of clearance. •A 4-5 fold decrease of the systemic exposure of dogs over the 1-year treatment, due to a time-dependent decline of the GI absorption during the chronic dosing. •A higher systemic exposure of the female test animals, except for dogs in the 1000 mg/kg dose group, due to better GI absorption.

It has been shown that if M2, Ro 40-1379/001 (Batch 4433.197), a potential metabolite of orlistat with the characteristic four membered lactone ring preserved (retains the lipase inhibitory activity of the parent drug) is formed, it is stable enough to be detected in rat, dog, and human plasma. After 4 months storage, recoveries are between 92-98% (stored at -70°C) or 43% in rat plasma, 81.3% in human plasma, and 98% in dog stored at -20°C for 4 months. Values were considerably less after 6 hrs at room temperatures or following freeze-thaw conditions.

The metabolic profile of orlistat was investigated in urine of different species, including man, after single intravenous or oral administration of Ro 18-0647/004 [¹⁴C-labeled (lactone ring) solution in glycofurol 75 (i.v); suspension in 5% skim milk rat, dog (p.o.); gelatin capsules, (p.o.) human]. Only about 1.2 (human) to 10% of the radio-labeled dose administered was found in the urine. The parent drug, orlistat, was metabolized extensively. Qualitatively metabolic profiles were similar in the various species and methods of administration. Four major metabolites were found in the urine in various proportions which included Ro 61-0593 (M13), a lactone form, Ro 61-0591 (M9), opened form, Ro 61-2257 (M10) and the glucuronic acid conjugate of Ro 61-0593. The glucuronic acid conjugate of M13 is found in human and dog urine only and M10 is missing in human urine samples. Thus the major metabolites found in human urine are M9, M13 and the glucuronic acid conjugate of M13. Polar metabolites accounting for less than 0.3% of administered dose were also found.

In another study after oral administration of ¹⁴C labeled orlistat (Ro 18-0647/004) to healthy human volunteers, radioactivity was mainly excreted in feces, and unchanged parent drug was the major drug-related compound isolated (~90% of the radioactivity extracted in n-hexane or in ethyl acetate).

In vitro binding studies have shown that in human and rat plasma, the concentration of orlistat was higher in the lipoprotein fraction than in albumin, indicating a strong affinity for lipoproteins. 40 to 50% of orlistat present in plasma was associated with the lipoproteins, in spite of their lower plasma concentration compared to albumin. Orlistat was highly bound to lipoproteins, independently of the concentrations studied (3.0 to 14.8 µg/ml). [Binding was determined by centrifugal ultrafiltration, and specific binding values might be overestimated due to adsorption to the material.]

Reprints: This submission also contained a number of reprints including in vivo and in vitro studies on the diverse properties of orlistat as well as various studies of clinical obesity and its sequelae. These also included the following:

A. Stark *et al.* [Digestive Diseases and Sciences, Vol. 40. No. 5 (May 1995), pp 960-966.] found that circular smooth muscle cell size in the distal colon was significantly increased following cellulose feeding but was less pronounced in the case of pectin. They concluded that fiber supplementation leads to morphological changes in the rat intestine including changes in length and tunica muscularis volume.

T. Oku [Nutrition Research Vol. 15, No. 9, pp 1355-1366, 1995] concluded that morphological changes induced by dietary fiber in the (rat) large intestine are reversible and might be one of the adaptations to response to the variable environments in the GI tract.

Labeling: Needs modification. See Comments section.

Summary, Comments and Conclusion:

NOTE: Considering the length of this NDA (some 120 volumes of pharmacology) and the numbers of reviews of IND [REDACTED] this section will be presented in the form of a summary with comments. Due to minimal absorption clinically, emphasis will be on ADME etc. [For further details regarding findings, numerical values etc. see table of contents for location of studies and individual reviews.]

KEY PRECLINICAL FINDINGS FOR ORLISTAT

This listing provides a summary for quick reference to key aspects of preclinical data for Orlistat. A more complete summary of each topic follows this listing.

1. For dose comparisons, the clinical dose of 120 mg TID = 360 mg daily. Based on a 70 kg human, this is approximately 206 mg/m². AUC for human exposure at the clinical dose is estimated to be 13 ng•h/ml. AUC determinations are based on total drug levels.
2. ADME: At high doses in animals, Orlistat is systemically absorbed. Systemic absorption is greater than dose proportional and appears to increase at a given dose with chronic exposure. Dose comparisons based on AUC are made on the basis of the early (lower) exposure ratios to provide a conservative estimate. In contrast, at clinical exposure levels, there is only a small amount of orlistat absorbed in humans. The increase in absorption seen in animals with chronic exposure does not appear to occur with clinical exposures. Systemically absorbed Orlistat is highly metabolized and bound ~ 99% to plasma proteins. Metabolic profile is qualitatively similar in all species.
4. Toxicology: Most of the findings were associated with the expected action of Orlistat. In rats, the following was observed: Increased fecal fat excretion (which led to loose or soft stools, unkemptness and oily fur). As body weights decreased, increases in food intake were observed. These increases did not compensate for the lost body weight. Hypertriglyceridemia, hyperbilirubinemia, increased plasma cholesterol, amylase and BUN were observed. Decreased liver concentrations of vitamins E and A were observed, sometimes accompanied by