Is Atipamezole better than Yohimbine for reversal of Xylazine in male C57BL/6 mice anesthetized with Ketamine/Xylazine?

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Alpha-2 agonist/antagonist pairs
• Ketamine/Xylazine is a mainstay of rodent anesthesia.
• Researchers are reluctant to introduce variables.
• Can we use the newer, better absorbed, more a-2 specific reversal drug to decrease anesthetic recovery time without forcing investigators to change the anesthetic regimen on which they have based years of data?
• Better for the mice AND the investigator.
Supporting Arguments

1. Reversal of xylazine with atipamezole has been a common practice in zoo/pet-exotic medicine since at least the late 1990’s*

2. Mouse dosages are available on formulary web pages of several laboratory animal or comparative medicine department websites.

3. Could not find a peer reviewed reference for mice or rats to give to PI’s in support of my recommendation.

Study Design

1. Animals: 18 C57BL/6J male mice, 5 weeks of age at delivery.
2. Anesthesia: ketamine 80mg/kg and xylazine 10mg/kg.
3. Reversal: atipamizole 1mg/Kg, yohimbine 1.5mg/kg, or saline administered 15 minutes after ketamine/xylazine.
4. Cross over study design.
5. All drugs diluted for delivery at 0.01mL/gm.
6. All drugs administered by IP injection.
7. Thermoregulation maintained using DeltaPhase Isotherm Pads (Braintree).
Data Collection

1. Time in minutes to return of righting reflex as judged by successful righting from recumbent 3 times within 60 seconds.

2. Heart rate logged q5 seconds using pulse ox (Kent Scientific) with data output to laptop. (Virtual COM on USB, Free Serial Port Terminal 1.0.0710)

3. Abrupt change from no whisker movement to frantic whisker twitch was noticed as the first indicator that the reversal drug was having an effect. This was noted as a datapoint on subsequent animals.
Kent Scientific Pulse Ox
Heart Rate, Saline Controls

Saline 999 Jan 9
Saline KKK Nov 28
Saline FFF Nov 27
Saline 666 Oct 31
Saline CCC Jan 11
Heart Rate, Atipamizole

Artificial spikes to mark Reversal time

Artificial spikes to mark Return of Righting Reflex

Artificial spikes to mark Whisker twitch
At left is Single factor ANOVA as run by Excel on times displayed as [mm]:ss but actually stored as time of day (12:14:06AM).

At right is the same single factor ANOVA run on times manually converted to minutes and decimal fractions of minutes, and then cross checked semi-manually.

Neither is the “Repeated Measures” ANOVA that is proper for a crossover study.

But, in both instances, P is very small, and F is much greater that F-crit. Therefore, statistical significance is not in question.

Plan to further examine data, specifically for “repeat measures” and to examine the biphasic appearance of the Righting Times.
Time from administration of reversal agent to return of righting reflex

- **SALINE**: 38.9 minutes, SD 6.8 minutes
- **Yohimbine**: 22.2 minutes, SD 4.4 minutes
- **Atipamizole**: 10.1 minutes, SD 6.6 minutes
Discussion

• Yohimbine is labeled for IV administration; therefore, delay of reversal may be due to poor absorption from IP injection site.

• Biphasic appearance of graphed “Time from reversal to recovery” may not be real, but may also represent IP v/s SQ or IM injection into body wall. Further analysis planned.

• Ensure adequate analgesia when A-2 agonist is reversed after painful procedures.
Conclusions

• If “Better” is defined as easier injection site, faster absorption, faster return of righting reflex, then Atipamizole can be recommended to investigators as “Better” than Yohimbine or spontaneous recovery without reversal.

• Recommendation to researchers to use atipamizole to recover mice that are currently not being reversed, or are being reversed with Yohimbine will have two results.
  – Reduction of many minutes of anesthesia per mouse, with associated physiologic benefits to perfusion, thermoregulation etc.
  – Cumulative reduction of man-hours of IACUC mandated observation of anesthetized mice by technicians and post-docs.
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