Research / Clinical Investigation

Effect of 50% enantiomeric excess bupivacaine mixture combined with pancuronium on neuromuscular transmission in rat phrenic nerve-diaphragm preparation; a pilot study

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ABSTRACT

Background and Aims: Local anaesthetics are drugs that are widely used in clinical practice. However, the effects of these drugs on the neuromuscular junction and their influence on the blockade produced by non-depolarising neuromuscular blocking drugs are still under investigation. The aim of this study was to evaluate, *in vitro*, the influence of a 50% enantiomeric excess bupivacaine mixture on neuromuscular transmission and neuromuscular block produced by pancuronium. **Methods:** Rats were distributed into three groups (n = 5) according to the drug studied namely, 50% enantiomeric excess bupivacaine mixture (5 μg/mL); pancuronium (2 μg/mL); 50% enantiomeric excess bupivacaine mixture + pancuronium. The following parameters were evaluated: (1) Effects of a 50% enantiomeric excess bupivacaine mixture on membrane potential (MP) and miniature endplate potentials (MEPPs); (2) amplitude of diaphragmatic response before and 60 min after the addition of a 50% enantiomeric excess bupivacaine mixture; the degree of neuromuscular block with pancuronium and pancuronium combined with a 50% enantiomeric excess bupivacaine mixture. **Results:** A 50% enantiomeric excess bupivacaine mixture did not alter the amplitude of muscle response (MP) but decreased the frequency and amplitude of MEPP. The block produced by pancuronium was potentiated by a 50% enantiomeric excess bupivacaine mixture. **Conclusion:** A 50% enantiomeric excess bupivacaine mixture used alone did not affect neuromuscular transmission, but potentiated the neuromuscular block produced by pancuronium. No action was shown on the muscle fibre, and alterations on MEPPs demonstrated a presynaptic action.

Key words: 50% enantiomeric excess bupivacaine mixture, animals, local anaesthetics, neuromuscular blockers, non-depolarising, pancuronium, rats

INTRODUCTION

The mechanism of action of local anaesthetics on neuromuscular transmission and their effects on synaptic and electrophysiologic components of the nerve cell are still under investigation. However, clinical and experimental evidences suggest that these drugs may interfere with the neuromuscular transmission and potentiate the effects of neuromuscular blocking drugs.[1-3] It is highly important to understand that neuromuscular blockers may interact with other drugs used simultaneously during anaesthesia. These drugs can alter the margin of safety of neuromuscular transmission and modify

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the effects of neuromuscular blocking drugs. This interaction may contribute to a higher incidence of the residual blockade and postoperative complications. Using genuinely Brazilian technology, enantiomeric manipulation of racemic bupivacaine components resulted in a new formulation containing 25% of the R-isomer (+) and 75% of the S-isomer (−) of bupivacaine. Although anaesthesia produced by the new formulation is comparable to that obtained with the racemic formulation, the presence of only 25% of the R (+)-isomer of bupivacaine conferred a lower risk of cardiotoxicity and neurotoxicity to anaesthetic activity.[4] Pancuronium is a long-acting amino steroid non-depolarising neuromuscular blocking drug. Its use is justified in prolonged surgical anaesthesia and intensive care units.[5] The aim of this study was to evaluate the effect of a 50% enantiomeric excess bupivacaine mixture (S75-R25) on neuromuscular transmission, and its influence on neuromuscular block produced by pancuronium, using an experimental model.

METHODS

This was an *in vitro* experimental study. Male Wistar rats (weight range: 180–250 g) were used. The animals were anaesthetised with urethane, 1.2 mg/kg intraperitoneally. Exsanguination was performed by sectioning of the neck vessels, to facilitate identification and removal of the left hemidiaphragm and a portion of the corresponding phrenic nerve. To evaluate the effect of a 50% enantiomeric excess bupivacaine mixture on neuromuscular transmission and its influence on neuromuscular block produced by pancuronium, a technique described by Bulbring was used.[6] Preparations were fixed in a vat containing 40 mL of tyrode nutrient solution, constantly aerated with carbogen (95% O_2 + 5% CO_2) and maintained at 37°C. The nerve was placed over platinum electrodes connected to a Grass S48® stimulator. The diaphragm was maintained under constant tension (5.0 g) by its tendinous portion through a wire connected to a load cell BG50 GMS isometric transducer. Indirect stimulation was applied at a frequency of 0.1 Hz and duration of 0.2 ms. Variations in tension produced by diaphragmatic contractions were recorded in a Gould RS-3400 physiographer. Study drugs were administered to the tyrode solution containing the preparation, with a graduated pipette. To evaluate the effect of drugs on neuromuscular transmission, either used alone or combined, three groups were formed (n = 5): Group I - R25-S75% (5 μg/mL); Group II - pancuronium (2 μg/mL); Group III - pancuronium (2 μg/mL) in a preparation previously exposed to R25-S75% (5 μg/mL). In Group III (R25-S75% - pancuronium), pancuronium was added to the preparation 30 min after the addition of R25-S75%. Muscle response to indirect stimulation was recorded during 60 min after addition of the drugs.

In the rat diaphragm, the effects of R25-S75% on miniature endplate potentials (MEPPs) and membrane potentials (MPs) were also studied. The following parameters were evaluated: (1) The amplitude of diaphragmatic response to indirect stimulation, before and 60 min after adding of R25-S75%; (2) the amplitude of diaphragmatic response to indirect stimulation, before and 60 min after the sole addition of pancuronium alone and in a preparation previously exposed to R25-S75%; (3) MPs and MEPPs (PPTM).

The results were expressed in means and standard deviations. The Wilcoxon test was used for analysis of muscle fibre MPs. To evaluate the reduction in amplitude of muscle response, a Student's *t*-test (normal distribution) was used. The level of significance was set at 5% ($P < 0.05$).

RESULTS

In the concentration studied, R25-S75% did not cause any reduction in the amplitude of muscle response to indirect stimulation, in a rat phrenic nerve-diaphragm preparation when used as the sole drug. Blockade produced by pancuronium alone was (54.9 ± 14.1%). In preparations previously exposed to R25-S75%, pancuronium produced a 100% block, with a significant difference (P = 0.015) compared to the block produced by pancuronium alone [Figure 1]. No significant effect of R25-S75% on MPs was

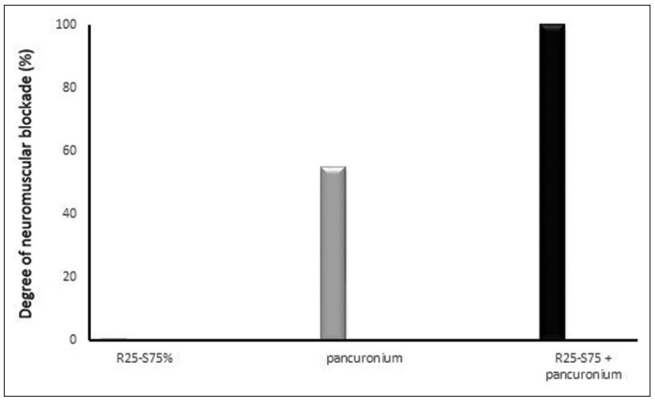

Figure 1: Degree of blockade with R25-S75 and pancuronium alone and in preparation previously exposed to R25-S75

observed [Figure 2]. The effects on MEPPs (PPTM) were characterised by a decrease in the frequency and amplitude until complete block [Figure 3].

DISCUSSION

In clinical practice, it is quite common for the practitioner to combine regional anaesthesia with general anaesthesia. In addition, other anaesthetic techniques are commonly performed with the use of local anaesthetics. Peak blood levels of local anaesthetics may be achieved, and neuromuscular transmission may be affected. Therefore, it is imperative to conduct studies into the probable mechanism of interaction and response, to explain the influence of local anaesthetics on neuromuscular blockers. Confirmation of this interaction is crucial. The effects of neuromuscular blocking agents should be closely observed and monitored, in particular when they are simultaneously used with other drugs or in situations where the safety margin of the neuromuscular junction is compromised. Synergism between local anaesthetics and neuromuscular blocking agents may produce residual curarization and prolonged neuromuscular block. Both are established risk factors for critical postoperative respiratory events and increased morbidity.[7] Residual paralysis is common at the time of tracheal extubation and arrival at the post-anaesthetic care unit, despite qualitative neuromuscular monitoring and use of neostigmine.[8]

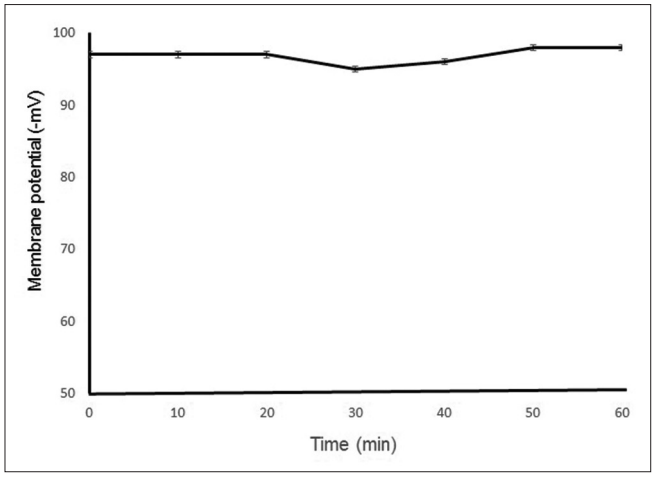

Figure 2: R25-S75 effect on membrane potential in the rat diaphragm preparation

Some authors have described that local anaesthetics may interfere with neuromuscular transmission, due to nerve conduction blockade. There is no evidence that local anaesthetics inhibit acetylcholine synthesis. However, low concentrations of these drugs may reduce the amplitude, without altering the frequency of MEPPs. The influence of local anaesthetics on these parameters may indicate a presynaptic action, consequently decreasing acetylcholine release or reducing the sensitivity of the postjunctional membrane to acetylcholine.[1,3,9-13] In our study, impaired neurotransmitter release was observed. This event was demonstrated by a progressive decrease in the frequency and amplitude of MEPPs. Results have

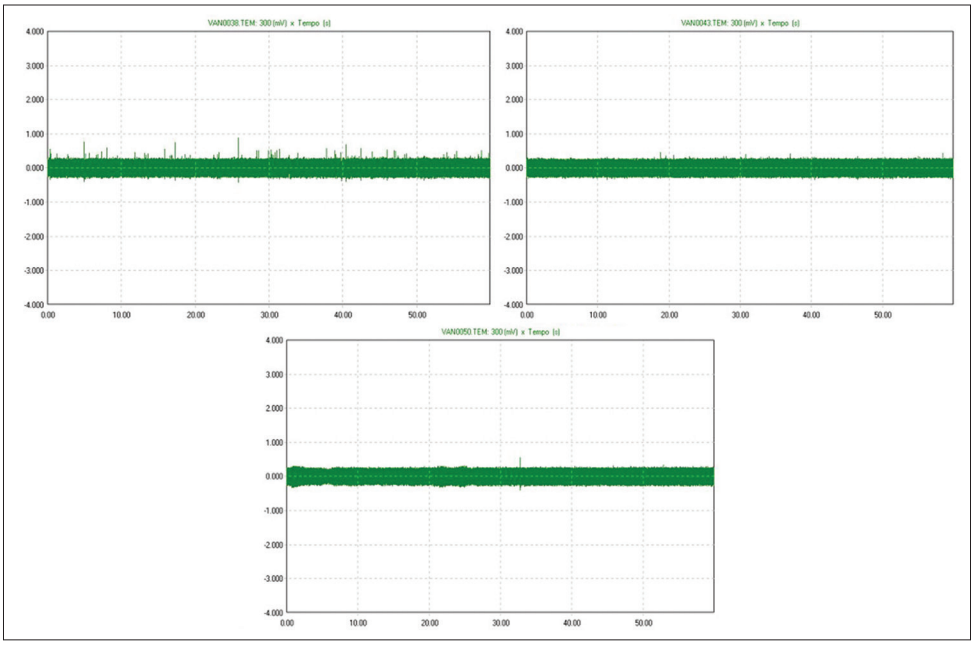

Figure 3: R25-S75 effect on miniature endplate potential in the rat diaphragm preparation

suggested that 50% enantiomeric excess bupivacaine mixture has a presynaptic action.

In addition to preventing the generation and propagation of action potential, local anaesthetics may selectively depress motor fibre conduction. Through postsynaptic action, binding to different specific acetylcholine sites may block acetylcholine-induced contractile response, temporarily occluding nicotinic receptor channels.[8,13-19]

Racemic bupivacaine, enantiomeric bupivacaine mixture, ropivacaine, levobupivacaine and mepivacaine, all have in common a piperidine ring containing a chiral centre that provides optical isomers with S (−) and R (+) configurations. Along with receptor affinity, this stereoisomerism is also a contributing factor in cell membrane fluidisation.[20-22]

In this study, we observed that a 50% enantiomeric excess bupivacaine mixture in the concentration used and administered alone in a rat phrenic nerve-diaphragm preparation, failed to affect neuromuscular transmission. Nevertheless, it significantly potentiated the block produced by pancuronium previously added to the preparation.

These results are in agreement with other experimental and clinical studies showing that local anaesthetic alone does not affect neuromuscular transmission. However, local anaesthetics may potentiate neuromuscular block produced by different neuromuscular blocking drugs.[1-3,13,23]

Similarly to other studies,[3] we observed that a 50% enantiomeric excess bupivacaine mixture in the concentration used did not alter MPs of muscle fibres. Muscle MP was maintained within normal limits.[14] From a study by Bowman,[14] it may be inferred that this drug does not exert a depolarising action on muscle fibres. The mechanism of action of these drugs on the neuromuscular junction is not related to stabilising the activity of the muscle membrane. However, the drug prevents the conduction of propagated action potentials, without affecting MP.

The structure and physicochemical characteristics of local anaesthetics may be determining factors for different effects. Interpretation of their mechanism of action should not be restricted to the importance of interaction between these drugs and lipids or directly with sodium channel proteins. A broader interpretation should exist, providing a more global view of their action on cell membranes.[24,25]

CONCLUSION

The results showed that R25-S75% in the concentration used, potentiated neuromuscular block produced by pancuronium. Changes in MEPPs exhibit a presynaptic action with modifications in quantal acetylcholine release. The lack of effect on MP demonstrates that it has no depolarising action on muscle fibre. The interaction between blockade potentiation and increased risk of residual curarization suggests that neuromuscular block monitoring is necessary whenever these drugs are used simultaneously in clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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Announcement

ANNOUNCEMENTS FOROM ISA NATIONAL SECRETARIAT

1. The Annual National Conference of ISA will be in November from 2016
   - Workshops 25th November
   - CME 26th November
   - Conference inauguration 26th November evening
   - Orations 27th November forenoon
   - AGBM 28th November Afternoon
   - Valedictory function 29th November noon
2. ISA Android App is live now. Members can download it from Google Play store by searching "ISA" in Google Play store Apps or by clicking the following link

   https://play.google.com/store/apps/details?id=dotweb.isa&hl=en

   Member can use their ISA login details for Login to App. (Your ISA No is log in ID & the password id same as ISA log in password)
3. Members are requested to visit www.isaweb.in regularly to get information on various ISA Awards, Fellowships, Conferences, member details, decisions of GC / GB etc.
4. SMS is sent from ISANHQ regularly to pass messages to members
5. Online election for ISA National President elect & GC members is from 8.00 a.m on 1st December 2015 to 5.00 p.m. on 5th December 2015.
6. ISA Protocols are published in www.isaweb.in Please go through.
7. ISA Member details is published in www.isaweb.in Please go through and update if required.
8. Members are requested to update their details (Branch Transfer, ALM to LM conversion & Address change etc) by logging in at www.isaweb.in
9. ISA membership is online only & membership Fee payment is also through online payment only.
10. All City & State Branch Office Bearers are requested to update their details with ISA National & also send their Bank details to transfer Grant in Aid.

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