3. Body Weight/Food Consumption (FC)
No significant dose related BW or FC responses .
4. Hematology /Bone Marrow
Decreased RBC, Hb, and Hct values for 3 of the 4 (one male, two female) in 100 mg/kg dose. This change was evident in days of 30 and 50 samples with RBC values ranging from 14.7 to 28.1% below day 1 levels.
Bone marrow from the right femur at study termination from a female dog in 100 mg/kg showed to be anemic. Microscopically the marrow was normal.
5. Serum Chemistry
At high dose, slight reductions in serum cholesterol noted for 3 of the four dogs; the lowest levels were noted on day 89 (approx. 50 mg/dl below pretest values). Slight elevated serum alanime transaminase (ALT) noted for 2 of the 4 on day 89. These dogs had pretest values of 29 to 36 u/l, the day 89 levels were 79 and 73 u/l.
6. Urinalysis
No dose-related changes at termination.
7. Organ Weights
Slight increases noted in liver (both sexes) and heart (females only) weights as % necropsy body wts in both 10 and 100 mg/kg groups.
Testicular wts were low for one of the two 100 mg/kg dosed males.
8. Gross and Histology Observations
Slight to mild subacute hepatitis noted for 3 of the four 100 mg/kg dose group, bile stasis was noted for one of these dogs. Also, bilateral testicular degeneration was noted for one of the two male 100 mg/kg dose recipients.

f. Three-Month Oral Toxicity with 1-month Recovery:
o Drug Dosage
[redacted] in a citrate-based [redacted] was administered orally for 90 (male) or 91 (female) days to beagle dogs. In addition to vehicle control (C), a daily dosage of 30 (L), 60 (M), and 150 (H) mg/kg free base of [redacted] was given. C consisted of 4 males and 4 females, and L, M, and H each consisted of 6 males and 6 females. In all groups, one half of the total daily dosage was administered in the a.m. and the other half in the p.m. approx. eight hours later.
o Results
1. Observed signs
Clinical signs associated with drug treatment included vomiting, diarrhea and soft stool, anemia manifested as a pale oral mucosa and abdominal distension. The frequency appeared dose-dependent.
2. Body Weight/Food Consumption (FC)/Water Consumption (WC)
No effect on appearance, BW, or FC was observed. Males in L, M groups, and females in M had a statistically signif. increase ($P < .01$) on some days during the studies in comparison with control. These findings were however considered incidental.
3. Ophthalmic Exams
No treatment effect
4. Laboratory tests

- o Hematology: A signif. ($P < .01$) reduction of Hct, Hb and RBC in all gps on days 30, 58 and 90 when compared to control and these decrease was dose-dependent. The changes in erythrocyte indicated a severe dose-related anemia and the type of which was normocytic and normochromic.
- o Serum Chemistry
  A reduction of serum albumin and total protein in all groups on days 30, 58 and 90; and increased BUN in H on day 30 and in M and H on days 58 and 90; an increased LDH level in M, H on day 30 and in all groups on days 58 and 90; increased serum CPK in L, M, and H on day 30, and in L and H on day 58. Serum cholesterol was lowered in H males and in all females with drug-treated groups on day 30, in all groups of both sexes on day 58, and in all females on day 90 as compared to controls. This cholesterol lowering was regarded as a possible pharmacological effect of drug. A higher Cl in H on days 30, 90, a lower K in M on day 58, and a lower Na in H on day 90 were also noted. These ionic changes were probably due to vomiting and diarrhea.

  No treatment-related abnormality was observed in urinalysis.

5. Organ Weights
   Increased adrenal and heart (all), kidney(H), liver (M,H) of soth sexes and decreased prostate (M,H) and splenic weight (M,H).
6. Pathological Findings
   Pathological findings were diverse which included an increased amount of clear abdominal effusion in some of the animals in H, and an enlarged liver which had ruptured in the 2 unscheduled sacrifice males from H, hydropericardium in all drug-treated groups of animals, hepatic congestion, epicardial mesothelial cell proliferation, chronic inflammation and thickening of the epicardium and extramedullary hematopoiesis in the liver and spleen in response to anemia in all gps, hypocellularity with dilated venous sinous in the marrow of the rib and and sternum of M, H males and H females, edema in the marrow of femur and serous atropy of fat in the H males, an excessive number of developing follicles in the ovaries and focal epithelial dysplasia of the mammary gland in all drug-treated female gps, atrophy of prostate in M and H, and excessive involution of the thymus in H animals. It was concluded the [redacted] was poorly tolerated due to multiple toxicities when given orally to dogs at a dosage of 30 mg/kg/d or higher for 3 months.

4. Monkey (Cynomolgus)
   a. 4-Day Oral Bioavailability Study:
   The objective of this study was to determine the bioavailability of [redacted] in monkeys after administrating four different oral formulas, one each successive day, in order to discern if the monkey could be a better animal model. Two females received 10 mg/kg and 2 females had 20 mg/kg of each formulation. Assays of the serum levels of drug disclosed that the solution administered on day 2 resulted in the highest serum levels in three of the four monkeys. The remaining monkey,

Monkey #2 (10 mg/kg), vomited shortly after dosing of [redacted] soln. In addition, a large degree of intersubject variability was present and no dose proportionality was noted when serum levels from Monkeys 1 and 2 (10 mg/kg) were compared to those from Monkey 3 and 4 (20 mg/kg). Thus, the findings indicated the monkey did not offer any improvement over the dog.

C. Mutagenicity Studies

a. Ames Assay:

[redacted] (dissolved in dimethylsulfoxide) was tested for bacterial mutagenicity using Salmonella typhimurium strains TA98, TA100, TA 1535, TA1537, and TA 1538. Doses for the assay were 250, 500, 1000 and 2000 ug/plate. The test results showed no evidence of bacterial mutagenicity at any dose, either in the presence or absence of an in vitro liver homogenate (S-9) metabolic activation system.

b. CHO/HPRT and AS52/XPRT Mammalian Cell Forward Gene Mutation Assays:

[redacted] was evaluated concurrently in the CHO/HPRT and AS52/XPRT (a genetically engineered cell line which is more sensitive to chromosome breakage than the CHO cell system). Dose levels, elected based on range-finding studies, were o.167, 0.50, 1.67, 5.00, 16.7, 50.0 167, 500, 1670 and 5000 ug/ml. The limit of solubility of U-72,107E was exceeded above 167 ug/ml, and cytotoxicity was noted in both cell lines in the absence of S-9. From this prescreen, doses of 25.0,50.0, 75.0, 100, 200, 300, 400 and 500 ug/ml with S-9 and 25.0, 50.0, 75.0, 100, 125, 150, 175 and 200 ug/ml without S-9 were evaluated. There were no dose-dependent or three-fold statistically significant increases in the average mutant frequencies of either cell line due to treatment with drug. Thus, [redacted] was concluded as nonmutagenic.

c. Unscheduled DNA Synthesis (UDS) Assay:

U-72,107E was assayed in the UDS to see if DNA repair occurs following chemically induced DNA damage. Two assays were made, each using rat primary hepatocyte cultures prepared by collagenase perfusion of the liver from a male F-344 rat. The cultures were fixed, washed, mounted on microscope slides and evaluated for UDS by autoradiography. In each test negative controls (dimethylsulfoxide-solvent- and untreated medium) and a positive control (2-acetylaminofluorene) were used.,The results did not induce a significant increase in UDS over the solvent control. Hence it was concluded that drug was not a genotoxic agent.

d. Micronucleus Test:

[redacted] was evaluated to determine its ability to induce the formation of micronucliated polychromatic erythrocytes in the bone marrow of treated animals. On the basis of the range-finding study the doses of 1250, 2500 and 5000 mg/kg were administered via i.p. to CD-1 (5/s/gp). Slides were prepared from the bone marrow cells collected at 30, 48 and 72 hrs after a single i.p dose and scored blind. The results were compared with negative (2% dimethysulfoxide) and positive (0.5 mg/kg triethylenemelamine) controls. There were no significant increases in the number of micronuclei in polychromatic erythrocytes at any dose tested. Thus, the drug was considered as nonmutagenic.

V. SUMMARY AND EVALUATION:

The current classification of diabetes mellitus divides patients into two major categories, insulin dependent (IDDM, Type I) and non-insulin dependent (NIDDM, Type II). Type I patients have little endogenous insulin whereas Type II patients have normal or even elevated insulin levels. Approximately 90% of the estimated five million diabetes in the United States are Type II.

Type II diabetics are frequently characterized by obesity and insulin resistance. Treatment is by either diet, oral agent, or insulin. Patients often fail to adhere to their prescribed dietary program. The oral agents often fail to provide continued therapeutic response and insulin fails to solve the problem of the diabetics' insulin resistance. Thus, the ideal therapeutic agent for these patients should act directly on the target tissues and alleviate the insulin resistant state. [redacted] is a thiazolidinedione and appears to function in several animal models by such a mechanism.

The results of the general pharmacological studies related to efficacy suggested that [redacted] lowers blood glucose by increasing glucose uptake and by reducing hepatic glucose production. The data also showed that this drug does not interact with any classical pharmacological systems and does not have any known non-specific actions that would be predicted to adversely affect other physiological systems. Although the underlying mechanism of the effect was not elucidated, it would appear to involve an enhanced responsiveness to insulin. The improved insulin sensitivity following treatment with drug resulted in a lowering glucose and insulin concentrations in non-insulinopenic diabetic animals but did not affect glucose levels in normal animals. As such, this drug appears to offer a novel treatment for diabetes without the potential for hypoglycemia of currently available therapy and with the potential to avoid the adverse consequences of long-term hyperinsulinemia.

Pharmacokinetic evaluations of pioglitazone in the dog indicated a terminal disposition phase half-life of 1.4-1.7 hr, with dose proportionality from 1-10 mg/kg for an oral solution containing citric acid and 5-75 mg/kg for the granulated formulation. Kinetics were linear and food obviously effects absorption. Three drug metabolites (M1, M2, M3) of unknown structure were observed in the serum of dogs and monkeys with M1 having a serum profile similar to the parent drug and M2 and M3 having longer disposition half-lifes. Accumulation of drug and M1 was not observed but was present for M2 and M3. In the rat, similar metabolites were observed. Their absorption disposition ,however, was not linear which indicates differences between species of animals.

Almost all drug metabolites were cleared from systemic circulation within 24 hr after a single i.v. or p.o. dose. Pioglitazone and metabolites showed potential accumulation in fat depots, liver, and adrenal glands in rats, although this was not confirmed by other species. Elimination of drug metabolites in animal models was primarily by biliary excretion.

The animal toxicity studies of pioglitazone [redacted] consists of studies conducted with either the acetate [redacted] or the HCl formulation [redacted]. All toxicological data generated with [redacted] utilized drug dissolved in citric acid, or, as a citrate-based granular powder due to an extremely poor solubility of U-72,107A. Because of such limited solubility of drug and death due to the toxicity of the vehicle occurred, the acute toxicity of [redacted] was not determined. In various repeated-dose toxicity studies, no insight into pioglitazone toxicity was gained from shorter-term testings; However, data from the 3-month studies showed some notable toxicity signs. In separate 13-wk oral studies in rats conducted [redacted] or [redacted], increased deposition of adipose tissue was noted at a dosage level of 30 mg/kg/d or higher. In contrast to an earlier observation from ciglitazone [redacted] in which cataracts were produced in Sprague-Dawley rats (not noted in Wistar rats) after 5 weeks of oral administration at 30 mg/kg/d or greater doses, no cataracts were induced in 3-month studies conducted in the same strain rat with U-72,107A or [redacted] at doses up to 300 mg/kg/d or in dog studies at doses up to 150 mg/kg/d. Anemia was not noted for [redacted]-treated rats at these doses but was noted for [redacted]-treated female rats at a dose of 100 mg/kg/d or greater. Other notable signs in rats at high ciglitazone doses(100 to 300 mg/kg/d) were elevated liver enzymes and increased organ weights. Increased heart wts were also noticed for rats treated with [redacted] (HCl and acetate) and increased liver wts were noted with [redacted] but the level of liver enzymes was not remarkable in either studies.

In dogs, dosages up to 10 mg/kg/d [redacted] for 1-month were well tolerated producing no drug-related effects. Preliminary results of the 3-month oral study conducted at lower dosages indicate a low incidence of anemia and hydropericardium at the 10 mg/kg/d dosage level. Increasing the dosage to 30 mg/kg/d or greater for 3 months induced multiple toxicities clinically and in multiple organ systems. The most unusual findings were anemia with hypocellularity of selected bone marrow and hydropericardium. The sponsor argued that since there is no known common cause of these 2 conditions in dogs, the pathogenesis can not be presented . Nevertheless, with exception of organ wt change, all toxic findings were found to be reversible within 1-month of drug withdrawal. It appeared from the data of relative ogran wts that these would reverse if given more time.

As is apparent from the foregoing discussion, [redacted] produces more toxicity in dogs than in rats; thus, the dog is considered more sensitive species for toxicity testing of this drug. The reason for this is not clear although the metabolism differences known at present would in part be an explanation. Since rats were administered drug once daily, and dogs on

the 3-month toxicity study were dosed twice daily, the rat would have a greater chance of clearing all the drug and related materials before the next dose as compared to the dog. Faster clearance could decrease toxicity. Studies to date indicate pioglitazone toxicity in rats and dogs consists of reversible anemia and hydropericardium (dogs only), and increased heart and liver wts. The NOEL for [redacted] administration over 3 months was set as 3 mg/kg/d in the dog which is approx. 100X the proposed initial clinical single dose (0.03 mg/kg for a 70 kg person).

Mutagenicity studies showed that pioglitazone is negative for genotoxic potential in the in vitro Ames, CHO/HPRT and AS52/XPRT mammalian cell and UDS assays, and in the in vivo micronucleus test in mice.

VI. CONCLUSION AND RECOMMENDATIONS

The preclinical investigations conducted by Upjohn and Taketa with the anti-diabetic drug, pioglitazone, in this IND are adequate to meet the FDA requirement for support of the proposed clinical studies (Phase I). The pharmacological spectrum of this drug in the animal models supports the potential efficacy in man for the therapy of non-insulin dependent Type II diabetes. However, this drug at dosages of 30 mg/kg/d or greater ,at least in dog, induced anemia, elevated liver enzymes and increased heart and liver weights. Admittedly the reported toxicology studies were of relatively short duration and therefore appropriate chronic studies will have to be performed ,by the use of same drug formulation for the clinical studies, [redacted] to further determine the extent of toxicity. It is, therefore, recommended that the following preclinical studies be conducted:

Letter to sponsor

1. In view of some indication for potential bioaccumulation of drug metabolites in dog over time when administered twice daily, a well-designed pharmacokinetic study is required to further elucidate this possibility along with full characterization of drug metabolites. We also need data on serum levels of the drug and metabolites under steady state conditions to determine the degree of similarity of rats and dogs to humans.

2. From the toxicity data submitted to date, it is clear that U-72,107A produces more toxicity in dogs than in rats, and increasing the dosage in dogs induced multiple toxicities clinically and in organ systems. As such, a careful consideration of long-term toxicity testing is very important and it is critical that ~~the maximum tolerated~~ a dose be used as the high dose to demonstrate overt toxicity 3. Please submit the results of the range finding studies and a rationale for the doses to be employed for the ~~chronic toxicity and~~ carcinogenicity studies.

rat & mouse

3. When available,

I [redacted]

Because of potential testis toxicity, Seg I repro studies should be initiated as soon as possible.

3. [redacted]

Teratology studies will find that the high dose should produce some maternal toxicity. for the

please keep in

/S/ [redacted]

Cheong (John) C. Chah, Ph.D.

/S/ [redacted]

11/16/89

CC: IND Arch
HFD-345
HFD-502/JWeissinger
HFD-510
HFD-510/Supv. Pharmacologist
HFD-510/CChah/11/09/89/Wang 0606p

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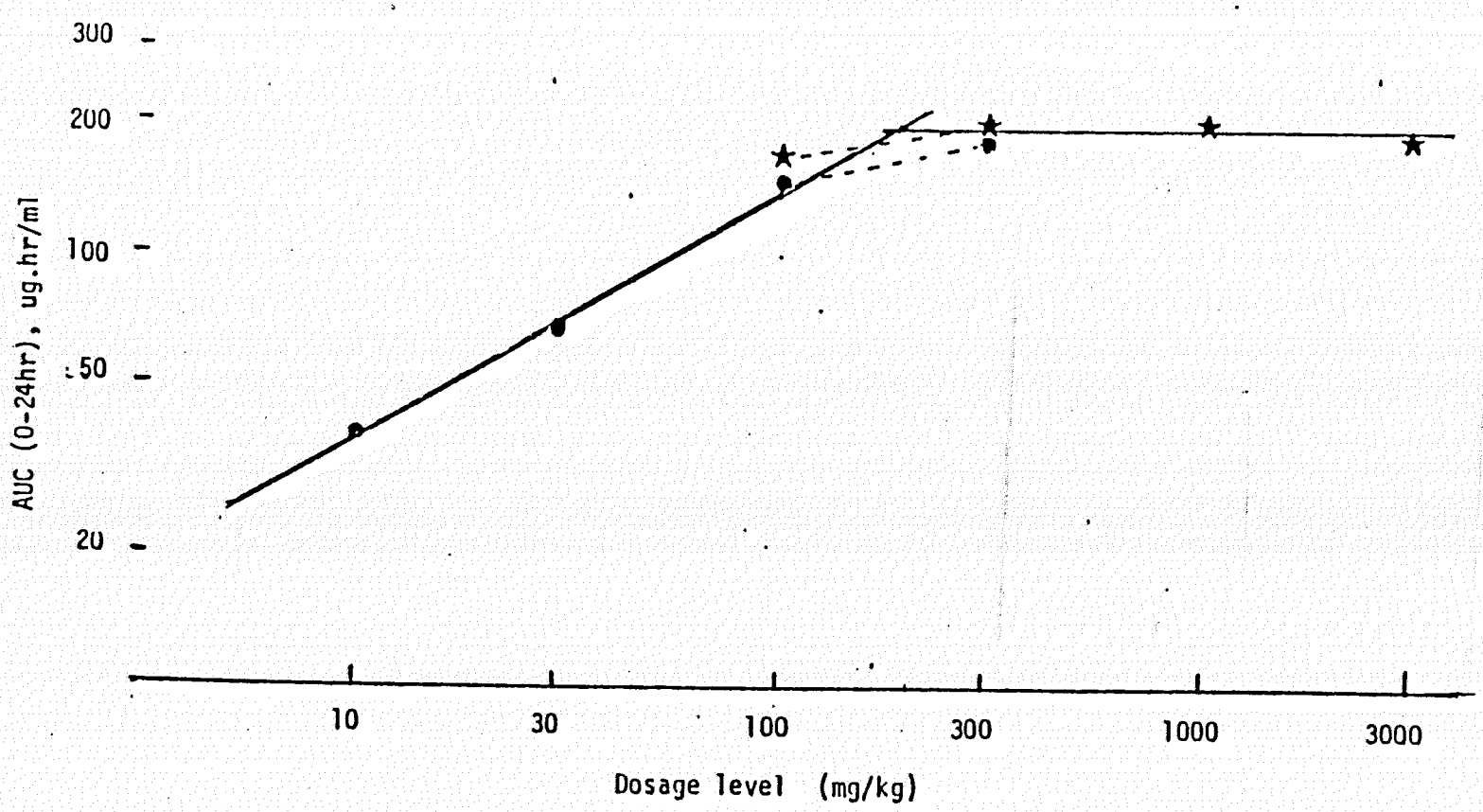

Fig.1 Relationship between AD-4833 dosage level and AUC(0-24) in rats

Data are expressed as the mean of three rats.

★---★—★ : results from the previous study.

JORDAN

IND# [redacted]

Sponsor: [redacted]

Review No. 3

Date of Review: 2/24/1991

AMENDMENT NO. 005

Date of submission: 1/17/1991

Drug: Pioglitazone HCl(U-72,107A)

Class: Oral hypoglycemic agent

Related: IND# [redacted]

Date of previous pharmacology reviews: 11/16/1989

This information amendment contains 15 specific technical reports. Among these only selected technical reports, which are relevant to drug safety, pharmacology and toxicology, will be reviewed briefly below.

1. TR No. 7250/90/024, CHARACTERIZATION OF PIOGLITAZONE EFFECTS ON H4-11-E CELLS; AN INSULIN RESPONSIVE CELL LINE

Effects of pioglitazone on H4-11-E cells, an insulin responsive rat hepatoma cell line derived from the Reuber hepatoma H-35, was investigated. Since pioglitazone is known to be an insulin sensitizer, the sponsor predicted that the drug would accelerate H4-11-E cells growth. Pioglitazone which was dissolved in DMSO at a concentration of 25 mM inhibited growth of the cells in a dose-dependent manner(fig 1). The cells exposed to a dose of 25 uM initially exhibit growth inhibition, however upon removal of the drug and an addition of fresh media, they follow a growth curve parallel to control. Analysis of the cell cycle revealed that pioglitazone causes a non-specific cell cycle arrest.

Comments: Cell growth inhibitory doses of pioglitazone were higher than those needed for in vivo pharmacological effects in diabetic model mice(KKAy). Inhibitory effects of this drug on other cells, particularly in vivo are needed to be established.

2. TR No. 7250/90/061, PIOGLITAZONE INDUCED CHANGES IN GENE EXPRESSION

The sponsor has previously demonstrated that pioglitazone enhances the insulin regulated differentiation of 3T3-L1 cells to adipocytes. In the present study the sponsor showed that the drug does similar effect the differentiation of 3T3-L1 cells induced by insulin-like growth factor-1(IGF). But, the drug does not elicit such differentiation in the absence of insulin or IGF. When triglyceride accumulation is measured as the end point of differentiation [illegible]

activities known to increase during differentiation were increased by pioglitazone over the activities seen with insulin or IGF alone. However, there was a lag period of 18 to 24 hours between the onset of drug treatment and increased activities. For induction of the mRNA it was required to incubate the cells with pioglitazone and insulin or IGF. The sponsor speculates that the induced genes encode regulatory proteins which enhance the response of cells to insulin.

Comments: Pioglitazone enhances either insulin or IGF action on 3T3-L1 cell differentiation, of which action could be extended to other tissues mediated by these hormones.

3. Technical Report No. 7250-90-062 THE ANALYSIS OF THE aP2 PROMOTOR; A POSSIBLE TARGET FOR AN INSULIN SENSITIZING AGENT

In an effort to understand the mechanism that pioglitazone enhances sensitivity of target tissues to insulin, the sponsor studied effects of the drug on production of mRNA of aP2(adipocyte specific fatty acid binding protein). Pioglitazone increased the aP2 mRNA 10-fold of control level within 4 hours of treatment in 3T3-L1 cells, which suggests the aP2 gene is a candidate for direct action of the drug. The sponsor also cloned aP2 promoter sequences by polymerase chain reaction. The promoter sequences(750 bp) and a truncated promoter fragment(250 bp) was placed into chloramphenicol acetyltransferase reporter gene. And the combinant was transfected into staged 3T3-L1 cells. Promoter specific expression and specific regulatory regions within the aP2 promoter sequence(e.g., glucocorticoid responsive element) were confirmed.

4. Technical Report No. 7250-90-063 PIOGLITAZONE INDUCTION OF aP2 mRNA In Vivo

The objective of this study was to investigate pioglitazone action on fat tissue aP2 mRNA in vivo, since the sponsor demonstrated that the drug induced directly the aP2 mRNA in vitro. Administration of pioglitazone(up to 71 mg/kg/day for six weeks) to ob/ob mice resulted in a dose-dependent increase in a P2 mRNA from epididymal fat with a 3-fold increase. In perirenal fat from KKAy mice the drug(20 mg/kg for 4 days) increased the aP2 mRNA by two-fold with proportional changes in blood glucose and insulin, suggesting certain relationships among these parameters(fig. 2).

Comments: Pioglitazone increased the aP2 expression in C57 mice without effecting blood glucose. The drug is not able to increase the aP2 expression in streptozotocin-treated rats, although insulin sensitivity is augmented by pioglitazone in the model. Pioglitazone improves insulin sensitivity in all tissues while aP2 mRNA induction may be an adipose-specific.

5. Technical Report No. 7250-90-032 RECEPTOR-COUPLED HIGH AFFINITY GTPase ACTIVITY IN HUMAN PLATELET MEMBFANES

As part of a study of the effects of pioglitazone on insulin action, the objective of this investigation was to determine the response of GTPase activity of human platelet membranes to insulin. High affinity GTPase activity was not affected by insulin(0.1 to 100 nM) added either simultaneously with GTP or up to 20 min prior to substrate addition. Low affinity GTPase activity was also negligibly affected by insulin. Pioglitazone did not alter high affinity GTPase activity measured in the absence or presence of insulin(30 nM). This study shows that human platelet membranes contain a high affinity GTPase that is unresponsive to insulin under a variety of experimental conditions where stimulation by other agonists is observed. This suggests that agonist-responsive GTPase activity is not be a useful index for insulin receptor-G protein interactions.

6. Technical Report No. 7250-90-039 EFFECT OF PIOGLITAZONE ON GLUCOSE UPTAKE IN L6 MUSCLE CELLLS AND RAT CARDIAC MYOCYTES

Both L6 skeletal muscle cell line and rat cardiac myocytes were used to determine the effect of pioglitazone on glucose transport, using radioactive 2-deoxy D-glucose and 3-O methyl D-glucose. Pioglitazone increased glucose transport in a time and dose dependent manner in differentiated L6 cells like insulin does. The insulin and pioglitazone doses for optimal glucose transport were 0.17 uM and 20 uM, respectively. The effect of insulin and pioglitazone was additive. Rat cardiac myocytes were also insulin responsive as measured by increased glucose transport with increased time in culture.

7. Technical Report No. 7250-89-051 PIOGLITAZONE TREATMENT LOWERS CIRCULATING CHOLESTEROL IN CHOLESTEROL-FED RATS AND REDUCES INTESTINAL ACAT ACTIVITY IN RATS AND DIABETIC MICE

The major cause of death in diabetes is coronary heart disease as a result of atherosclerosis. The exact cause(s) of premature atherosclerosis in diabetes is unknown, although diabetic patients have many risk factors such as hypertension, altered circulating lipids and increased blood viscosity. Pioglitazone reduces circulating triglycerides in several species. This drug also reduces total and beta-cholesterol in rats fed a moderately hypercholesteremic diet, although it may not reduce circulating cholesterol in aminals on a normal diet. The reduction in circulating beta-cholesterol is known to associate with a reduction in both intestinal and hepatic microsomal acyl-CoA: cholesterol acyltransferase(ACAT) activity. Pioglitazone produced a dose-dependent decrease in the intestinal ACAT activity of the diabetic ob/ob mouse without affecting circulating cholesterol. These data suggest that ACAT might be under the control of insulin so that insulin deficiency might contribute to higher intestinal ACAT activity. The objective of this present study was

to re-examine the effect of pioglitazone on circulating cholesterol concentrations.

Pioglitazone reduced circulating triglycerides in most of tested animals(except ob/ob mice), but had little effects on circulating cholesterol in animals on a normal laboratory diet(Table 1). Pioglitazone also reduced circulating triglycerides and insulin, which might be related to an improved sensitivity of the peripheral tissues to insulin. Pioglitazone treatment tended to decrease ACAT activity in the gastrointestinal microsomes of rats. The fact that pioglitazone treatment had no effect on cholesterol levels in the animals on a normal rodent diet suggests that an effect of pioglitazone treamtnet might be to affect cholesterol absorption. Intestinal ACAT, an enzyme thought to be essential for absorption of dietary cholesterol, appears to be under the negative control of insulin.

7. Technical Report No. 7250-89-089 EFFECT OF [redacted] ON INSULIN TOLERANCE AND MEAN STIMULATED RESPONSE IN NORMAL DOGS.

The objective of this study was to determine whether a pioglitazone dose selected by extrapolation from mouse data was efficacious in dogs. The effect of pioglitazone on insulin sensitivity was assessed by two methods: (1) insulin tolerance test profiles measuring the response of serum insulin and glucose to an exogenous insulin bolus and (2) pre- and postprandial testing of serum samples for the insulin, glucose, triglycerides, and non-esterified free fatty acids response to endogenous insulin. Two groups of six lean mongrel dogs were studied using a placebo controlled with a crossover design with a 2 week washout period separating the 7-day treatment phases. A placebo or pioglitazone was administered once daily as a citrate formulation in a gelatin capsule. No statistically significant effects were observed for any parameter in response to pioglitazone. Serum drug levels were determined in samples from representative dogs obtained immediately prior to the low dose insulin tolerance tested. These data indicate that pioglitazone is less effective in young, healthy dogs than in their aged, obese counterparts when administered.

8. Technical Report No. 7250-89-062. THE EFFECT OF PIOGLITAZONE HCl ON TOTAL SERUM T4 AND T3 LEVELS IN THE DOG.

Symptoms, such as anemia, pericardial effusion and hepatomegaly, which resemble those occurring in hypothyroidism were observed in dogs treated with pioglitazone. Thus, the purpose of this study was to examine the effects of pioglitazone on serum levels of T4 and T3 in dogs. Six male and 6 female dogs were treated with pioglitazone at doses of 30, 60 and 150 mg/kg/day for 90 days. By one month serum T4 and T3 levels had decreased in all pioglitazone treated dogs to levels about 2/3 control levels. T4 and T3 in treated dogs remained low during the treatment period with one exception. Serum T3 levels in the 30 mg/kg group increased after one month and by 90 days were no longer

significantly different from control. This study suggests that pioglitazone appears to have a negative effect on thyroid hormone economy and the reduced serum T4 and T3 levels observed in the treated dogs could have contributed to those side effects resembling to symptoms of hypothyroidism.

9. Technical Report No. 7256-90-004 PIOGLITAZONE BIOAVAILABILITY COMPARISON IN THE DOG; ORAL SOLUTION, PATH/TOX FORMULATION, AND CLINICAL FORMULATION(PROTOCOL 89-235).

In animal models pioglitazone-HCl has been shown to have enhanced bioavailability when administered with citric acid. However, the total amount of citric acid and the drug to citric acid ratio necessary to achieve optimal bioavailability have not been completely defined. The objective of this study was to evaluate the potential change in pioglitazone bioavailablity for formulation modification in the beagle dogs. Four dogs each received four pioglitazone formulations: an oral solution containing citric acid and 10 mg pioglitazone; the granulated formulation containing 10 mg pioglitazone; two 5-mg clinical formulation capsules(high clinical); and two 2-mg clinical formulation capsules(low clinical). Pharmacokinetic evaluation of the serum concentration-time profiles showed no apparent difference in pioglitazone relative bioavailability for the three solid formulations compared to the oral solution. Statistical evaluation of various pharmacokinetic parameters(AUC, Cmax, Tmax, apparent terminal rate constant and mean residence time) suggested that no significant differences between the four formulations. This result indicates that the bioavailability of the pioglitazone(phase 1 clinical formulation) is similar to the formulation used in animal safety studies.

RECOMMENDATIONS: None.

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted]

3/13/91

cc: IND, HFD-510, HFD-345
A. Jordan/H. Rhee

Table I

Summary of Effects of Pioglitazone on Circulating Lipids in Various Animal Models

Animal	Dose mg/kg	Study	Cholesterol (mg/dl)		Triglycerides (mg/dl)	
			Control	Pioglitazone	Control	Pioglitazone
KKA^y Mouse	24.5	1	142 ± 12	121 ± 6	817 ± 76	363 ± 16*
ob/ob Mouse	50	2	269 ± 10	290 ± 9	228 ± 23	237 ± 17
db/db Mouse	100	3	164 ± 9	181 ± 5	429 ± 43	220 ± 29*
Chinese Hamster	94	4	147 ± 7	107 ± 4*	808 ± 107	389 ± 51*
Normal Rats	100	5	-	-	200 ± 26	90 ± 8*
Zucker Rat	10	6	-	-	426 ± 55	100 ± 9*

Data are Mean and SEM; * = Statistically significant versus Control group.

[redacted]