



CONFIDENTIAL

To: Tim Mulcahy
Chair, All Campus ACUC

From: [REDACTED]
RARC

RE: Dr. Terasawa and Protocol G00146

Date: July 10, 2003

Enclosed please find my report of the review of the clinic histories of Dr. [REDACTED] animals on protocol G146 for the last three years. Also enclosed is a memo from Dr. [REDACTED] reporting the results of her own investigation of laboratory notebooks and clinic records.

As you can see, there have been a variety of deviations from her approved protocols over this period of time. There have also been more negative outcomes than expected for the animals on the push pull perfusion studies, especially for aged animals. I understand that the All Campus ACUC will decide whether she can continue to do animal research here at the university, and may allow the Graduate School ACUC to lift her suspension and approve her most recent protocol at their discretion. If this is the case, I submit the following ideas to prevent future reoccurrences of these types of problems.

First, the [REDACTED] veterinary staff should review the clinical records of all of her current animals on a regular basis and report to the Graduate School ACUC any events of concern (once a month or once every two months). Second, someone should review her current laboratory notebooks and report to the ACUC as above (perhaps [REDACTED] from the [REDACTED]). Third, a letter should be crafted to Dr. [REDACTED] explaining to her that further serious violations will result in loss of ability to perform animal research at the university. Last, Dr. [REDACTED] should reply in writing that she understands and accepts these conditions.

I believe a period of one year of close observation should be sufficient to assure compliance and understanding by Dr. [REDACTED] and her staff.

Research Animal Resources Center

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To: Christine Parks
RARC
From: ██████████
RE: Protocol G00146
Date: July 1, 2003

██████████ and ██████████ cross-compared the protocols (see below) and records obtained from all possible sources (e.g., colony records, laboratory note books, check sheets) and compiled a list of discrepancies between approved protocols and actual records. The apparent and potential violations are summarized below. The period of this investigation is from March 2000 to present. The tabulated information in detail is available upon request.

Protocol approval date: 3/13/00 with final amendment on 4/19/00 Major amendment approval date: 10/15/01 Minor amendment date: 05/20/02 Renewal approval date: 04/14/03

1. Apparent Violations

- Bret left animals during the push-pull-perfusion (PPP) experiment for lunch 4 times. The protocol states that animals will be attended continuously. One animal died in the chair while Bret was at lunch.
- In 24 PPP experiments in 11 animals, 4 different drug challenges were given. The 3/13/00 protocol did not contain this, but in the 10/15/01 protocol the maximum number of drug challenges was added to be three.
- Five drugs (the ATP sensitive potassium channel blockers, tolbutamide and glybenclamide; the purinergic receptor blockers, PPADS and suramin; and the ATP hydrolysor, apyrase) that had not been stated in the 10/15/01 protocol were examined with PPP experiments.
- In 15 PPP experiments ATP doses exceeded the dose range stated in the protocols.
- In 2 animals more than 12 PPP experiments were conducted (1 animal with 13 times; 1 animal with 15 times). The 3/13/00 protocol did not contain this, but in the 10/15/01 protocol the maximum number of PPP was added to be 12.
- One c-section resulting in a 4th major surgery (the 3/13/00 protocol stated that the maximum number of major surgeries was 3. This confusion occurred because the protocol stated that "the animals used for the project 1 (puberty project) with PPP experiment can be used for breeding females in project 2, in which 3 c-sections were allowed".

2. Inadvertent Violations

- Five animals received reheadcap surgeries. This procedure was not described in the 3/13/00 protocol, but it was added in the 10/15/01 protocol.
- In 15 animals blood draws were conducted on project 2, in which no blood draw was described. In projects 1 and 4 blood drawings are described.

- In 12 animals more than 4 ml of bloods were drawn in one week. The protocol stated that taking more than 4 ml/week of bloods requires CBC. (See also related problems in No.3).
- Forty-two animals received higher doses (up to 20 mg/kg) of ketamine. The protocol stated that ketamine dose will be 10-15 mg/kg.
- Antibiotics were given to 18 animals after PPP experiments. In most cases a staff veterinarian approved the treatment, but consent was not in writing. In fact, Carol Emerson suggested antibiotics after PPP experiments on a regular basis.
- Prophylactic antibiotics were given before and after surgeries, such as cranial pedestal implantation as well as cranial pedestal repair and removal, in 59 animals. Staff veterinarians knew of this practice, but we failed to describe it in the protocol.

3. Errors due to Reproductive Services for Breeding in Our Animals

- In 7 animals Reproductive Services took blood samples up to 14 ml in a week under our protocol.
- Reproductive Services conducted ultrasounds in 8 animals under our protocol. Ultrasound examination was not described in the protocol.

4. Record Errors

- Fourteen initials/signatures are missing from check sheets.
- Forty-one hourly check sheets for Chairing/PPP experiments conducted by a former postdoctoral research fellow (Dr. Shinichiro Nakamura) are missing. Since the check sheets had to be displayed on the door of room [REDACTED] when the experiment was conducted, they must have been filled out. However, at this time we cannot locate these records.
- Errors in computer records are found in the following animals:

r94070	6/15/01	Remark by a veterinary assistant says "in chair". However, this animal did not have cranial pedestal at this time and there is no chairing record. So, there is no way that she was in chair.
r96130	7/10/00-7/14/00	Record says she was in chair after cranial pedestal removal. This is not possible.
r96112	8/8/00	The animal should be 96119. Record has been corrected.
r99046	6/12/01	The animal should be 97046. Record has been corrected.
r98003	6/12/01	The animal should be 98033. Record has been corrected.
r98040	7/10/01	The animal should be 98049. Record has been corrected.
r84108	10/3/00	This monkey does not belong to this protocol, because of a male. To take blood Reproductive Services assigned to our protocol.
rhaa56	10/3/00	This monkey does not belong to this protocol, because it is a male. To take blood Reproductive Services assigned to our protocol.
r87100	12/8/00	This monkey does not belong to this protocol, because it is a male. To take blood Reproductive Services assigned

		to our protocol.
rhaq36	4/30/01	Cranial pedestal removal surgery was stated as major surgery.
r93061	3/13/01	Cranial pedestal removal surgery was stated as major surgery.
rh1935	10/8/01-10/11/01	Chairing is not stated in colony record.
rh2116	05/14/01	Record states "out of chair on 5/14". But it should be 5/4, because there is no way that she was in chair for 13 days.
rh2118	04/25/01	PPP experiment was conducted on 4/26/01. The date of the out of chair should be 4/26/01.
r93058	12/10/02	PPP experiment was conducted on 12/11/02. The date of the out of chair should be 12/11/02.
r96082	11/27/00-11/29/00	Chairing is not stated in colony record.
r98033	1/6/03-1/8/03	Chairing is not stated in colony record.
r99031	2/18/01 and 2/19/01	Two records of cannulation. 2/19/01 is correct, based on chairing record.
r99031	03/12/02	Cannulated three weeks after last time by accident. However no PPP experiment was conducted.
r99077	09/10/01	Record says that pedestal repair and cannula implantation. Only cranial pedestal repair surgery was conducted.
r01050	10/14/02	There is no record saying that ketamine was injected.
rhYO3	05/12/01	Cannulation date should be corrected to 14 th , based on the chairing record and the PPP experiment, which was conducted on the 17 th .

5. Others

- Three animals had skin lesions after chairing.

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All "Apparent Violations" were recognized during the review process for the renewal of recent protocol (approved on 04/14/03) and discussed with the Graduate School IACUC. Also, my letter dated 6/5/02 in response to the Dr. Eric Sandgren's letter dated 5/13/03 dealt with some of the issues. Thus, they have been resolved.

Most of the "Inadvertent Violations" are due to unrealistic descriptions in the previous protocol or absence of our recognition, e.g., the activity by the Reproductive Services Unit. We will submit an amendment to correct these errors:

1. A series of 3 ml blood draws up to 12 ml in an experiment will be added to project 2 and project 4.
2. Ketamine dose will be changed from 10-15 mg/kg to 10-20 mg/kg. p. 12, 13, 20, 23
3. Prophylactic antibiotic treatments before and after surgeries such as cranial pedestal implantation, cranial pedestal repair and removal, will be added to the protocol. p. 25
4. Activities of the Reproductive Services Unit, such as blood draw up to 14 ml/week, ultrasound examination and possible artificial insemination, will be added to the protocol.

Examination of clinical records from 2000- 2003 by Christine Parks and Amanda Crumbaugh.

1.) Unusual number of death/ serious illness in animals used late 2001 – mid 2002 (23 monkeys used; events of use included even if outside above date range.)

Some of the animals were aged. The following are summaries/quotes from the clinical records:

- a). rhap53- Died 6/17/02, 6 days after experiment (push pull perfusion). Necropsy report in clinical record said : “ The infarct and thrombus most likely occurred during the experimental procedure 6 days prior to death. They could account for the altered mentation reported clinically, but the ventricular hemorrhage and acute brain swelling are the most probable reason for the monkeys rapid clinical decline and demise.”
- b). rhao21 – In 2001, animal had problems such as lethargy, inappetence, lying down in cage after experiment, cannula implant infection, holding hand stiffly, ulcers. Was noted to have a guarded prognosis but recovered (3/2001). Used again in late 2001 and January 2002; ulcers on 1/29/2002.
- c). 95105 – 3/11 cannulated On 3/15/2002 – poor recovery after experiment (vomiting, lethargy, laying down in cage, dehydrated). 5/28/02 cannula placed. 5/31/2002 –“ lethargic and not eating post procedure; evolved to apnea; good hr and pulse; no spontaneous respiration; pc02 61.4 mmtg; pupils fixed and dilated; elected euthanasia - by i.b.” Necropsy: Cerebral/cerebellum edema but no histological diagnosis due to accidental freezing of tissue.
- d). rh2116 – 3/06/2002 - “received emergancy (sic) call that chaired animal had died; dead at least 5 minutes (no crt), attempted chest compressions; no heart beat/blood pressure; called pathologist on duty, left message - by [REDACTED]. Three moderate to severe ulcers on caudal thigh, pressure point in chair; blood on ulcers - by [REDACTED].” Blood clot in heart found on necropsy: probably throwing thrombi into circulation. Laboratory notebook notes that something unspecified is “bloody” or “pinkish” while in the chair.
- e). 00046 - 11/08/2002 “in chair on experiment; ulcer (1cm) noted on left foot; cleaned and applied ointment; some swelling of foot, foot is warm; able to use foot and is eating; plan-observe - by [REDACTED]” “removed from cahir (sic) procedure 11-7 p.m.; left ankle appears irritated with open sore present on lateral surface; appears bright, alert, responsive and hydrated with good attitude; discuss treatment with vet- by j.b.”
- f). 00040 –12/20/2001 – “post push-pull observation-animal is lethargic”
12/21/2001 – “down in cage; eyes open; will move if touched; push-pull procedure ended 12/20; antibiotic started 12/20; plan- collect cbc/chemistry; monitor closely; [REDACTED]” Lethargic for a few more days but improved by