UBC ANIMAL CARE COMMITTEE

SOP for the Use of Neuromuscular Blocking Agents (NMBs) In All Species

Standard Operating Procedure

Steps for Using Neuromuscular Blocking Agents:

1. The appropriate amount of anesthetic must be first defined on the basis of results of a similar procedure that used the anesthetic without a blocking agent. In order to ensure that the planned anesthetic regimen will be effective during the entire period of time that subsequent animals will be paralyzed, a pilot study must be completed. The goal is to demonstrate that the administration of the proposed anesthetic regimen to un-paralyzed animals prevents them from experiencing pain or distress when they are exposed to procedures identical in duration and invasiveness of those proposed for paralyzed animals.

2. Anesthetize animal. Attach monitoring equipment such as temperature probe, pulse oximetry, heart rate monitor, blood pressure monitor and capnograph.

3. Prior to administration of an NMBA ensure:

    a. The animal has reached a surgical depth of anesthesia with species-specific indicators. For example, the toe pinch in rodents.

    b. The animal has reached a stable, surgical depth of anesthesia. This is determined by evidence of a stable physiological state. This period must also be used to establish and validate the physiological signs/values that will be monitored under paralysis.

4. A surgical depth of anesthesia must be maintained during the entire time that the neuromuscular blocking agent is being administered and operative.

5. Artificial ventilation must be initiated prior to the administration of the neuromuscular blocking agent.

6. If the surgical procedure requires multiple doses of a NMBA, if possible, each dose should be allowed to wear off or the NMBA should be reversed to ensure that the animal is still in a surgical depth of anesthesia sufficient for the actual procedure. Exceptions must be justified to the ACC. Non-depolarizing agents can be reversed with neostigmine,

pyridostigmine, edrophonium or sugammadex (for rocuronium- or vecuronium-induced neuromuscular blockade). Note that depolarizing agents have no reversal agent.

7. During the period of paralysis, multiple physiologic indicators of waking, pain and stress appropriate for the species must be monitored at a minimum every 15 minutes and recorded on the intra-operative record.

8. The following alterations to physiological variables can indicate that an animal is returning to a lighter depth of anesthesia:

    a. Increase in heart rate

    b. Increase in arterial blood pressure

    c. Salivation, lacrimation, dilation of pupils

    d. Defecation, urination

    e. Increase in end-tidal CO_2 on capnograph

    f. Muscle twitching, especially around the head (e.g. curling tip of tongue), muscle movement of the limbs

9. A good rule of thumb to indicate that an animal is returning to a lighter depth of anesthesia and needs deepening is an increase of >20% in any one or combination of monitored parameters without other explanations.

10. All of the currently used non-depolarizing muscle relaxants have cardiovascular effects. Please be aware of the effects of the NMBA and all other drugs on the cardiovascular system or other systems being used for monitoring surgical depth. For example, pancuronium moderately increases heart rate (e.g. by 9% in rats). To account for this increase, note the degree of increase from the baseline heart rate when the NMBA is fully functional. Use this rate as your new baseline.

11. Use of injectable anesthetics such as Ketamine and Xylazine can be more challenging because they are unreliable for maintaining animals at a surgical depth for long periods of time. Researchers must be familiar with the duration of effect of these drugs and be prepared to provide additional doses appropriately. Gaseous anesthesia is preferred.

12. Core temperature and fluid balance must be maintained within normal levels during the period of paralysis. Hypothermia potentiates block. If animals will be paralyzed for long periods of time (e.g. greater than 4 hours) provision must be made for periodic voiding of the urinary bladder.

13. Monitoring of electroencephalography (EEG) may also be helpful for evaluating changes in depth of anesthesia but it can be unreliable. However, the normal EEG appearance differs with different types of anesthetics or drugs or physiological parameters. For example, agents such as atropine, or a rise in carbon dioxide tension can reduce the

reliability of EEG. Thus, confirmation of an anesthetized state may not always be possible based on the EEG. Therefore, the investigator should be thoroughly familiar with the expected EEG pattern for the particular anesthetic, drug/s and species used.

14. The details on the specific physiologic measures to be monitored and means of documentation will be determined on a case-by-case basis. The use of automated monitoring devices cannot substitute for direct monitoring of the animal by a human observer.

Steps for Recovering Animals

1. Care should be taken to ensure that there is no residual effect of the NMBA when recovering the animal: the animal must recover control of respiration and locomotion before it is returned to the home cage or pen. Residual neuromuscular blockade results in serious adverse complications: hypoventilation, airway obstruction, and hypoxia.

2. Quantitative methods for evaluating recovery from a NMBDA are preferred. Visual assessments of the quality of spontaneous muscular activity such as ventilation are associated with more complications because all muscle groups do not display the same sensitivity to NMBDA and thus duration of blockade. In particular, the diaphragm recovers earlier than other more peripheral muscles.

3. Drugs used to reverse the neuromuscular blockade can be used to accelerate patient recovery and to reduce the incidence of severe morbidity and mortality. Choice of reversal agent will depend on NMBDA used.

Reversal Agents:

   a. Cholinesterase inhibitors: neostigmine, edrophonium, or pyridostigmine. Due to their mechanism of action, cholinesterase inhibitors do not fully reverse deeper levels of neuromuscular blockade and they have a number of other side effects such as bradycardia, bronchoconstriction, and hypersalivation. They are often used in combination with atropine to reduce these effects.

   b. Sugammadex is a selective relaxant-binding agent (SRBA) that has been designed to reverse the steroidal neuromuscular blocking drug rocuronium. It has been shown to minimize the side effects associated with cholinesterase inhibitors.

Quantitative Methods for Evaluating Neuromuscular Blockade

1. A strategy to minimize the risk of residual effects of the NMBA is to monitor the degree of blockade prior to recovery from general anesthesia. One method is to use a peripheral nerve stimulator (electronic peripheral twitch monitoring device). This can be used to monitor the effectiveness of a neuromuscular blockade during anesthesia and as an aid in recovering animals. Electrical stimulation of peripheral nerve will cause skeletal muscle contraction served by that nerve if the neuromuscular junction is intact. If an NMBA is present on the receptors the muscle will not contract. A common approach is to provide "train of four" (TOF) impulses at 2Hz: without blockade, the muscles twitch 4 times (T_1,

T_2, T_3, T_4) with equal strength over a period of 2 seconds. As the block takes stronger effect, the last twitch disappears (T_4), then the 3rd (T_3), then the second (T_2) and lastly the first (T_1) twitch. When the block wears off, the twitches return in the reverse order (T_1, T_2, T_3, T_4). When the NMBA is completely gone, all twitches return to equal.

2. Place the electrode on the facial, adductor pollicis, peroneal/fibular or ulnar nerve on the pelvic limb (allow the limb to lie in a relaxed position). The nerve used will depend on the species. Apply a pulse of 2 Hz, 60 mA (typical value but may need to be determined), and pulse duration 0.2 of milliseconds. Stimulate the nerve for several minutes with TOF every 15 seconds to allow for stabilization and twitch potentiation and achieve a baseline. When baseline values are obtained administer NMBA and monitor TOF starting in about 15 minutes.

    a. Track ratio of response of 4th stimulus to 1st stimulus (T_4/T_1) = TOF Ratio
    b. With administration of NMBA the following pattern should be seen:
        i. T_4 disappears: 75% of receptors are blocked T_4:T_1 = 75%
        ii. T_3 disappears: 80% of receptors are blocked T_4:T_1 = 80%
        iii. T_2 disappears: 90% of receptors are blocked – T_4:T_1 = 90%
        Start procedure at this point.
        iv. T_1 disappears: 100% of receptors are blocked
        v. Monitor the TOF every 15 seconds.
        vi. Additional injections (25 – 33 % of the loading dose) are needed if block wanes before surgery is completed.
    c. With recovery from NMBA blockade:
        i. T_1 reappears
        ii. T_2 reappears
        iii. T_3 reappears
        iv. T_4 reappears

Evaluation of Blockade for Recovery:

1. **Use of Reversal Agent:** The reversal is administered after at least two twitches (preferably all 4) have returned on the peripheral nerve stimulator after muscle stimulation. Early reversal can deepen the block.

2. Recovery from block is considered complete when TOF ratio ≥ 70-90% or as soon as the TOF ratio equals or exceeds baseline values for at least five consecutive readings.

3. Judge adequacy of breathing: observe chest excursions, measure tidal volume, measure amount of negative pressure generated on inspiration. Compare to what is expected in the species used.

4. Another method to assess neuromuscular blockade is the use of acceleromyography where acceleration of the contracting muscle is measured.

Note on Fish

In Zebrafish MS222 (Tricaine) can be used as a neuromuscular blocking agent: Tricaine anaesthesia at normal doses (e.g. 0.61 mM;; 160 mg/L in zebrafish) do not paralyze skeletal muscle. However, at higher concentrations (3.2 mM in zebrafish) tricaine can also blocks muscle action potentials, thus acting as a NMBA (Attlia and Hughes 2014).

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