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Effects of Multimodal Analgesia on the Success of Mouse Embryo Transfer Surgery

John M Parker, Jamie Austin, James Wilkerson, and Larry Carbone*

Multimodal analgesia is promoted as the best practice pain management for invasive animal research procedures. Universal acceptance and incorporation of multimodal analgesia requires assessing potential effects on study outcome. The focus of this study was to assess effects on embryo survival after multimodal analgesia comprising an opioid and nonsteroidal antiinflammatory drug (NSAID) compared with opioid-only analgesia during embryo transfer procedures in transgenic mouse production. Mice were assigned to receive either carprofen (5 mg/kg) with buprenorphine (0.1 mg/kg; CB) or vehicle with buprenorphine (0.1 mg/kg; VB) in a prospective, double-blinded placebo controlled clinical trial. Data were analyzed in surgical sets of 1 to 3 female mice receiving embryos chimeric for a shared targeted embryonic stem-cell clone and host blastocyst cells. A total of 99 surgical sets were analyzed, comprising 199 Crl:CD1 female mice and their 996 offspring. Neither yield (pups weaned per embryo implanted in the surgical set) nor birth rate (average number of pups weaned per dam in the set) differed significantly between the CB and VB conditions. Multimodal opioid–NSAID analgesia appears to have no significant positive or negative effect on the success of producing novel lines of transgenic mice by blastocyst transfer.

Abbreviations: CB, carprofen–buprenorphine; ES cell, embryonic stem cell; ET, embryo transfer; NSAID, nonsteroidal antiinflammatory drug; VB, vehicle–buprenorphine.

Surgical transfer of mouse embryos (embryonic transfer, ET) to surrogate dams is currently standard procedure in producing transgenic mouse models for research; the technique also is used to reestablish pathogen-free stocks of mice.[25] The procedure is invasive, in that it penetrates the peritoneal cavity and reproductive tract, often requires bilateral flank incisions through skin and muscle (each equivalent in length to approximately 10% of the snout–anus length), entails externalization and traction of internal organs, and requires wound closure. The ideal analgesia regimen for rodent ET would safely manage pain in the recipient female mouse without adversely affecting the quality or number of offspring from implanted embryos. Studies to date have found no effect of either the opioid buprenorphine or the nonsteroidal antiinflammatory drug (NSAID) flunixin on the number of embryos surviving after ET when compared with those after untreated or saline-placebo mice.[17,20] Furthermore, no study to date has looked at whether a multimodal combination of opioid and NSAID might either improve or decrease embryo survival.

In the present study, we sought to compare reproductive outcomes of NSAID–opioid analgesia compared with opioid alone in embryo-transfer recipient mice by comparing outcomes from surrogate dams treated with carprofen and buprenorphine (CB) with those from dams treated with vehicle and buprenorphine (VB). We performed our analgesic comparison in the context of ongoing work in the University of California–San Francisco Transgenics Core. The Core receives targeted embryonic stem (ES) cell clones from laboratories and microinjects them into 8-cell embryos obtained from superovulated B6D2F1/Crl and Swiss Webster mice. The Core then surgically places these embryos into 1 to 3 recipient female mice, resulting in the generation of mice chimeric for the targeted ES cells and host blastocyst cells. To minimize any effects of learning curves, disruption of routine, and variability to ongoing research, the surgeon was instructed not to deviate from Core protocol in regard to animal anesthesia, handling, or housing. Therefore, the only change was to substitute buprenorphine with either carprofen–buprenorphine or vehicle–buprenorphine.

Measured outcomes were the average number of weaned offspring produced per embryo implanted in the surgical set (that is, yield) and the average number of weaned offspring produced per dam in the surgical set (that is, birth rate).

Materials and Methods

The program at the University of California–San Francisco is fully AALAC-accredited and the protocol was approved by the facility's Institutional Animal Care and Use Committee. All animals used in this study were part of ongoing work in the institution's transgenic mouse core facility. No changes were made to the ongoing protocol other than the preoperative analgesics administered.

Animals were housed in ventilated cage rack systems (Lab Products, Seaford, DE) plumbed with UV-sterilized, filtered, dechloraminated, purified water, fed Purina irradiated breeder chow 5080 (LabDiet, Purina Mills, St Louis, MO), and maintained on paperchip bedding (Shepperd Specialty Paper, Richland, MI). The light:dark cycle was 12:12 h. The ambient room temperature and humidity were maintained at 68 to 70 °F (20.0 to 21.1 °C) and 30% to 40%, respectively. Cages were changed every other week. All routine sentinels in a soiled-bedding sentinel program were negative throughout this study. The sentinels were screened for epizootic diarrhea of infant mice, mouse hepatitis virus, mouse parvovirus, minute virus of mice, Theiler murine encephalomyelitis virus, *Mycoplasma pulmonis*, ectromelia virus, pneumonia virus of mice, Sendai virus, fur mites, and pinworms.

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Laboratory Animal Resource Center, University of California, San Francisco, California.
Corresponding author. Email: larry.carbone@ucsf.edu

Donated embryos. All host embryos were derived initially from either B6D2F1/Crl or Swiss Webster CFW (both from Charles River Laboratories, Wilmington, MA) mice. Donor female mice (age, 6 wk) were superovulated by intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (Sigma-Aldrich, St Louis, MO) at 2 d precoitus followed 46 to 48 h later by 5 IU IP of human chorionic gonadotropin. (Calbiochem, EMD Biosciences, San Diego, CA) and bred to stud male mice of the same stock. Female mice with copulatory plugs were identified the following day and euthanized by CO_2 inhalation 48 h after vaginal plug detection for collection of 8-cell staged embryos (age, 2.5 d postcoitus). Embryos (n = 20 to 70) were injected with 8 cloned ES cells. The number of embryos receiving ES cells ultimately depended on the quality of the ES cells provided by the laboratories. Standard general microinjection technique was followed, although the cell line, handling, manipulations, and overall quality of the grafted ES cells varied by laboratory and were not standardized. Once microinjected, embryos were expanded in vitro to blastocyst-staged embryos (age, 3.5 d postcoitus), of which only appropriately developed viable blastocysts were transferred into pseudopregnant recipient dams at 2.5 d postcoitus.

Recipient female mice. Crl:CD1 female mice (n = 199; age, 5 wk; Charles River Laboratories) were used in this study. Pseudopregnancy was induced by mating female mice with Crl:CD1 vasectomized males (Charles River Laboratories), and ET was conducted at 2.5 d postcoitus in surgical sets of 1, 2, or 3 recipient female mice. The number of recipient female mice, as well as the overall number of embryos transferred per surgical set, ultimately was determined by the quality and quantity of ES cells provided by the laboratories.

In total, 99 surgical sets of female mice were assigned nonrandomly to either the CB group (n = 55) or VB group (n = 44). On any given surgery day, the first set of mice was assigned to a group based on date (CB on odd-numbered days, VB on even-numbered days); subsequent sets of mice that day were assigned to groups in alternate fashion (for example, on an odd-numbered day, the first set of mice would be assigned to the CB group, the second set would be VB, third set would be CB, and fourth VB). Recipient female mice were housed either 1 or 2 per cage, with no mixing of female mice from different sets.

Surgery. All anesthesia, analgesia, and surgeries were performed by a single experienced surgeon, who was blind to whether the animals received CB or VB analgesic. Recipient female mice were anesthetized by a single intraperitoneal injection of tribromoethanol (250 to 400 mg/kg; Sigma-Aldrich). Prior to incision, anesthetized animals received either CB or VB analgesic by subcutaneous injection. After the mouse became unresponsive to paw pinch, sequential bilateral flank incisions approximately 1 cm in length were made, allowing each uterine horn to be exteriorized. Blastocysts were introduced into each uterine horn through a 27-gauge needle under the guidance of a stationary magnifying lens. Recipient mice received 15 to 28 blastocysts divided equally (±1 when receiving an odd amount) between the right and left horns, abdominal anatomy was restored, and the incision closed in a single layer by using one wound clip for each incision. Recipient female mice were implanted in sets of singletons, pairs, or triplets depending on the availability of viable mutant blastocysts of a given genotype to be administered.

Treatments. Mice received either CB or VB by subcutaneous injection prior to surgery. Simple coding of the vials enabled the administrator to remain blinded regarding which analgesic was administered. CB animals received a preemptive–preventive dose of a combination of 5 mg/kg carprofen (Pfizer, New York, NY) and 0.1 mg/kg buprenorphine (Reckitt Benckiser, Bristol, UK). VB mice received the same preemptive–preventive dose of buprenorphine, with a volume of propylene glycol vehicle equivalent to the volume of carprofen received by CB group.

Postsurgical care. Postoperative mice were allowed to recover individually in clean cages, which were set on top of a warming plate, and visually monitored until they regained responsiveness and awareness. Once recovered, mice were returned to standard housing either singly or paired. Pups per surgical sets were counted and weaned at 21 to 28 d after parturition by the surgeon who had performed the procedures while remaining blind to which animals were in the CB and which in the VB group. Surgical sets of recipient female mice that failed to give birth were scored as having 0 pups and included in the analysis.

Statistical analysis. All data were analyzed (StataCorp, College Station, TX) by surgical sets. The primary question was whether condition (that is, CB or VB) affected yield (defined as the number of offspring per number of implanted embryos per set) and birth rate (defined as the number of pups weaned per dam per set). Statistical significance was defined as $P < 0.05$.

Results

In total, 199 Crl:CD1 female mice, comprising 99 surgical sets (either singletons, pairs, or triads) served as recipients, from which 996 offspring were weaned. All recipient female mice recovered unremarkably from surgery.

Although the sets of female mice were not randomized by condition, the 2 groups were not different in terms of potential confounders: donor strain (that is, B6D2F1 or Swiss Webster), the number of female mice per surgical set (that is, 1, 2, or 3), the number of embryos per set, and the average number of embryos per female mouse.

The number of embryos implanted per recipient mouse ranged from 15 to 28 (mean, 21.6; median, 21; 1 SD, 2.6). The approximate overall yield (number of pups per number of embryos implanted) ranged from 0 to 0.9 (mean, 0.23; median, 0.20; 1 SD, 0.13). The mean yield of VB dams was 0.21 (median, 0.20; 1 SD, 0.10), compared with 0.24 (median, 0.21; 1 SD, 0.14) for CB dams; these differences were not statistically significant ($P > 0.05$; Figure 1). The approximate overall birth rate (no. of pups weaned per dam) ranged from 0 to 18 (mean, 4.9; median, 4.5; 1 SD, 2.7). The mean birth rate of CB dams was 5.2 (median, 4.5; 1 SD, 3.1); the mean birth rate of VB dams was 4.6 (median, 4.0; 1 SD, 2.1); these differences were not statistically significant ($P > 0.05$; Figure 2).

Because yield and birth rate were count data, Poisson regression was used to test the relationships between condition and yield and between condition and birth rate.[2] Analyses were adjusted for potentially confounding variables (that is, number of embryos per set, the number of dams per set, strain, and estimated number of embryos per dam). For both analyses, goodness-of-fit χ^2 tests confirmed the model fit the data reasonably well. The Poisson regression model with yield as the dependent variable and condition and potentially confounding variables as predictors was statistically significant (likelihood ratio χ^2, –47.0; df = 5; $P < 0.01$). Strain was the only statistically significant ($P < 0.05$) predictor of yield. The Poisson regression model predicting birth rate from condition and the potentially confounding variables was statistically significant (likelihood ratio χ^2, –214.8; df = 5; $P < 0.01$). Strain was the only statistically significant ($P < 0.05$) predictor of birth rate.

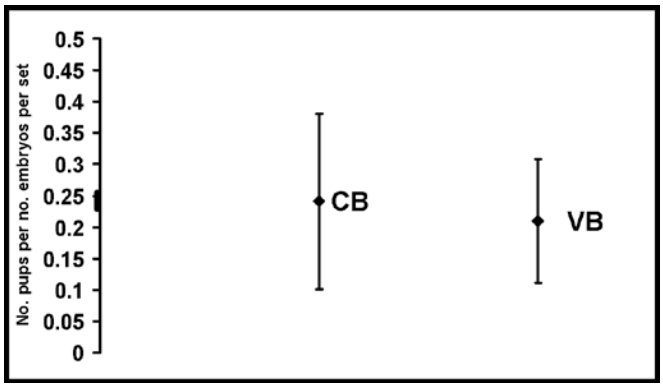

Figure 1. Number (mean ± 1 SD) of offspring weaned per embryo implanted into the surrogate dam, by surgical set. Carprofen–buprenorphine (CB; $n = 55$) and vehicle–buprenorphine (VB; $n = 44$). Difference was not statistically significant.

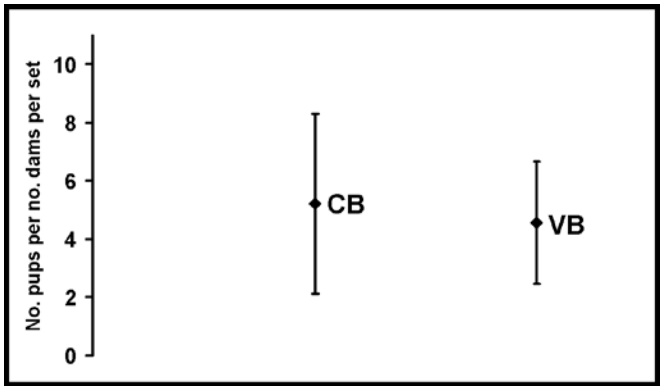

Figure 2. Birth rate (mean ± 1 SD) of pups weaned divided by the number of surrogate dams per surgical set. Carprofen–buprenorphine (CB; $n = 55$) and vehicle–buprenorphine (VB; $n = 44$). Difference was not statistically significant.

Discussion

Various authors have attempted to quantify pain after laparotomy in mice. Previous studies have not used standardized pain assessment methods, standardized time of assessment, or standardized strain of mouse. Although precise findings have varied, there is no indication that mouse laparotomies are pain-free.[1,6,7,10,16,29,36] The United States Department of Agriculture, Public Health Service of the United States, American Association of Laboratory Animal Science, and American College of Laboratory Animal Medicine share the position that "[u]nless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain and distress in other animals."[34] This principle, coupled with the available literature, suggests that ET by laparotomy should be considered as potentially painful in mice.

Multimodal analgesia should be considered for surgeries of predicted moderate to severe intensity, to increase the efficacy and safety of analgesia.[11] Several professional organizations endorse preventive pain management through multimodal analgesia for surgeries of moderate intensity,[3-5,11,33] and it is the standard of veterinary care at our campus. Available classes of analgesic drugs include opioids such as buprenorphine, NSAID, and local anesthetic analgesics (lidocaine and bupivacaine). Opioids and NSAID have each been evaluated separately as sole analgesics in either ET or 'mock ET' surgeries by laparotomy in rodents,[8,16,17,20,32] but assessments of opioid–NSAID multimodal analgesia for this procedure have not been published. In addition, assessment of local anesthetics as adjunct analgesics for mouse ET has not been published to date.

The goal of the current study was to assess whether NSAID–buprenorphine multimodal analgesia would alter weaned pup outcomes compared with those after buprenorphine alone. Conducting this study in the Transgenics Core laboratory provided a 'real-world' evaluation of the effect of multimodal analgesia on mouse embryo transfer success. In doing so, however, we compromised the sensitivity to which we were able to detect differences, given the degree of variability that accompanied this study design. Although the moderate degree of variability may have obscured subtle difference between conditions, we interpret our results to indicate that NSAID–buprenorphine analgesia has no meaningful effect on, and therefore is safe for use in, transgenic mouse production

Prostaglandins have a critical role in female reproductive processes in various mammalian species.[9,12,13,26,31] In mice, targeted disruption of the gene for COX2, but not COX1, has been reported to result in failure of essential events such as ovulation, fertilization, blastocyst emergence, endometrial preparation, and implantation.[15,28,35] Selective blocking of COX2 activity on the third and fourth day of pregnancy led to dose-dependent inhibition of implantation in mice.[23] Consistent with these findings from mice are human studies that suggest prostaglandin synthesis is critical at various stages in pregnancy, including implantation and parturition.[27] As a result, excessive use of NSAID, which disrupt prostaglandin formation, has been identified as a pregnancy risk factor due to reports of spontaneous abortion.[27]

In contrast to work suggesting that the use of NSAID during ET would negatively affect reproductive outcomes, early studies in humans found NSAID to have no effect on pregnancy outcomes of in vitro fertilization, and more recent findings support beneficial effects on pregnancy rates.[14,19,24] Endometrial activation of COX can adversely affect outcome, and low-dose aspirin administered at implantation resulted in significant improvements in pregnancy rates with human in vitro fertilization compared with nonaspirin controls.[30] Meta-analyses of the use of low-dose aspirin for in vitro fertilization are equivocal.[21] One group of authors reported the NSAID piroxicam was beneficial for implantation and pregnancy rates in women who underwent embryo transfers.[24]

In addition to direct effects of prostaglandin production or inhibition on pregnancy success, we recognize that untreated perioperative pain and inflammation themselves might affect outcome. One study found no difference in success of ET when buprenorphine was compared with no-analgesia control.[20] Another group found no difference in ET success with either buprenorphine or flunixin used as sole analgesics, compared with saline placebo.[17] Buprenorphine–carprofen may act synergistically to better control pain in rodents than either drug alone,[22] so it seemed plausible to us that ET success in mice could improve with more aggressive pain management. In the current study, carprofen–buprenorphine was neither better nor worse for successful ET, as determined by numbers of weaned offspring.

Two methodologic points of the current study merit further discussion. First, the observed null effect may have resulted in part from the lack of intentional randomization, although no significant differences emerged with regard to potential confounders. Further work should involve randomization, include only one ES donor strain, and hold constant the number of dams per set and number of embryos per dam and per uterine horn. Second, consistent with the Transgenics Core protocol

and to avoid disruption of newborn litters, pups in the current study could not be assigned to particular dams but rather were allocated to sets of dams; therefore the reported differences for birth rate and yield by condition should be considered rough approximations of the per-dam outcomes.

Assessments of the clinical analgesic efficacy of preventive multimodal analgesia for the perioperative pain of ET in mice have yet to be published. Although nonsurgical ET has been described, it is not yet standardized or widely implemented;[18] therefore the potential pain of ET surgeries must be anticipated, assessed, and managed. We believe that investigators should consider strongly the use of multimodal analgesia (opioid, NSAID, incisional block). Our study indicates that carprofen–buprenorphine multimodal analgesia has little or no effect in either direction on the success of ET for transgenic mouse production and should be considered for routine use in surgical ET protocols.

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