

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CNPRC**

EH&S USE ONLY

PROTOCOL: 10371
EXPIRES:

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:			
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus Monkeys	2	CRPRC

Project Title	Effects of an insulin sensitizer on glucose, insulin and lipids		
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.

After a 1 week baseline period, 2 hyperglycemic monkeys will receive daily subcutaneous (SQ) injection of insulin sensitizer drug provided by Allergan Pharmaceuticals. For the first week the animals will receive a dose of 3mg/kg/day and during the second week they will receive 10mg/kg/day. Food will be provided ad libitum for the 3 weeks (baseline + treatment) and food intake will be monitored. 8AM fasting and 2PM postprandial blood samples will be collected 4 times.

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.

Throughout the 3 week experiment, ad libitum food intake will be monitored. Drug will be administered SQ for 2 week. Monkeys will be fasted for 16 hours before fasting blood draws.
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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: Previously approved? Yes No
 Is the project already funded? Yes No Previous protocol number (if any):

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

Lab Animal Health Clinic (2-0514) California Primate Research Center (2-0447)
 VMTH Large Animal Field Service (2-0292) Another Veterinarian

If you checked "Another Veterinarian", please provide:

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

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@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.

Allergan Pharmaceuticals has developed a compounds that may improve insulin sensitivity. We propose to examine the effects of this compound on serum lipids, T3, T4, glucose and insulin in 2 hyperglycemic monkeys.

b) Procedures employed in this project:

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

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

**** 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.)

General Experimental Protocol: There will be a 1 week baseline, a 2 week drug treatment period.

General Feeding and Body Weight Protocol: During the 3 week experiment, animals will be weighed weekly. They will be fed their usual chow diet ad libitum and food intake will be monitored twice daily. The 2 animals selected for this study have recently been identified as hyperglycemic, but do not require insulin treatment. The blood lipid, glucose and insulin levels of these animals may be improved by administration of the insulin sensitizer drug. These parameters are also positively affected by an energy restricted diet. Ad libitum feeding ensures that none of the animals are in negative energy balance. Therefore any positive results during dosing can be attributed to the drug and not to the effects of energy restriction.

Drug Administration: Following the 1 week baseline, animals will receive 1 subcutaneous injection/day of the drug for 2 weeks. During the first week the animals will receive 3mg/kg of the drug and during the second week, 20mg/kg.

Fasting Hormone and Lipid Blood Collection: On the 4 days designated below, fasting blood samples will be collected at 8AM and postprandial samples will be collected at 2PM. Eight ml of blood will be collected for fasting serum lipids, triiodothyronine (T3) and tetraiodothyronine (T4), and clinical chemistry. Another seven ml of blood will be collected in EDTA for fasting glucose and insulin analysis. Total blood collected at each of these eight collection times is 15 mls. Blood sampling volumes will conform to CRPRC guidelines.

Schedule for treatment and blood draws

Timeline

Days 1-7

Day 7 8AM

Day 7 2PM

Days 8-14

Day 14 8AM

Day 14 2PM

Day 15-21

Day 21 8AM

Day 21 2PM

Day 22

Day 22 8AM

Day 22 2PM

PROCEDURE

Baseline with ad libitum feeding and food intake recording

Fasting blood collection

Postprandial blood collection

3mg/kg drug treatment

Fasting blood collection

Postprandial blood collection

20mg/kg treatment

Fasting blood collection

Postprandial blood collection

No treatment

Fasting blood collection

Postprandial blood collection

- 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	Drug - Insulin Sensitizer	2	1

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>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.</p>
2	<p>Minor stress or pain of short duration</p> <p>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</p>
3	<p>Moderate to severe distress</p> <p>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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>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.</p>

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?

Nonhuman primates are considered to be more relevant models of human physiology and nutrition than rodents. This is a pilot study. If the tested drug produces positive effects in the 2 hyperglycemic animals, a new study will be proposed to test the drug in more animals.

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

Building: 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?

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 Allergan with this insulin sensitizer drug, no adverse effects have been observed in rodents at these doses.

No adverse effects resulting from the procedures are anticipated beyond those associated with blood sampling such as bruising at the site of blood collection or injection.

It is possible the ad libitum feeding may cause the animals to attain body weights greater than 15 Kg. The upper weight specification for their 6.2 square foot (floor space) cages is 15 Kg.

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 weekly during the trial. These measurements will allow us to determine if animals are experiencing any adverse effects of the drug. If an animal decreases its food intake by more than 50% during any week, loses more than 20% of body weight (the animals are currently obese), or shows clinically relevant adverse changes in serum chemistry 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 blood collection and injection should be temporary and minimal.

We do not expect that animals that attain a BW above 15 Kg will experience discomfort in the standard size cages. The additional weight gain caused by ad libitum feeding will increase body fat, but not increase body frame size. It may also cause the animal to become less active. Therefore the 6.2 cubic foot cage should still be adequate for the animals that gain weight.

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 [X] 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:

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search?

6-20-02

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
PubMed	1965-2002	Insulin sensitivity, obesity, lipid profile, insulin resistance, monkeys
ISI Web-of-Science	1975-2002	Insulin sensitivity, obesity, lipid profile, insulin resistance, monkeys

What were your findings with respect to alternative methodologies?

There are no alternative methodologies to examine the effects of these compounds 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?

All animals will be returned to colony.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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