

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CRPRC**

EH&S USE ONLY

PROTOCOL # 9642**EXPIRES: _____**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone:		Phone:	
Fax:		Fax:	

Species (common names):	Number:	Source:
Rhesus Monkey	7	CRPRC

Project Title	Effects of a PPAR Agonist on Serum Lipids and Insulin in Obese Rhesus Monkeys		
Overnight housing location::	CRPRC	Day use only :	CRPRC
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Blood samples will be collected from 7 obese rhesus monkeys before and during 4 weeks of oral administration of a Peroxisome Proliferator-Activated Receptor (PPAR) agonist provided by Merck that may have effects to lower cholesterol and triglycerides. Food will be provided ad libitum intake will be monitored. A DEXA scan to assess body composition will be performed before and at the end of the 4 week dosing period. The obese animals have high plasma insulin concentrations indicative of insulin resistance and the PPAR agonist may also improve insulin sensitivity and lower insulin levels.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

None, except that <i>ad libitum</i> food intake will be monitored and blood samples will be collected from overnight (16 hr) fasted monkeys.
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Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	Merck	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pcstillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Compounds that activate PPAR may be useful for treating hyperlipidemia and/or insulin resistance. We propose to examine the effects of a PPAR agonist developed by Merck on serum lipids (cholesterol, triglycerides, and free fatty acids) and circulating insulin levels in 7 adult male rhesus that have become obese and insulin-resistant from eating an ad libitum diet supplemented with sugar-containing beverages.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|---|--|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

**** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.**

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Experimental Design:

The seven animals in the study will first be treated during a baseline period with vehicle (sweetened Kool-Aid PO) for 14 days followed by 28 days treatment with the Merck PPAR agonist administered at a dose of 3 mg/kg given twice daily dissolved in the sweetened Kool-Aid vehicle. The animals are already accustomed to drinking sweetened Kool-Aos which will be offered for them to drink from a syringe. A blood sample (15 ml) for CBC and serum chemistry panels and for measuring serum lipids, insulin, and leptin will be collected after an overnight (16 hr) fast twice during the baseline period and at 7, 14, and 28 days during treatment with the PPAR agent. Blood samples (3 ml) for plasma drug analysis will be collected on day 1 of dosing, and a second time between day 21 and day 28 of dosing. Samples will be collected at 0.0, 0.5, 1, 2, 4, 8 and 24 hours after dosing on the designated days. The animals will be weighed weekly in a transfer cage placed on a scale during the baseline and treatment periods of the experimental protocol. The animals do not need to be anesthetized in order to be weighed. Food intake will be ad libitum and measured twice daily. Animals will be transported to the WHNRC DEXA facility (in the Ragle Human Nutrition Research Center) and DEXA scans for assessment of body composition (fat vs lean body tissue). The animals are transported in a transfer cages in a CRPRC van used for animal transport. The animals will be anesthetized with ketamine (10-15 mg/kg IM) immediately before the DEXA scans are performed. DEXA scans will be performed once prior to treatment at the end of the baseline period and once at the conclusion of the treatment period on or near day 28 of treatment.

d) **Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	Males	7	2

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

Rationale for species: Nonhuman primates are considered to be more relevant models of human physiology and nutrition than rodents. Merck has previously tested the PPAR compound in rodents but it is necessary to determine whether it will have similar effects in a nonhuman primate model (rhesus monkeys). Based on our previous measurements of serum lipids and circulating insulin and leptin in rhesus monkeys, we will be able to detect a 20-30% change in these parameters with a sample size of 7 animals.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

The project does not involve surgery

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus monkey	Ketamine	10-15 mg/kg	IM	Injection prior to DEXA scan for measuring body composition.

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

In previous studies conducted by Merck with the PPAR agonist has been administered acutely to several species (mouse, rat, dog, monkey) at dosages as high as 15 mg/kg with no adverse effects noted. The compound has been administered chronically (periods up to 14 days) to several species including hamsters, mice and rats at dosages up to 30 mg/kg with minimal to no side effects. A moderate increase in liver weight was observed with chronic administration in diabetic (ZDF) rats however, this finding was not observed in diabetic mice (dbdb), obese mice (obob) or obese hamsters. Based on pharmacokinetic parameters, we anticipate the total drug exposure in rhesus monkeys dosed at 3 mg/kg b.i.d. will be substantially lower than the exposure in rodents dosed at 30 mg/kg/day. . In addition, no adverse effects were observed after administration of a single dose to rhesus monkeys (n=2) at 0.5 mg/kg IV 2.0 mg/kg PO in methocel or in Kool-Aid. For this reason, we do not expect that rhesus monkeys will exhibit adverse reactions to the drug.

No adverse effects resulting from the procedures are anticipated beyond those associated with brief ketamine anesthesia and blood sampling, i.e., slight discomfort associated with the injection of ketamine and bruising at the site of blood collection.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Food intake will be assessed daily, body weight weekly, and CBC and serum chemistry analysis will be performed every one to two weeks during the trial. These measurements will allow us to determine if animals are experiencing any adverse effects of the drug. If an animal decrease its food intake by more than 50% during any week, loses more than 30% of body weight (the animals are currently obese), or shows clinically relevant adverse changes in serum chemistry/hematology parameters, it may be removed from the trial at the discretion of the CRPRC Veterinary Staff in consultation with the Principal Investigator.

The discomfort associated with the procedures should be temporary and minimal. Analgesics can be provided at the discretion of the CRPRC Veterinary Staff.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not

appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

July 1999

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Melvyl Medline	1995-present	PPAR, obesity, serum lipids, insulin, monkeys
Promed	1995-present	PPAR, obesity, serum lipids, insulin, monkeys

What were your findings with respect to alternative methodologies?

There are no alternate methodologies for testing the effects of a PPAR agonist compound in monkeys.

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

The study has not been previously conducted.

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

Animals will be euthanized if they become ill or debilitated such that the veterinary staff of the CRPRC feels that euthanasia is indicated.

l) Methods of euthanasia: Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	Route
Rhesus Monkey	Overdose	Pentobarbital	60 mg/kg	IV

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

The animals will be returned to the colony. They may be used for subsequent dietary or pharmacological studies after a suitable "washout" period, of at least 30-60 days.

