Variability of Injectable Anesthesia in Germ-Free Mice: Evaluation of Ketamine Cocktails

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ABSTRACT

Tribromoethanol (TBE) (100–300 mg/kg i.p.) is used in our gnotobiotic facility as an injectable anesthetic for Germ Free (GF) and Defined Flora (DF) Swiss Webster mice undergoing embryo transfer (ET) and vasectomy procedures. Due to the unavailability of pharmaceutical grade TBE as well as its variable anesthetic efficacy, Taconic Biosciences performed a study to evaluate use of a ketamine/xylazine/acepromazine cocktail (65–100 mg/kg ketamine, 13–20 mg/kg xylazine, 2–3 mg/kg acepromazine i.p.) as an alternative for use during ET. Data collected included weight of animal, volume/dose given, time to induction of a surgical plane of anesthesia, and time to recovery as well as whether animals received an additional dose of anesthetic, and if any adverse or unusual events occurred during anesthesia or after recovery. Spontaneous mortality during use of this cocktail was unacceptably high (13 of 40 [32.5%] GF animals and 4 out of 35 [11.4%] DF animals anesthetized with the cocktail) compared to 113 out of 4741 [2.4%, combined GF and DF] during nine months' use of TBE at Taconic's facility. Furthermore, a higher percentage of animals (4 out of 40 [10%] GF and 2 out of 35 [5.7%] DF) failed to achieve a plane of surgical anesthesia than is typical with TBE at our facility (0.34%). Provision of analgesia in the study group was unchanged from Taconic's usual procedure (buprenorphine 0.01–0.05 mg/kg i.p. administered after induction of anesthesia). These results demonstrate variable effects of different injectable agents for anesthesia of germ-free mice and highlight the need for evaluation and comparison of different injectable anesthetic protocols.

PROBLEM/APPROACH

- Replace Tribromoethanol (TBE) with pharmaceutical grade injectable anesthetic for Germ Free (GF) and Defined Flora (DF) embryo transfer and vasectomy surgeries in Swiss Webster mice.
  - Regulatory concerns and published potential for lot-to-lot variability and adverse effects.
- Alternatives evaluated: ketamine/xylazine/acepromazine, ketamine/xylazine, and ketamine/dexmedetomidine with and without atipamezole reversal.
- Data collected: weight of animal, volume/dose given, time to induction of a surgical plane of anesthesia, time to recovery, adverse events.
- All animals were administered 0.01-0.05 mg/kg buprenorphine HCl i.p. after induction of anesthesia, prior to surgery for analgesia.

OBSERVATIONS

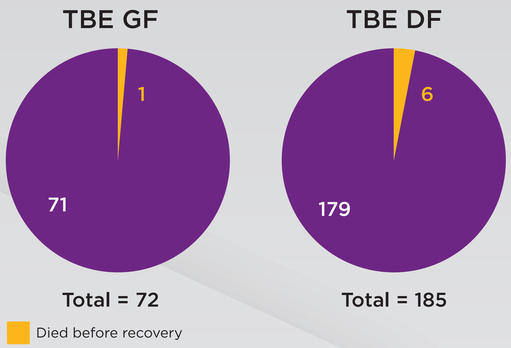

Figure 1. Incidence of spontaneous mortality under TBE anesthesia (100–300 mg/kg i.p.) for all embryo transfer surgeries performed during two weeks in 2018.

- Spontaneous mortality for Swiss Webster mice undergoing embryo transfer surgery under TBE anesthesia is 1.4% for GF, 3.2% for DF.

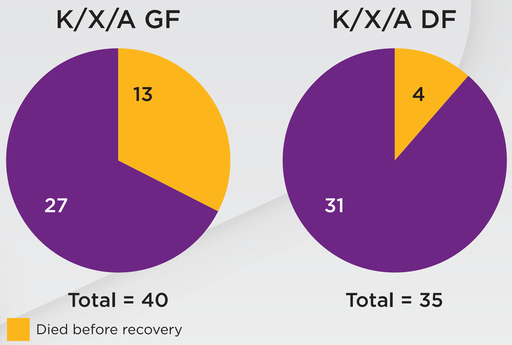

Figure 2. Incidence of spontaneous mortality under ketamine/xylazine/acepromazine anesthesia (65–100 mg/kg, 13–20 mg/kg, 2–3 mg/kg i.p., respectively) for vasectomies (GF) and embryo transfer (DF) surgeries performed.

- Ketamine/xylazine/acepromazine was trialed as an alternative to TBE. Spontaneous mortality was unacceptably high: 32.5% for GF and 11.4% for DF.

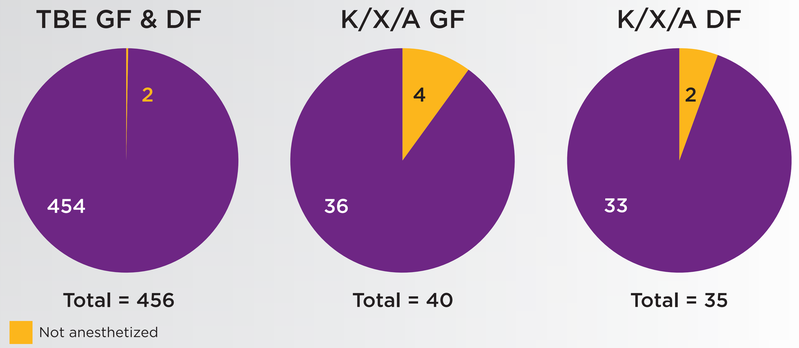

Figure 3. Incidence of animals that failed to achieve a surgical plane of anesthesia after a single dose of anesthetic agent. Dosages of TBE and K/X/A as previously listed. Embryo transfer surgeries and vasectomies were performed.

- An increased number of animals failed to achieve a surgical plane of anesthesia after a single dose of the ketamine/xylazine/acepromazine combination compared to a single dose of TBE.

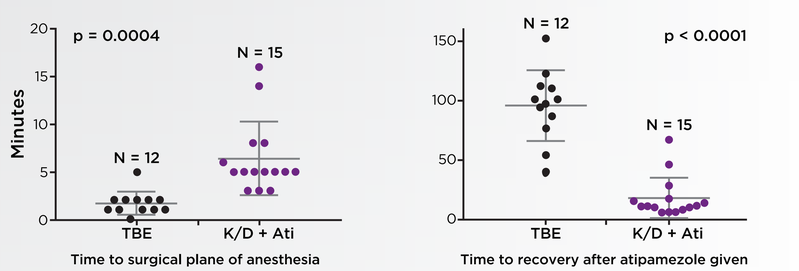

Figure 4. Times to reach surgical plane of anesthesia and recovery after atipamezole (1 mg/kg s.c.) administration, ketamine/dexmedetomidine (75 mg/kg/0.5 mg/kg i.p, respectively). All mice DF, embryo transfer surgeries performed.

A small pilot study of ketamine/xylazine (130 mg/kg/8.8 mg/kg i.p.) was not completed as the first three mice anesthetized (all DF) died before recovery.

- Ketamine/dexmedetomidine (75 mg/kg/0.5 mg/kg i.p.) produced good quality anesthesia without any adverse events, however two DF mice anesthetized were awake, but still not ambulatory 3 hours after dosing.
- Atipamezole (1 mg/kg s.c.) reversal of dexmedetomidine after surgery enabled shortened recovery times with no adverse events in embryo transfer surgeries.

CONCLUSIONS

- Ketamine/xylazine/acepromazine resulted in much higher mortality than TBE in both GF and DF animals.
- A higher percentage of animals failed to achieve a surgical plane of anesthesia with ketamine/xylazine/acepromazine than TBE.
- Ketamine/dexmedetomidine provided good quality anesthesia with no adverse events, however mice exhibited notably longer recovery times than with TBE.
- Reversal of dexmedetomidine with atipamezole significantly shortened recovery time of ketamine/dexmedetomidine by an average of 80 minutes compared to TBE, providing a practical alternative for embryo transfer surgeries.

REFERENCES

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