

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

EH&amp;S USE ONLY

**PROTOCOL # 9902  
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	20	CRPRC

**Project Title** Effects of Phosphatase Inhibition on Insulin Sensitivity in Monkeys

Overnight housing location::	CRPRC	Day use only :	CRPRC
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

**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 for the measurement of insulin and other hormones and substrates involved in carbohydrate/lipid metabolism will be collected from obese rhesus monkeys before and during a dose-escalation trial. Three doses (1, 3, and 10 mg/kg) of two different antisense compounds (10 animals per compound) developed by ISIS that inhibit a specific phosphatase enzyme will be administered by subcutaneous injection. Each dose will be administered over a three week period. Food intake and body weight, CBC, serum chemistry, and urine creatinine clearance will be monitored. The animals will be chair-trained. An IV glucose tolerance test will be performed in chaired animals before and during each of three doses.

**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 food intake will be monitored and blood samples will be collected from overnight (16 hr) fasted monkeys.

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:	ISIS Pharmaceuticals	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](mailto: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 target a specific phosphatase may be useful for treating insulin resistance and type-2 diabetes. We propose to examine the effects of two antisense compounds developed by ISIS on circulating insulin levels and glucose-tolerance in 20 obese adult rhesus monkeys that are insulin-resistant due to their obesity.

**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:**

There will be 10 animals per group. All animals will be chair-trained. Body weight will be assessed (weekly) and food intake (daily) throughout the study. During the baseline period three (4ml) blood samples (5 minutes between samples) will be collected following an overnight (16 h) fast from an arm vein on two separate occasions to establish stable fasting insulin and other hormone concentrations. The samples will be collected from animals in their home cages to assure that basal insulin levels are measured. At the same time on both these days, another 6 ml sample will be obtained to measure serum lipids, CBC and serum biochemistry parameters and a urine sample will be collected to assess renal function by creatinine clearance.

On different day than those of the the fasting blood draws, a baseline IV glucose tolerance test (IVGTT) will be conducted in chaired-animals. An IV catheter is inserted in each arm vein. Three baseline samples (3 ml) are collected from one catheter at -10, -5 and 0 minutes. Then 600 mg/kg of 50% dextrose is administered in the contralateral catheter. Additional 3 ml blood samples are collected at 1, 3, 5, 10, 15, 20, 30, 40, and 60 minutes. All blood sample volumes collected will conform to CRPRC guidelines. We have previously performed the IVGTT procedure in chaired rhesus monkeys (Protocol #7248). Less blood/sample will be collected for the IVGTT than for the fasting blood draws because of the fewer analyses (glucose and insulin) required for the IVGTT samples.

Following the baseline data collections, each group of 10 animals will receive a different ISIS antisense compound. Each compound will be given subcutaneously in the interscapular region at a dose of 1 mg/kg on 3 alternate days during the first week, and one time each week for the next 2 weeks. During the second week of injections, fasting samples will be collected as described above. An IVGTT will be performed during the third week. After these three weeks the protocol will be repeated as described with 3 injections the first week and 1 weekly injection during the next 2 weeks, but at a dose of 3 mg/kg. The resulting insulin samples will be analyzed and if no pharmacological effect is observed, the protocol will be repeated at a dose of 10 mg/kg. 10 mg/kg will be the upper limit dose for the study.

See schedule below:

## Experimental Schedule:

Week 1: Baseline fasting hormone (3 X 4ml) and lipid blood draw (6ml) at 0,5,10,15 minutes

Week 2: Day 1: Baseline fasting hormone (3 X 4ml) and lipid draw (6ml) at 0,5,10,15 minutes

Day 3: Baseline IVGTT (12 X 3ml blood samples) at -10,-5,0,1,3,5,10,15,20,30,40,60 minutes.

Week 3: Day 1: ISIS antisense compound injection (1mg/kg)

Day 3: ISIS antisense compound injection (1mg/kg)

Day 5: ISIS antisense compound injection (1mg/kg)

Week 4: Day 1: ISIS antisense compound injection (1mg/kg)

Day 3: Fasting hormone (4ml) and lipid draw draw (6ml)

Week 5: Day 1: ISIS antisense compound injection (1mg/kg)

Day 3: IVGTT (12 X 3ml blood samples)

Week 6: Day 1: ISIS antisense compound injection (3mg/kg)

Day 3: ISIS antisense compound injection (3mg/kg)

Day 5: ISIS antisense compound injection (3mg/kg)

Week 7: Day 1: ISIS antisense compound injection (3mg/kg)

Day 3: Fasting hormone (4ml) and lipid draw draw (6ml)

Week 8: Day 1: ISIS antisense compound injection (3mg/kg)

Day 3: IVGTT (12 X 3ml blood samples)

Week 9: Day 1: ISIS antisense compound injection (10mg/kg)

Day 3: ISIS antisense compound injection (10mg/kg)

Day 5: ISIS antisense compound injection (10mg/kg)

Week 10: Day 1: ISIS antisense compound injection (10mg/kg)

Day 3: Fasting hormone (4ml) and lipid draw draw (6ml)

Week 11: Day 1: ISIS antisense compound injection (10mg/kg)

Day 3: IVGTT (12 X 3ml blood samples)

Weeks 12-28 Washout Period

**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	ISIS Phosphatase AS Compound A	10	1
2	ISIS Phosphatase AS Compound B	10	1

#### Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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. ISIS has previously tested the AS compound in rodents but it is necessary to determine whether it will have similar effects in an insulin-resistant nonhuman primate model (obese rhesus monkeys). Based on our previous measurements of serum circulating insulin in rhesus monkeys, we will be able to detect a 20-30% change in fasting insulin levels with a sample size of 10 animals.

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

Building:

N/A

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 ISIS with these compounds, no adverse effects have been observed in rodents or in monkeys at these doses.

No adverse effects resulting from the procedures are anticipated beyond those associated with blood sampling or with injection of the compounds and bruising at the site of blood collection. The short 2 hour period of chair restraint is not considered to be adverse in chair-trained animals.

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 three weeks 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 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	"name of specific phosphatase", antisense, obesity, insulin resistance, monkeys
Promed	1995-present	"name of specific phosphatase", antisense, obesity, insulin resistance, monkeys

What were your findings with respect to alternative methodologies?

There are no alternate methodologies for testing the effects of the phosphatase antisense 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?

The animals will be returned to the colony. They may be used for other studies after a suitable "washout" period, of 120 days.



