

2004 award totals: \$132,345

1. Thea Brabb, DVM, PhD, University of Washington “Analgesic Mitigation of Pain Associated with Embryo Transfer”

The focus of our study was to determine if embryo transfer causes pain that can be relieved with analgesics without affecting the yield of viable pups. To determine if pain was present following embryo transfer, we videotaped mice for ten minutes per hour for the first nine hours following embryo transfer and evaluated those videotapes for behavioral signs of pain. We used the standard anesthetic protocol used in the University of Washington Transgenic Facility (ketamine (130mg/kg) and xylazine (8.8 mg/kg), i.p.) for the surgery and the usual strain of recipient mice (CD-1). Mitigation of pain through analgesia administration of buprenorphine (0.05 mg/kg), oxymorphone (0.2 mg/kg), carprofen (5 mg/kg) or saline at the time of embryo transfer was explored. In addition, the number of normal fetuses present in the uterus post embryo transfer was determined.

Our study indicates that buprenorphine, oxymorphone and carprofen do not interfere with the success of embryo transfer. We found no difference between the numbers of normal pups, the number of resorbing fetuses, or the percent of mice that became pregnant between the different groups of animals. No malformed fetuses were noted.

By behavioral scoring, we saw no change in mobility, grooming, digging, twitches, or stretches in mice that underwent embryo transfer performed by our experienced surgeon. When the surgery was altered to make it more traumatic (as would be produced by an inexperienced surgeon), we did find decreased movement and decreased overall grooming in these mice ($p < 0.01$) when compared to mice which had an equally poor surgery but received carprofen (5 mg/kg SQ), anesthetized mice which had no surgery, or mice which received a normal surgery.

In summary, we saw no detectable signs of pain in CD-1 mice during the first 9 hours following embryo transfer performed by an experienced surgeon. However, signs of pain were observed, and ameliorated by a one time dose of carprofen, in mice that received traumatic surgery.

2. Melanie Ihrig, MS, DVM, Texas A&M University “Incorporation of Analgesics into Established Rodent Studies: Assessing the Effects on Experimental & Reproductive Outcomes”

This research encompassed two sets of experiments, one set involved rats, the other mice. The purpose of both was to determine the effects of analgesics on experimental outcomes in ongoing studies in our collaborators’ laboratories.

Rat Study

At the outset, the analgesic drugs that were to be evaluated in the rat study were ketoprofen and marcaine. Ketoprofen was administered perioperatively in association with an ovariectomy and marcaine was administered topically as part of a stereotaxic procedure. The ovariectomy was performed three weeks prior to the stereotaxic surgery. The initial experimental design in the rat study included 72 young Sprague Dawley female rats, each weighing approximately 250 grams. Twelve animals were in each of 6 treatment groups; group 1) ketoprofen administered to **no surgery** controls, 2) surgery with placebo pellet and saline controls 3) surgery with placebo pellet and ketoprofen only, 4) surgery with placebo pellet, ketoprofen and marcaine, 5) surgery with estrogen replacement pellet and ketoprofen only and 6) surgery with estrogen replacement pellet, ketoprofen and marcaine. The placebo and estrogen replacement pellets were included as factors in our collaborator’s research.

After 36 of the 72 rats had been used in the study as initially planned, 8 additional animals were added to explore problems that arose during the study. Thirty-two of these 44 animals received ketoprofen, either as controls or in conjunction with surgery. Of the 32 rats that received ketoprofen, 19 (60%) were either found dead or showed clinical signs and were euthanized within seven days after the surgery. Necropsy of the affected rats revealed significant gastrointestinal ulceration. Because of this unanticipated pathology, which was suggestive of nonsteroidal anti-inflammatory drug toxicity following ketoprofen administration, a follow-up study was performed in an attempt to identify factors responsible for this unexpected outcome. No adverse effects of ketoprofen in Sprague Dawley rats at the dosage being used had been reported in the literature previously. After eliminating numerous potential explanatory factors including contamination of the bottle of ketoprofen in use, improper preparation or calculation of the ketoprofen dose, improper administration of the drug or possible drug interactions, we hypothesized that the effect was attributable to a phenotype specific to the Sprague Dawley rats acquired from this particular vendor.

To test this hypothesis, twelve rats from each of two vendors were subjected to bilateral ovariectomies and received either ketoprofen or saline postoperatively. Rats were monitored for 10 days post-surgery. No clinical signs were observed and no lesions were noted at necropsy in either group. At the completion of this study it was discovered that although the rats were acquired from the same vendor as in the initial study, they had come from a different barrier than those used in the first study.

A second follow-up study was conducted to reconcile these conflicting results. Twelve rats acquired from each of the two barriers used in the earlier studies from the original vendor were subjected to bilateral ovariectomies and received either ketoprofen or saline postoperatively. Rats were monitored for 10 days post-surgery. Six rats from each barrier received ketoprofen and six rats from each barrier received saline. Of the six rats from the original barrier that received ketoprofen, three (50%) had gastrointestinal lesions similar to those observed in the initial study. The six rats from the second barrier that received ketoprofen had no lesions on gross necropsy. Histopathology revealed inflammatory gastroenteritis, ulcerative and necrotizing gastroenteritis with bacterial septicemia, and acute hepatic necrosis in the three rats from the original barrier.

We concluded that outbred rats acquired from a single vendor, but from separate barriers may develop differing sensitivities to a drug. This may be due to either environmental factors unique to a barrier, or genetic differences that develop within a barrier over time.

Mouse Study

The analgesics under evaluation in the mouse study were ketoprofen and buprenorphine. They were administered perioperatively to mice undergoing embryo transfer surgery. Recipient female mice were divided into five treatment groups: 1) no surgery/ketoprofen, 2) no surgery/buprenorphine 3) surgery/saline, 4) surgery/ketoprofen, 5) surgery/buprenorphine. Each experiment consisted of five mice, one mouse in each of the five named groups. The three surgical mice in each experiment underwent either a uterine or oviduct embryo transfer.

Thirty-eight uterine transfer experiments were performed and 15 oviduct transfer experiments were performed, for a total of 265 experimental females. The mice that did not undergo surgery, yet were administered analgesics, were observed to determine the effects of the drugs alone, when no stimulation was provided by surgery. Three major outcomes were measured to determine the effects of administering analgesics in association with reproductive surgeries in mice: 1) number of litters overall, 2) number of pups born and 3) number of pups that survived to weaning.

In the 38 uterine transfer experiments, 114 recipient females underwent surgery, 76 acted as no surgery controls. In the 15 oviduct transfers, 45 females underwent surgery, 30 served as no surgery controls. A chi square test was done to determine if there was an effect on litter success with the administration of either ketoprofen or buprenorphine when compared to saline. There was no association found between analgesics and loss of litter in either the uterine or the oviduct transfer procedure, regardless of the analgesic used ($p=0.880$ (ketoprofen), 0.480 (buprenorphine) for the uterine procedure, and $p=0.890$ (ketoprofen), 0.648 (buprenorphine) for the oviduct procedure).

Analyses of Variance (ANOVAs) were performed to determine the significance of the effects of analgesics on reproductive outcomes. The results from these analyses indicate that the number of pups born is not affected by the pre-operative administration of buprenorphine or post-operative administration of ketoprofen when compared with the administration of saline as a control ($p=0.9526$ (uterine transfer), $p=0.6876$ (oviduct transfer)).

In analyzing the effects of analgesics on the number of pups weaned, weight of the recipient was included in the analysis as a covariate because weight of the females varied substantially and because weight of the mother can be an important factor in reproductive success. Once again, the results indicate that the administration of buprenorphine or ketoprofen, when compared with the administration of saline as a control, has no effect on the number of pups surviving until weaning age ($p=0.6697$ (uterine transfer), $p=0.9526$ (oviduct transfer)).

The underlying assumption of this study was that the analgesics used, ketoprofen and buprenorphine, alleviate pain and distress in mice and therefore have a positive effect on the welfare of the animals. The results from this study led us to conclude that the administration of either of these analgesics to mice does not adversely affect the reproductive yield following embryo transfer. Therefore, we recommend the use of analgesics when performing reproductive surgeries in mice.

3. Atul J. Shukla, PhD, University of Tennessee “Controlled Release of Analgesics in Mice” The objectives of this project are to evaluate biodegradable injectable gels for delivery of buprenorphine in vitro and in vivo.

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Objective: The objective of this study was to develop a long acting formulation of buprenorphine. **Methodology:** To ascertain analgesic concentrations of buprenorphine in mice, a dose of 2.4 mg/kg of buprenorphine hydrochloride was injected into the mouse tail vein. Both analgesia (tail flick method) and plasma concentrations of buprenorphine were measured at each time point. The compatibility of the drug in three different vehicles namely, Na CMC solution, Acetyl triethyl citrate (ATEC) and sesame oil were evaluated. Buprenorphine suspensions prepared from the aforementioned three vehicles were subcutaneously injected into mice. Both analgesia and plasma concentrations of the drug were determined up to 5 days. **Results:** The onset of analgesia following intravenous injection was 15 minutes with 53.7% MPE. It peaked at 60 minutes with 100% MPE and remained for approximately 7 hours. The analgesic effect dropped to 54% MPE at 9 hours with a plasma concentration of 5.2 ng/mL. No analgesia was observed at 12 hours. The drug was compatible in all the three vehicles for at least 30 days. Analgesic effect in mice was maintained for at least 4 days, while the buprenorphine plasma concentrations ranged from 39 to 261 ng/mL following subcutaneous injections of the three buprenorphine suspensions. **Conclusion:** Long-acting buprenorphine formulations were successfully developed for mice.

4. Stephen T. Kelley, MS, DVM, University of Washington “Flaviviral Screening & Seroepidemiology in Macaques: West Nile & Japanese Encephalitis Viruses”

Renee Rosemary Hukkanen and Stephen T. Kelley

West Nile virus (WNV) is an encephalitic virus belonging to the genus *Flavivirus*, family *Flaviviridae*, and the Japanese encephalitis virus serogroup. Since the North American emergence of WNV along the Eastern seaboard in 1999, the virus has been detected in every continental state, all but one Canadian province, and most of northern Mexico. A recent report showed that WNV infection rates may exceed 30% in out-door housed macaques within endemic regions. Animals from these colonies are routinely transported to the Washington National Primate Research Center (WaNPRC), located within a region currently free of WNV (Seattle, WA). Confirmation of WNV infection in non-human primates (NHP) traditionally requires the use of a gold standard assay, the plaque reduction neutralization test (PRNT). The PRNT assay requires a high level of biological safety (BSL3), which may not be readily available in all institutions housing NHP. Therefore, we developed and validated a simple, fast, species-independent enzyme-linked immunosorbent assay (ELISA) assay for use in screening macaques for WNV. The ELISA is based on a non-structural (NS1) viral protein of Kunjin virus, a subtype of WNV. As such, it detects viral replication and patent infection. The ELISA was validated through correlation with PRNT assays. This correlation allows for interchangeable use of the ELISA and PRNT assays.

Using the developed ELISA assay, WNV antibody isotype, titers, and persistence in macaques were studied using banked plasma samples from 1999 to 2004. ELISA seropositivity to WNV was demonstrated to persist for up to 36 months in out-door housed macaques within WNV endemic regions. Persistent antibody titers may be related to continued environmental exposure and to the presence of co-circulating flaviviruses. Western Blot techniques were employed to discern between WNV and St. Louis Encephalitis Virus (SLEV), a co-circulating flavivirus. Banding patterns were compared between blots generated from WNV or SLEV infected Vero cell lysates. The relative banding strength of the viral envelope protein allowed for distinction between viruses. For each animal, NS1 ELISA seropositivity and PRNT tiers were correlated with Western blot banding patterns and found to be 88% and 76% concordant respectively.

As the ELISA assay was developed for use at the WaNPRC, seven commercially available human diagnostic flaviviral assays were further compared with the validated ELISA. Assays were evaluated for detection of WNV seroconversion in macaques and included: West Nile Virus IgM Capture, West Nile Virus IgG Indirect, and Arbovirus Screen (USA) IFA Slides (PanBio Inc., Columbia, MD), West Nile Virus IgG DxSelect, West Nile Virus IgM Capture DxSelect (Focus Diagnostics, Cypress, CA), West Nile Detect IgM and West Nile Virus IgG analyte specific reagents (ASR) (InBios International, Inc., Seattle, WA). PanBio Arbovirus Screen (USA) Slides demonstrated high sensitivity (100%) and predictive value (positive: 88%, negative: 100%) with low specificity (50%). As such, they would provide adequate diagnostic screening for animals entering a colony where general flaviviral reactivity is of concern. The PanBio IgG ELISA demonstrated high sensitivity (88%) and specificity (92%) for WNV with strong predictive value (positive: 92%, negative: 88%). As such, the PanBio IgG ELISA is a useful secondary, possibly confirmatory, WNV diagnostic immunoassay for colony viral screening.

In summary, we have developed a sensitive and specific ELISA for WNV screening at the WaNPRC. This assay may be performed under BSL-2, or BSL-1 conditions with heat-inactivated serum. Additionally, we have developed a Western blotting technique to differentiate WNV from SLEV infection. We have further validated two commercially available human WNV assays for use in macaques. These assays are simple, BSL-2 (or BSL-1 with heat inactivation)

compatible, cost-effective means of screening for WNV. These assays are ideal for use in facilities which house macaques, but can not perform in-house virologic analysis at the BSL-3 level of containment.

5. Jennifer K. Pullium, MVB, Emory University “Evaluation of Current Standards for Frequency of Wire Bar Lid and Filter Top Sanitization in Rodent Caging.”

Curtis W. Schondelmeyer, DVM; Dirck L. Dillehay, DVM, PhD; Sonji K. Webb; Michael J. Huerkamp, DVM; Deborah M. Mook, DVM; Jennifer K. Pullium, MVB

Frequent sanitation of wire bar lids and filter tops is an expensive part of caring for and housing rodents. *The Guide for the Care and Use of Laboratory Animals* states “In general, enclosures and accessories, such as tops, should be sanitized at least once every two weeks,” however there is no published scientific justification for this recommendation. It is also unclear if these guidelines are meant to minimize organic contamination, bacterial contamination, or both. Furthermore, there are several studies that support less frequent sanitation of these caging accessories. The measurement of adenosine triphosphate (ATP), expressed in relative light units (RLU), is a new method of measuring organic matter and provides a more accurate measurement of cleanliness. The ATP method is now frequently used in food production facilities, state health laboratories, drug companies, and as a method of cagewasher effectiveness in laboratory animal facilities. In addition, current microbial evaluation programs suggest evaluating cages for sanitation based on assessment of different morphological groups rather than specific organisms. The most important and useful being Gram negative morphology. Currently there are no standards stating the acceptable levels of RLUs and CFUs for caging accessories. Our study did not attempt to define standards of acceptable levels of RLUs and CFUs, but set out to demonstrate that there was no significant difference between cage accessory contamination at two weeks versus several months, thus questioning the requirement of bi-weekly sanitation of caging accessories. Our hypothesis was that organic contamination (adenosine triphosphate, (ATP) and the number of bacterial colony forming units (CFU) on caging accessories did not differ significantly at 2 weeks versus several months of use. The study examined four groups, mouse and rat ventilated and static wire bars with or without filter tops (n=10/per group). Cages were evaluated for ATP levels using Charm Firefly® swabs expressed as relative light units (RLU) and bacterial CFU on RODAC plates. The cages were evaluated at several time periods from 2 weeks to 6 months.

When examining the amount of organic material (RLU) that accumulates on caging accessories over a 180 day period, the majority of the equipment examined showed no significant difference between 14 and 180 days. The amount of organic material (RLU) is important because it measures cleanliness (including food residue and biofilm from humans and animals), as well as replicating bacteria. The CFU data show that the numbers of Gram negative bacterial CFU are quite low: in all but one case they are less than 50 Gram negative CFU per RODAC plate. Current American Public Health Association standards for judging RODAC plate counts from patient room floors describe CFU counts of 0-25 as good and 25-50 as fair. Although these guidelines refer to both Gram positive and Gram negative bacteria, others have suggested that the Gram negative bacteria are most clinically important. Due to the expense associated with using large populations of rodents, institutions have been forced to reevaluate how they care and house such animals. Less frequent sanitation can have a direct effect on labor and operating costs for laboratory animal facilities. These include decreases in the number of caging accessories needed on-hand, less storage space to contain housing accessories, and reduced labor costs. All of these factors have the ability to control and potentially decrease costs associated with cage accessory sanitation.

In conclusion, our CFU and RLU data show that bi-weekly sanitation of filter tops and wire bar lids for laboratory mice and rats is not necessary. All filter tops and wire bars can be left

unsanitized with no significant change in the amount of organic material or Gram negative bacterial contamination for at least 90 days.

6. ER Griffin Research Foundation Grant: Jeanette E. Purcell, DVM, DACLAM, Tulane University/Tulane National Primate Research Center “Shedding of Herpes B Virus: A Comparison of SIV-positive and SIV-negative Rhesus Macaques (*Macaca mulatta*)”

The Simian Immunodeficiency Virus (SIV)-infected rhesus macaque is the premier model utilized in HIV/AIDS research. While the NIH has made funding available for the production of SPF rhesus macaques to support these studies, it is available only to participating Centers. As a result, a large number of rhesus macaques currently used for SIV studies are of either of B Virus positive or unknown status. SIV in the rhesus macaque usually results in immunosuppression and illness from opportunistic agents. We thought it possible that B Virus, which generally remains latent during infection, could be shed more often in SIV-infected when compared to uninfected rhesus macaques. If SIV infection were to cause an increased shedding of B Virus, this would be important from an occupational health perspective. To investigate this question we collected mucosal swabs for B Virus PCR from 27 B Virus-positive SIV-positive and 23 B Virus-positive SIV-negative rhesus macaques every third day (days 1,4,10,13,16,19,22,25 and 28) over the course of one month. Blood was collected on days 1, 16, and 28 for B Virus antibody titer and CD4+/CD8+ determinations. No SIV-positive animals were found to shedding B Virus at any time point during the study. Four SIV-negative animals were found to be shedding B Virus by PCR at one or more time point. All animals remained B Virus antibody positive throughout the course of the study. No herpetic lesions were identified on any animals at any time point. Our study indicates that SIV infection does not appear to increase shedding of B Virus from B Virus antibody positive animals. The animals on the study were not moved prior to the initiation or during the course of the study. Interestingly, three of the four animals that shed B Virus were housed in a building where other animals were transitioned from outdoor housing before being moved to the main research facility. It may be that this location, which tended to be socially unstable, played a role in the shedding of B Virus in these animals.