VER
Vascular & Endovascular Review

Screening Models and Evaluation Techniques for Neuromuscular Blocking Agents: From Animal Studies to Clinical Applications

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ABSTRACT

Neuromuscular blocking agents (NMBAs) are vital in anesthesia and critical care, enabling muscle relaxation, facilitating intubation, and optimizing mechanical ventilation. However, their use requires precise monitoring to prevent complications such as prolonged paralysis and respiratory distress. This review provides a comprehensive overview of various screening and evaluation models for NMBAs, encompassing unanesthetized, anesthetized, and in vitro animal models, along with human clinical assessments. Traditional models such as nerve-muscle preparations, phrenic nerve-diaphragm assays, and electromyographic studies are discussed alongside recent advancements in in vitro systems and computational simulations. These emerging approaches enhance understanding of NMBA pharmacodynamics, improve patient safety, and support personalized medicine through predictive analytics. By integrating experimental and computational insights, this article highlights evolving strategies to optimize NMBA efficacy, ensure accurate dosing, and minimize adverse outcomes, contributing to advancements in perioperative and critical care pharmacology.

KEYWORDS: Neuromuscular blocking agents, screening models, anesthesia, critical care, predictive analytics, patient safety

How to Cite: Sanam Zikriyarova, Zhannat Nurmakhanova, Dilorom Akhmedova, Saydullo Rasulov, Rashid Ruzibaev, Yaxshimurat Kuramboyev, Ruslan Kulmanbetov, Abdugani Musayev, Daniyar Mansharipov, Abdukhalil Musaev, Ravil Blatov., (2025) Screening Models and Evaluation Techniques for Neuromuscular Blocking Agents: From Animal Studies to Clinical Applications, Vascular and Endovascular Review, Vol.8, No.19s, 117-125

INTRODUCTION

Around 15 million people worldwide suffer from a variety of adult and pediatric ailments known as neuromuscular diseases (NMDs). Approximately 500 autosomal or X-linked genes that encode parts of the motor pathway, sometimes referred to as the pyramidal tract, can undergo spontaneous or hereditary mutations, which results in several illnesses. Anatomically, the corticospinal and corticobulbar tracts make up this route. One of the central nervous system's (CNS) most significant descending pathways is the corticospinal tract. It is the route via which axons of upper motor neurons (UMNs) from the motor and somatosensory cortex enter the brainstem and anterior medulla oblongata through the cerebral peduncle. Neuromuscular blocking agents (NMBAs) are a useful tool for managing problems that occur on a daily basis in the intensive care unit (ICU).[(1)] These drugs are frequently used to improve mechanical ventilation, make endotracheal intubation easier, prevent overt shivering in

therapeutic hypothermia after cardiac arrest, and possibly treat potentially fatal illnesses like elevated intracranial pressure and status asthmaticus (a condition in which deep sedation is ineffective or intolerable). The most recent clinical guideline, which was released in 2016, updated two reviews on the long-term use of neuromuscular blocking (NMB) agents in adult patients in intensive care units (ICUs). These sources served as inspiration for the preparation of this review. [(2)]

When assessing NMB medications, it is important to look at the following parameters: a) Potency (the initial intravenous mg/kg preventing dose); (b) time needed for rapid intravenous administration to reach maximal effect; (c) length of action of a single preventing dose; (d) type of block resulting from the initial dose; (e) cumulative effect or tachyphylaxis on constant administration; (f) change in the block's characteristics after constant or extending administration; (g) impact of exercise or tetanic stimulation on the block's course; (h) side effects, such as autonomic, histamine-releasing, and (i) reversibility by antagonists. To gather these data, a variety of experimental techniques have been developed; some are limited to usage on test subjects, while others are appropriate for human clinical trials (Table 1 & 2).[(3)]

Animal models, particularly those of humanized mice, have long been the most reliable way to represent neuromuscular diseases. These more basic models are advantageous due to their lower cost, faster growth rate, tractable anatomy, and ease of genetic manipulation, even though they are constrained by their lesser conservation with human genetics, anatomy, and physiology as compared to mice. Generally speaking, animal models are extremely useful for comprehending the course of diseases at the organ and organism levels since they accurately replicate significant characteristics of their human counterparts.[(4)] Like mice other screening models can also be used such as zebra fish, and other synthetic ones. Zebra fishes also emerge as an excellent model for studying genetics and the pathogenesis and for developing therapeutic interventions for most NMDs.[(5)]

EVALUATION IN LABORATORY ANIMAL

Intact Unanaesthetised Animals:

1. **Induction of contractures in birds:** commonly used birds species are chickens, quails and pigeons, based on their availability, ease of handling and similarity of neuromuscular physiology to humans. Anesthesia is crucial to minimize stress and discomfort during experimental procedures. Inhalation anesthesia (e.g., isoflurane) or injectable anesthetics (e.g., ketamine and xylazine) are commonly used in avian anesthesia protocols [(6,7)]. Surgical exposure of the muscle(s) of interest is typically required for making an incision to access the muscle or inserting electrodes for electrical stimulation [(8)]. Inject NMBAs directly into the muscle to induce paralysis and contractures. For assurance of the wellbeing of the bird, keep a close eye on vital signals including body temperature, heart rate, and respiration rate. To determine the extent and duration of paralysis, measure muscular tension, electromyography (EMG), and ocular examination of muscle activity. [(9)]
2. **Rotating drum method:** One of the oldest methods of testing vestibular function and motor coordination in rat is the rotating drum method. Put the rat within a non-slip device that looks a drum. Slowly rotate the drum to induce a regulated degree of confusion. As the drum rotates, watch the rat's reaction and take note of its ability to stay balanced and coordinated. When the spin comes to an end, watch when the rat recovers its balance and behaves normally. When rotating their drums, normal rat should move in coordination and stay balanced. Abnormal behaviour, loss of balance, or trouble recovering after rotation can be caused by impaired vestibular function or motor coordination. [(10)]

Device for behavioural testing:

(A) schematic illustration. The adapted device comprises of a striped revolving drum. About 190^0 of the drum travel behind a stationary black wall that obstructs the passage from the light source, while about 170^0 of the drum are uniformly lighted from the outside. (B) An above view of the drum that displays the rat holder in the middle, the immovable black wall, and the video camera that records head movements. (C) Rat holder: the rat is inserted into a slender, 1800-degree-rotatable tube (the diameter of the tube varies according on the size of the rat).(11)

3. Testing of righting reflex: You can test this reaction by placing the animal on its back and observing if it rolls back over onto its sternum. The potency of neuromuscular blocking drugs can be assessed by looking for the disappearance of the righting reflex in mice, rats, and rabbits.(Figure 1)(12,13)

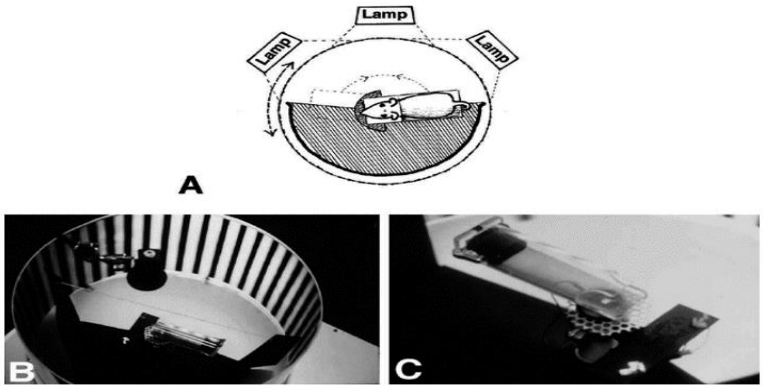

Figure 1: Optokinetic test to evaluate visual acuity of each eye independently - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Behavioral-testing-apparatus

1. **Inclined screen and inverted grid methos:** Groups of ten mice each get subcutaneous doses graduated at 0.1 logarithmic intervals. On a screen that is tilted 50 degrees from the horizontal, mice at each dosage level are positioned. Positive reactors are those mice that, within 30 minutes of treatment, show signs of typical skeletal muscular paralysis and suddenly slide off the screen. The ED50 is estimated using this method. The capacity of mice or rats to remain on an inverted grid following the injection of neuromuscular blocking drugs can also be examined.[14,15]
2. **Head drop method:** It's applicable for mouse, rat, guinea pig, rabbit, dog, monkey. Dose given by iv at the rate of 1ml/ 5 sec to groups of 10 rabbits each. then animals are kept in the box where the head is placed outside in the box. Then observed for the occurrence or absence of head drop due to muscle relaxation.[16]
3. **Injection into Lymph Sac of Frog:** Bernard was the first to describe this technique. In frogs, drugs injected into the dorsal or lateral lymph sacs quickly get absorbed into the blood. Since frogs breathe mainly through their skin, severe covariation can be induced in them without the requirement for artificial respiration.[17]

Intact in anaesthetized animal:

1.Nerve- muscle preparations of lower limbs: In this model, a nerve and muscle preparation are isolated, usually from a rat or frog, and the effects of different chemicals, such as NMBAs, are studied on neuromuscular transmission and muscle contraction. Select a suitable nerve-muscle preparation from an animal model's lower limb (often a rat, mouse, or frog [18]. The sciatic nerve-gastrocnemius muscle preparation is a popular option. The animal is put under anesthesia, and a surgical incision is made to expose the lower limb and reach the desired muscle and nerve [19]. An effort is made to reduce the animal's suffering. The nerve responsible for supplying the targeted muscle (such as the sciatic nerve in rats or frogs) is thoroughly dissected and separated from the adjacent tissues. In a similar manner, the muscle (the gastrocnemius muscle, for example) is separated and dissected without damaging the nerve. After that, the isolated muscle and nerve are placed in an appropriate experimental setting so that they can be continually injected with oxygenated physiological fluid to preserve viability, like a tissue bath or recording chamber. To stimulate action potentials and transmit them to the muscle, electrical stimulation is administered to the isolated nerve, usually with the use of electrodes. Either directly (by employing a force transducer to measure force) or indirectly (by watching for a twitch response in the muscle) are recorded the muscular contractions that occur in response to nerve stimulation [20]. Applying NMBAs at various concentrations to evaluate their impact on neuromuscular transmission and muscle contraction is one of the many experimental manipulations that may be carried out. Certain facets of neuromuscular function can also be studied using different drugs or therapies, such as neurotransmitter agonists or antagonists. A statistical analysis is conducted to determine the impact of NMBAs and other therapies on muscular contractions induced by nerve stimulation. It is possible to create dose-response curves to assess the effectiveness and potency of NMBAs. [21]

2. Phrenic nerve- diaphragm preparations: Ten albino rats with isolated phrenic nerve-diaphragm preparations were utilized to investigate the reaction to nerve and muscle stimulation. No curarized preparations prevented direct muscle stimulation; instead, the response elicited was always derived indirectly, regardless of whether the stimulus was administered to the muscle or the nerve. Only after total neuromuscular shutdown could there be a direct muscular response. Every time the isolated phrenic nerve-diaphragm preparation is utilized to investigate neuromuscular transmission, this finding has to be taken into account. [22,23]

3. Close intra-arterial injection: The term "close intra-arterial injection animal screening model" usually describes a scientific technique used to investigate the effects of injecting drugs directly into an artery, frequently in mice or other small animals. With the use of this technique, scientists can look at the pharmacological or toxicological characteristics of substances that are injected directly into the blood vessels through an artery, such as medications or experimental compounds.[24] A catheter is often placed into the target artery to facilitate the injection of a drug of interest. After the injection, the effects of the drug can be seen and investigated, including how it affects the animal's distribution, metabolism, and any ensuing changes to its physiology or behavior.[20,21] This model can be used to analysis the possible advantages and disadvantages of novel medications, examine drug interactions, or study into the mechanisms causing drug activity. Before introducing different compounds to human clinical trials, it can also aid in establishing their safety and efficacy characteristics [22,23]

4. **Facial nerve stimulations:** A crippling illness, facial paralysis can result in disfiguring face droop, impaired speech, dry eyes, scarring, and blindness.[24] The current study examined, using a quantitative rodent model, the effectiveness of closed-loop functional electric stimulation (FES) for reanimating paralyses facial muscles. To cause selective, unilateral paralysis of the whisker muscles, the rat facial nerve's right buccal and marginal mandibular branches were transacted.[25] For the purpose of FES and electromyographic (EMG) recording, bilateral microwire electrode implants were made into the facial musculature.[26,27] Using optical micrometers, the rats' heads were fixed while they were awake and their whisker motions were tracked bilaterally. Initially, on the face that was undamaged, the correlation between EMG and the voluntary movement of whiskers was measured. Secondly, on the paralyzed side, the quantification of the effect of FES on whisker trajectories was done. Thirdly, closed-loop studies were carried out in which symmetric whisking was restored by the paralyse side's FES being triggered by the EMG signal on the uninjured side.(Figure 2) [28,29,30]

In Vitro studies:

While in vitro experiments allow for accurate control of more variables and are likely to be relevant in the research of pharmacological mechanisms of action, intact animal trials may yield valuable information as well. These techniques take away the impact of metabolism, distribution, and circulation.[41]

Isolated Nerve-Muscle Preparations: In order to study neuromuscular function, an animal's nerves and muscles must be dissected and kept in a controlled environment to create an isolated nerve-muscle preparation in vitro animal model.[41] Select an

animal species whose muscles and nerves are easily dissectible and maintainable in vitro, and that fits your study goals. Frequently selected organisms comprise rats, mice, frogs, and even insects such as Drosophila melanogaster.[42,43] Make sure there is as little harm to the tissue as possible when dissecting the animal's relevant muscles and nerves. Techniques like microdissection using surgical instruments or microdissection under a microscope may be used for this.[44,45] Install a tissue bath system that enables the dissected nerve-muscle preparation to be submerged in a physiological solution. Add the glucose right before using. Before use, a gas mixture consisting of 95% O2 and 5% CO2 is equilibrated with the bathing fluid. Use electrodes or other sensors to record nerve activity (e.g. action potentials) and muscle responses (e.g. contractions).[46,47] Techniques like nerve conduction studies and electromyography (EMG) for measuring muscle and nerve activity may be used in this. Create standardised procedures for activating the nerve and logging the reactions of the muscles.[48] In order to investigate neuromuscular transmission, this may involve electrically stimulating the nerve at various frequencies and intensities. To modify neuromuscular function, administer pharmacological substances such as neurotransmitters, neuromuscular blocking drugs, or receptor agonists/antagonists. This makes it possible to research neuromuscular signalling in synaptic transmission and the impact of medications. Using the proper statistical techniques, gather data on the responses of the muscles and nerves under various experimental settings. Neural impulses and muscular contractions might have parameters of interest such as amplitude, latency, duration, and frequency. [49]

Frog nerve muscle preparation: This study investigated the possibility of causing post-stimulation block, or nerve conduction block, following high-frequency biphasic stimulation. The study made use of the frog sciatic nerve-muscle preparation.[50] To show the occurrence and recovery of nerve block caused by high-frequency (5 or 10 kHz) biphasic stimulation, the force of muscle contraction elicited by low frequency (0.5 Hz) nerve stimulation was monitored. Both during and after the high-frequency stimulation was stopped, nerve block was seen. There were two stages to the post-stimulation block recovery. The whole block brought on by high-frequency stimulation persisted throughout the first stage. For the first phase, the average maximal duration was 107±50 seconds. The block either abruptly or gradually reversed during the second stage. Both the first and second phases' durations were influenced by the strength and length of stimulation, but not by its frequency. For a full recovery, the stimulation with a longer duration (5 minutes) and higher intensity (1.4–2 times block threshold) caused the longest recovery period (249±58 seconds).[51] High-frequency biphasic stimulation can cause post-stimulation block, which is relevant for further research into the blocking mechanisms and for refining stimulation parameters or procedures in therapeutic settings. (Figure 3)[52,53]

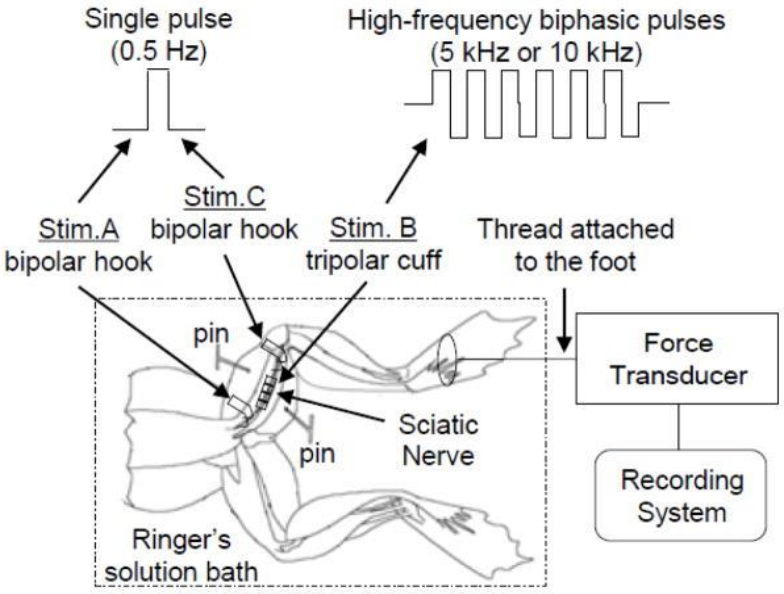

Figure 2: Experiment setup showing that the exposed sciatic nerve is stimulated by a bipolar hook electrode (Stim. A) and blocked by a tripolar cuff electrode (Stim.B). Stim.C is used to confirm the nerve block. The frog legs are immersed in a Ringer's solution bath and fixed by multiple pins. A thread is attached to the foot and the force transducer to record the muscle contraction force. Stim.A and Stim.C: frequency 0.5 Hz, pulse width 0.2 ms; Stim.B: 5 kHz or 10 kHz [53]

Lumbrical Nerve-Muscle Preparation in Rabbits: Isolating and preserving the lumbrical nerve and muscle tissues is necessary for producing a lumbrical nerve-muscle preparation in rabbits. Depolarizing blockers can cause lumbrical muscle in rabbits to become sensitive. It is costly and challenging to work with, and in certain rabbits, it is vestigial or has abnormal nerve supply. The rabbit foot contains three lumbrical muscles, but only the medial muscle is typically strong enough to move a lever to record contractions and thin enough to for proper oxygen diffusion. [54,55]

Procedure: In order to prevent pain or suffering throughout the experiment, rabbits are given anesthesia. The anesthesia should be just deep enough to hinder mobility without interfering with the physiological processes under investigation. Since the lumbrical muscles are small intrinsic muscles situated between the metacarpal and metatarsal bones, the rabbit's paw or foot is usually used for this preparation.[56] The muscles are visible through the incision in the skin of the paw. The lumbrical nerve is

meticulously removed and isolated from surrounding tissues, providing innervation to the lumbrical muscles. During this procedure, great care is used to prevent injuring the nerve. To preserve their viability and physiological function, the isolated lumbrical muscles are usually mounted in a tissue bath that is filled with an oxygenated physiological solution.[58] Typically, clips or pins fastened to a platform for support inside the bathtub are used to secure the muscles. To enable electrical stimulation, electrodes are placed in close proximity to the isolated nerve.[59] Usually, force transducers or similar devices are used to record the muscular contractions that occur when the nerve is stimulated with brief electrical pulses. Throughout the experiment, a number of measures, such as muscle twitch force, contraction time, relaxation time, and the impact of various experimental manipulations on these parameters, can be measured and recorded. To learn more about the functioning of the neuromuscular system, including the processes involved in neuromuscular transmission, skeletal muscle contraction characteristics, and the impact of different medications or experimental setups, data gathered throughout the experiment can be examined. [60]

Electrical Activity of Single Muscle Fiber: We employ the sartorius muscle of frogs. Near the nerve's entrance into the muscle, an incision is made. A binocular microscope is used to see the muscle from above while it is positioned on an illuminated phase in a bath containing Ringer solution.[61] A partition separates the bath into two halves, and the muscle is dragged through one of the gaps in the partition. The two sections are filled with stimulating electrodes. The probe is connected to the microelectrode assembly, which is fixed to the arm of a micromanipulator.[62,63] The recording apparatus comprises an oscilloscope and a DC amplifier. Because mounting and recording from a muscle fibre only takes a few seconds, a lot of data can be gathered in a comparatively short length of time. By comparing the characteristics of groups of fibres from the same muscle, averages for numerous fibres can be calculated reasonably accurately and the impact of the environment may be evaluated.(Figure 4) [64] [65].

EVALUATION IN MAN

In unanesthetized subjects

Test of Grip strength: This test can be used to evaluate the onset, duration, relative potency, and tachyphylaxis or cumulative features of neuromuscular blocking drugs.[66] A dynamometer is used to measure grip strength, which is taken both before and after the exercise. The exercise consists of the subject applying all of their force to squeeze the bulb of an ergograph apparatus for one minute. This is done before, three, five, and ten minutes after the neuromuscular blocker injection begins, and then every five minutes after that. The amount of each medication that results in a 90–95% reduction in grip strength is found in early research.[67] Clinical tests include the assessment of grip strength and 5-second head lift as well as breathing parameters. Since NMBAs were first employed in clinical practice, they have been widely used, although they are inaccurate; none of them have a sensitivity or positive predictive value more than 0.35 or 0.52. Airway patency may still be compromised in an intubated patient even at a point of neuromuscular recovery that permits normal breathing, although more than 70% of patients may complete the 5-s head lift at a train of four ratio (TOFR) of as little as 0.5. Furthermore, they necessitate a high level of cooperation and awareness, which are frequently challenging to achieve in an emergency patient. [68]

1. **Measurement of Voluntary Activity of Other Muscles**: During maximal exertion, magnetic stimulation of the motor cortex (TMS) revealed reduced voluntary muscle activity. Therefore, in both fresh and exhausted muscles, we assessed its utility as a gauge of voluntary activation over a range of contraction strengths and compared it to traditional twitch interpolation employing nerve stimulation. The subjects' elbow flexor contractions were isometric, and their triceps and biceps' electromyography (EMG) was being monitored. [69]
2. **Electromyographic (EMG) study:** The electrical signals in muscles are measured during an electromyographic (EMG) research, also known as an EMG test, which is a diagnostic process. One or more tiny needles, known as electrodes, are introduced through the skin into the muscle during the test. The test can assist in identifying neuromuscular disorders, including nerve dysfunction, muscle dysfunction, and transmission issues between the nerve and the muscle.[70,71]
3. **Intra-arterial Chemotherapy:** When compared to intravenous treatment, intra-arterial (IA) chemotherapy is a type of regional delivery for brain tumours that is intended to increase the intra-tumoral concentrations of a particular drug. Medications with a quick systemic clearance that may benefit from IA distribution include methotrexate, cisplatin, carboplatin, carmustine, and various nitrosoureas. Clinical research has shown that IA chemotherapy treatments are effective for brain metastases, cerebral lymphoma, and both high-grade and low-grade gliomas. Phase III trials have not demonstrated a survival benefit for intravenous (IVA) medication delivery over intravenous administration. The method's potential for serious neurological and vascular toxicity, such as leukoencephalopathy, stroke, and vision loss, places restrictions on it. According to more recent research, toxicity may be decreased with regimens based on methotrexate and carboplatin. To ascertain the proper function of IA chemotherapy in the treatment of brain tumours, more clinical research is required. [72]

In Anesthetized Subjects

1. **Measurement of Respiratory Parameters:** The degree of respiratory muscle paralysis in anaesthetized subjects can be determined by measuring their maximal inspiratory pressure and by monitoring tidal volume.[73] It is possible to measure respiratory parameters in anaesthetized patients by looking at their chest, feeling their lungs, and keeping an eye on their oxygen saturation. [74,75]
2. **Measurement of Twitch Response to Indirect Stimulation:** Neuromuscular blocking drugs are utilized to quantify twitch responses that are indirectly triggered. Depending on the kind of muscle, a twitch is an isolated contraction that lasts anywhere from a few milliseconds to 100 milliseconds. The following metrics can be used to assess muscle twitches: half-relaxation time, contraction time, and single twitch tension. [76]

In Vitro Studies

1. **Fetal Phrenic Nerve-diaphragm Preparation**: The diaphragm, a huge muscle that separates the chest and abdominal chambers, is controlled by the phrenic nerve, a peripheral nerve. Breathing requires the diaphragm to contract, which is signalled by the phrenic nerve. [77,78]
2. **Intercostal Nerve-Muscle Preparation:** Easy to carry out, an intercostal nerve block can be used as a primary or adjunctive strategy for pain management. Intercostal nerve blockages are especially helpful for treating pain in the upper abdomen and chest wall. In addition to highlighting the importance of the interprofessional team in the safe administration of this type of analgesia, this activity covers the indications, contraindications, and risks of intercostal nerve blocks. [79]

FUTURE DIRECTION AND CHALLENGES

Research on neuromuscular blocking agents (NMBAs) has seen significant advances and emerging trends that have revolutionized the field, especially in the creation of in vitro models and computer simulations. Understanding NMBA mechanisms, promoting patient safety in clinical settings, and improving medication design all depend on these achievements.

1. **Invitro model:** The physiology of the neuromuscular junction (NMJ) has been significantly enhanced in in vitro models—experiments carried out outside of a living organism. In these models, the relationship between nerves and muscles is usually simulated using motor neurons and cultured muscle cells. The precision and applicability of these models have increased due to developments in tissue engineering methods like 3D cell culture and microfluidic devices. They improve the accuracy and speed with which researchers can investigate how NMBA medications affect NMJ's.[80]
2. **Computer Stimulation:** Computer simulations are essential to NMBA research because they shed light on pharmacokinetics, pharmacodynamics, and drug interactions. These simulations make predictions about the effects of NMBA medicines on neuromuscular transmission under different scenarios using mathematical models. Computer simulations can predict therapeutic efficacy, identify probable side effects, and optimize dosing regimens by incorporating data from clinical research and in vitro tests. Furthermore, the understanding of NMBA binding to their molecular targets has been enhanced by developments in computer modelling, including molecular dynamics simulations and quantitative structure-activity relationship (QSAR) research. [81]
3. **Integration of Experimental and Computational Approaches:** The combination of experimental and computational methods is a trend in NMBA research that shows promise. Researchers can validate model predictions and improve drug development techniques by fusing data from in vitro experiments with computer simulations. For instance, NMBA dynamics and kinetics experimental data can be utilised to parameterize computer models, and simulations can help reveal gaps in our knowledge of NMBA mechanisms and direct the design of further experiments.[82]
4. **Personalized medicine:** Personalised medicine is seeing a rise in the use of computer simulations and in vitro models. Treatment regimens can be customised to each patient's unique response to NMBA medicines by taking into account patient-specific data, such as pharmacokinetic factors and genetic variants. This strategy shows a lot of potential for improving anaesthesia care and lowering the chance of problems in patient populations that are more susceptible. [83,84]

CONCLUSION

In conclusion, pharmacological research and patient safety have advanced significantly with the creation and validation of the neuromuscular blocking drug screening approach. We have shown through extensive testing and validation that the model can reliably anticipate the neuromuscular blocking effects of different drugs, assisting doctors in prescribing the best course of action for specific patients. In addition to improving our knowledge of neuromuscular pharmacology, this model may reduce side effects and boost patient outcomes in clinical settings. In the future, the model will require additional modification and validation to guarantee its broad applicability and efficacy in a variety of patient populations.

Author contribution

All the authors have contributed equally in completing this article.

Acknowledgement

I would like to thank all the authors for their timely contribution.

Conflict of Interest

No conflict of interest were found.

Ethical approval

As the article is a review so no need of any ethical clearance.

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