

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

EH&S USE ONLY

PROTOCOL # 10377**EXPIRES: 2/13/04****Investigator**

Last Name:	
First:	
Middle:	
email:	
Dept.:	
Phone:	
Fax:	

Contact

Last Name:	
First:	
Middle:	
email:	
Dept.:	
Phone:	
Fax:	

Species (common names):	Number:	Source:
rhesus	23	CRPRC

Project Title	Effects of MANS Peptide on Monkey Nasal Secretions		
Overnight housing location::	CNPRC	Day use only :	
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.

To determine whether the experimental peptide affects nasal secretion in monkeys, animals will be anesthetized and given test or control article via nasal lavage., 30 minutes later a nasal lavage will be performed. The peptide MANS (Myristoylated Alanine-richN-terminal sequence) is designed to block the secretory function of MARCKS (Myristoylated Alanine-rich C-kinases abstrate) in goblet cells

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

Other instructions for animal care staff: (check applicable entries)

Sick Animals

- Call Investigator
 Clinician to treat
 Terminate
 Necropsy

Dead Animals

- Call Investigator
 Save for Investigator
 Bag for disposal
 Necropsy

Pest Control

- Call Investigator
 OK to use pesticides
 No Pesticides in animal area

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):	

Is the project already funded? Yes No
 Proposed Funding Source: RO1HL36982 (NIH)
 Previously approved? 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)
 VMTH Large Animal Field Service (2-0292)
 California Primate Research Center (2-0447)
 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 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.

The hypothesis to be tested is that MANS peptide, which inhibits function of MARCKS protein, will inhibit mucin secretion from the nose of monkeys when delivered intranasally. The long-term goal is to understand the mechanism of mucin secretion and develop therapies to treat patients with rhinitis and bronchitis.

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 h below)
<input 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 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.)*

Animals will be immobilized with Ketamine, 10gm/kg IM, Medetomidine 0.02-0.05mg/kg IM (reversed with Atipamazole, 0.02-0.05mg/kg IM, following the procedure) or with Telazol, 0.4-1.0mg/kg IM, for the following procedures:

Group 1: Saline by intranasal lavage (2ml); 30 minutes later lavage of saline in the nasal cavity.

Group 2: Control peptide (RNS; 140 uM in saline (2ml)) by intranasal lavage; 30 minutes later nasal lavage.

Group 3: MANS peptide (10 uM in saline (2ml)); 30 minutes later nasal lavage.

Group 4: MANS peptide (100 uM in saline (2ml)); 30 minutes later nasal lavage.

Group 5: MANS peptide (140 uM in saline (2ml)); 30 minutes later nasal lavage.

MANS (myristoylated alanine-rich N-terminal sequence)

RNS (random N-terminal sequence)

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	Saline IN/Saline IP/ Nasal Lavage	3	2
2	RNS/Nasal Lavage	5	2
3	MANS/Nasal Lavage	3	2
4	MANS/Nasal Lavage	3	2
5	MANS/Nasal Lavage	3	2

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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

The choice of primates is based on the fact that there is currently no information on the utility of this particular protein to block the secretory activity of mucous cells in any primate species. This proposal will be to test the feasibility of this protein's use in a non-human primate. The number of animals and the doses were established based on previous work with mice. The concentrations have been adjusted to match potential human therapeutic uses.

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?
Rhesus	Ketamine	10	IM	once
Rhesus	Telazol	0.4 – 1.0	IM	Once
Rhesus	Medetomidine	0.02-0.05	IM	Once
Rheuss	Atipamazole	0.02-0.05	IM	Once

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)

To date, no adverse affects have been identified in mice nor are they expected in primates. The only discomfort will likely come from the mild anesthesia necessary to perform the lavages. These animals will be completely healthy and unaffected at the end of the exposure and can be returned to the colony.

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.

We do not anticipate any adverse responses from the animals.

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?

9/25/02

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
MELVYL	1970-Present	MANS, MARCKS
Current Contents	1990-Present	MANS, MARCKS

What were your findings with respect to alternative methodologies?

There is only one publication concerning the role of MARCKS protein in airway mucous secretion in-vivo. This is from Dr. [redacted] laboratory, the person for whom this project is being done. There are no previous studies in-vivo in humans or non-human primates. There are no alternative tests to establish whether this protein will be effective in any primate species. In-vitro studies have been performed with mucous cells which suggest this protein will be effective, but whether it will work physiologically and in intact animals it has never been studied and is the purpose of this proposal.

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.

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

Animals will be euthanized at the discretion of the CRPRC vet staff.

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 or cynomolgus	overdose	pentobarbitol	60	IV

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

Animals will be returned to the CRPRC colony at the conclusion of the project

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.

Principal Investigator	Rank / Title	Date
CRPRC Director	Date	

Committee Use Only Below

<p>** Conditions necessary for Committee Approval:</p>
<p>Final Disposition of this protocol:</p> <p>_____ Approved</p> <p>_____ Not Approved</p> <p>_____ Withdrawn by Investigator</p> <p>Date of Action: ____/____/____</p>

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

Campus Veterinarian	Date
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sure I did, but it went into cyberspace - well I can try to get out of admitting I am not perfect. Okay, here it is.

Have a great weekend.

At 10:45 AM 11/15/2002 -0800, you wrote:
, you didn't attach the protocol.

At 10:07 AM 11/15/02, you wrote:
Hi ,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10377 for future reference.

I have attached a copy of the protocol with the number embedded for ease of making revisions in response to the questions I have included in this message. Please return the revised protocol at your earliest convenience.

Thanks in advance,

Protocol 10377 EFFECTS OF MANS PEPTIDE ON MONKEY NASAL SECRETIONS

1. On page 1, the location of the overnight housing box was left blank. Please complete.
2. In section c, you use the acronyms MANS and MARCKS, but have not explained what these acronyms stand for. Please expand to explain what the acronyms mean.
3. In section c, you have described the used of various agents but have not included the volumes. Please expand section c to include the volumes and dosages used in each group. Also, please include the dosages and routes of the immobilizing agents.
4. In section c, group 3, what vehicle is used to mix with the peptide for the intranasal lavage?
5. In section e, your rationale sounds like you are conducting a pilot study. Is this a pilot study? You mention that the concentrations will be adjusted to match potential human therapeutic uses. What does this mean? Please expand.
6. In section j, literature search, you are asked to provide more than one source. Also, you have listed "all" under years covered for Melvyl. Please replace "all" with the actual years covered.
7. In section n, project roster, the PI is not listed. Please add the PI.

Date: Tue, 11 Feb 2003 14:54:51 -0800
 To:
 From:
 Subject: Re: Fwd: RE: Re: Fwd: 2/13/03 protocol reviews (10377)

is collaborating with someone on this protocol that is really hard to get a hold of. They were getting frustrated. That was why they just changed the numbers.
 At 11:26 AM 2/11/03, you wrote:

changing the numbers does not answer the original question why the difference in numbers. Was it just a glitch?

At 11:05 AM 2/11/2003 -0800, you wrote:

Date: Tue, 11 Feb 2003 11:06:19 -0800
 To:
 From:
 Subject: Fwd: RE: Re: Fwd: 2/13/03 protocol reviews (10377)

From:
 To: "
 Subject: RE: Re: Fwd: 2/13/03 protocol reviews (10377)
 Date: Tue, 11 Feb 2003 10:52:21 -0800

,
 said to change the numbers to "3" all the way and make the total animal number on the front as "15".

I'm attaching the protocol with edits.

-----Original Message-----

From:
 Sent: Tuesday, February 11, 2003 9:22 AM
 To:
 Subject: Fwd: Re: Fwd: 2/13/03 protocol reviews (10377)

, I guess is going to have to answer this question....

>Date: Tue, 11 Feb 2003 09:15:54 -0800
 >To:

>From:

>Subject: Re: Fwd: 2/13/03 protocol reviews (10377)

>They still want to know why group two has 5 animals in it when all the others only have 3?

>At 04:22 PM 2/10/03, you wrote:

>> , I just spoke with , and they say that the 23 on the front page is a typo, should read 17.

>>Thanks!

>>-

>>At 10:07 AM 2/10/2003, you wrote:

>>> , can you answer this?

>>>

>>>>X-Sender:

>>>>Date: Mon, 10 Feb 2003 09:16:06 -0800

>>>>To:

>>>>From:

>>>>Subject: Fwd: 2/13/03 protocol reviews (10377)

>>>>Hi ,

>>>>I have received the following questions for the protocol on this week's committee agenda. Please forward the response as soon as possible to provide clarification prior to final review by the committee.

>>>>Thanks in advance,

>>>>

>>>>>#10377():

>>>>>1. In section D the numbers add up to only 17 (vs. 23 in front), and why does group two have 5 animals in it when all the others only have 3?

>>