



Evaluation of the Effect of Early Age Anesthesia for Genotyping of Mice on Body Weight

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Abstract

The use of tail biopsy is a widely accepted method of sampling tissue for genotype determination of mice. It is often argued that use of anesthesia for this procedure in young mice is either not necessary or may affect the vigor of the mice as they grow. This is a critical issue because for many studies it is required that the mice be genotyped as young as possible. In this study, we evaluated the use of administration of isoflurane anesthesia for tail biopsy in neonatal mice at various time points after birth (1, 3, 5, 7, 9 and 11 days). The 1-day time point was especially important to determine if sufficient DNA could be extracted from the tail biopsy at this early age. Three treatment groups were used; mice biopsied with the use of isoflurane; mice biopsied without the use of isoflurane; and control mice that had underwent neither biopsy nor isoflurane anesthesia. Body weights of the 3 groups were monitored over a 10-week period. There were no statistically significant differences in mean body weights between the treatment groups. Further, we found that sufficient DNA for genotype analysis was procured from tail biopsies in animals as young as 1 day of age. These findings have significant implications as they contend that the determination of the genotype can be undertaken at a very early age of the animal, with no discernible long-term harm to the animal if anesthesia is withheld.

Introduction

Our standard of tissue biopsy for genotyping was to take the samples upon weaning the mice at day 21. The mice were identified with a metal ear tag and an ear punch was made on the opposite ear for a genotyping sample. However, as projects were introduced that utilized mouse strains possessing genetic modifications that manifest as early onset of disease, the investigators needed to be able to genotype litters at a much earlier age. Our current method is applicable for pups as young as 14 days of age, but pinnae on the younger mice are not yet developed enough for the placement of an ear tag, thus we were unable to identify the pups should we take a sample. Ideally, the genetic identification of the litter these strains is no later than day 5 to allow for disease treatments to be initiated on day 5. To meet this deadline, tissue samples must be obtained by day 3 or earlier. It was determined that tail biopsies and toe tattooing would be the best option on the neonates. However, the need for anesthesia to manage potential pain and long term distress caused by these procedures had not been established for neonatal mice. A literature search revealed several options for anesthetizing neonatal mice; inhalant anesthesia, topical anesthesia, local injectable anesthesia, and submersion in ice-cold ethanol [1]. Despite published disadvantages on the use of isoflurane on neonatal animals: potential for hypoglycemia[2] and apoptosis of brain cells [3] we concluded that it appeared to be the least harmful to the pups' overall well-being. However, because it has been determined that the tail vertebrae do not mature with end plates until day 17 in mice [4] we questioned if anesthesia was necessary for the health and well-being of the neonates as they matured and are placed on study.

Materials and Methods

Anesthesia

Equipment:

- Isoflurane
- Bell Jar with platform and screen
- Cotton ball

Method:

- Place cotton soaked with Isoflurane into the bottom of the jar
- Place platform and screen into jar
- Place the mouse in the jar.
- Carefully monitor the mouse. On average it takes 20 seconds for the neonates to be fully anesthetized.

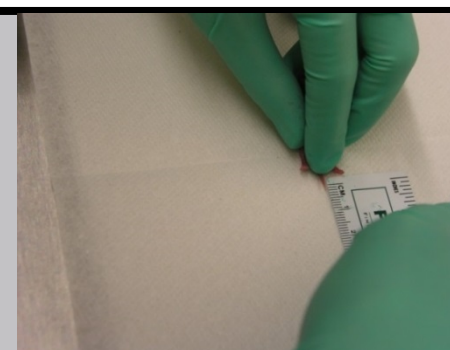


Tail Biopsy

Equipment:

- #11 scalpel blades
- 3-4 Paper Towels

Cautery pen
Ruler



Methods:

- Stabilize the tail and push down and draw the scalpel blade across the tail removing a maximum of 3 mm of tissue.
- Grasp the tail, blot any blood, and depress the switch for the cautery. When the cautery tip glows orange, touch it to the cut surface of the tail for an instant.
- Inspect for hemostasis.

Toe Tattooing

Equipment:

- Goldenrod Animal Lancet 4.0 to 5.0 mm
- Tattoo paste - green is recommended
- Holder for a dab of the paste.

Method:

- Restrain pre-weaned pups cupped in the hand.
- The animal is restrained to expose the chosen toe or toes for tattooing.
- The Goldenrod Animal Lancet tip is dipped in the tattoo paste.
- The desired toe is poked with the Goldenrod lancet. The skin must be punctured to introduce the paste into the skin leaving a mark.
- Poke the same spot 3 times to insure proper penetration
- The spot is then gently blotted on the wipes/towel to remove excess paste.



Photo taken by K J Diven, P Karavedas-Matos, and N Desir, courtesy of MEDipoint, Int. Inc.

Procedures

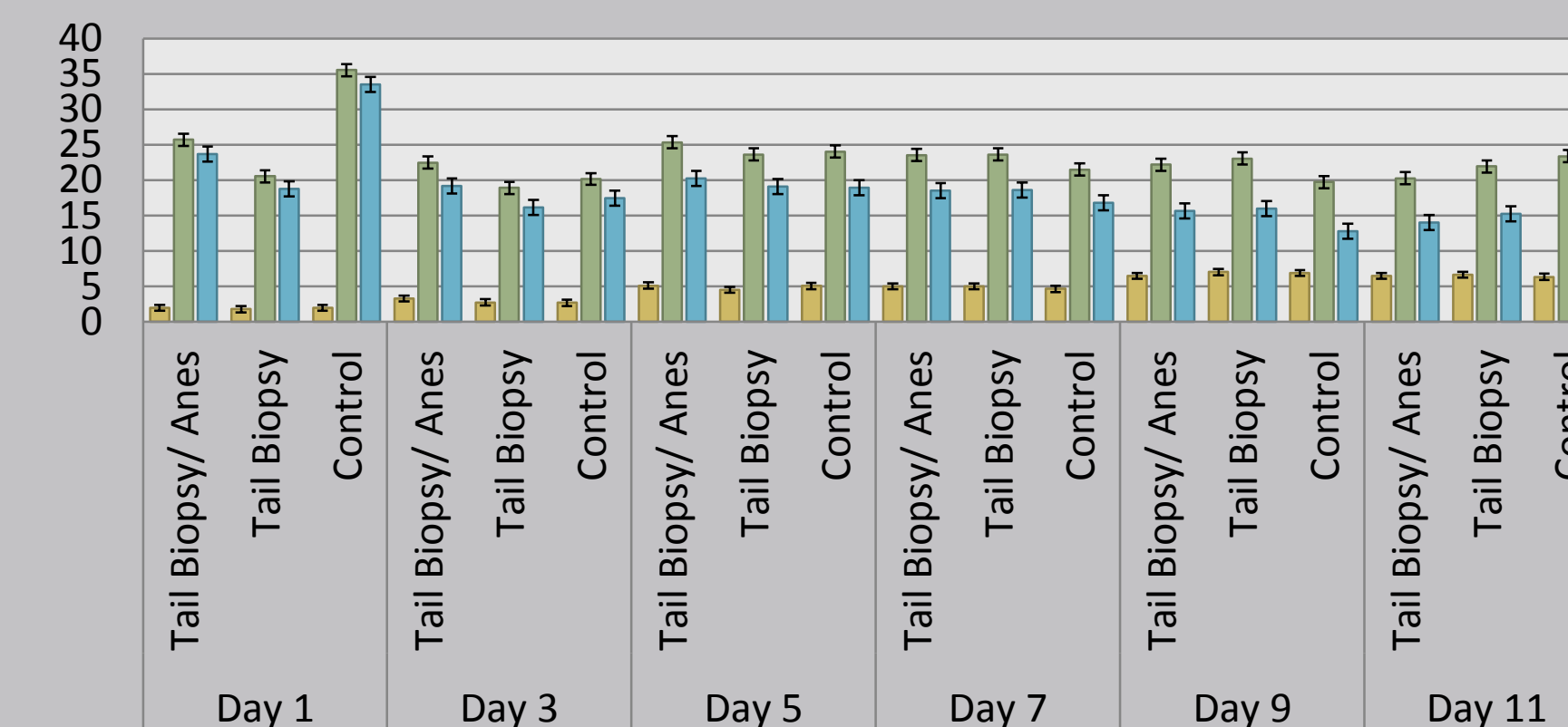
A protocol was established to test the long term effects of the tail biopsy with and without the use of Isoflurane. The age of the litters were at days 1,3,5,7,9 and 11 days of age. The litters were divided into three cohorts; tail biopsy and toe tattooing with anesthesia, tail biopsy and toe tattooing without anesthesia, and toe tattooing only. All pups were removed from the dam together and return one by one as their treatment was completed. On average, the mice were away from the dam for 5 minutes. All pups were weighed weekly until 10 weeks of age.

Results

Weight in Grams

- beginning weight
- final weight
- weight gain

Weight Gain of Pups



Discussion

Statistical analysis utilizing the Two-Way ANOVA with multiple comparisons of the initial weight, the final weights and the weight gain within each time point demonstrates that there is no significant differences in the growth weights of the pups in any of the treatment groups. All pups thrived after the procedure and appeared to have no ill affects for the use of or the absence of anesthesia. Because there is no apparent benefit to using anesthesia, we feel that the additional 20-30 seconds induction time for the anesthesia does not justify the time spent away from the dam. Thus for mouse pups under the age of 14 day, we do not use anesthesia for tail biopsies.

References

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4. Hankenson FC, Garzel LM, Fischer DD, Nolan b, Hankenson KD. JAALAS 47:10-18.