Myocardial contractility impairment with racemic bupivacaine, non-racemic bupivacaine and ropivacaine. A comparative study

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ABSTRACT

PURPOSE: To study racemic bupivacaine, non-racemic bupivacaine and ropivacaine on myocardial contractility.

METHODS: Isolated Wistar papillary muscles were submitted to 50 and 100 mM racemic bupivacaine (B50 and B100), non-racemic bupivacaine (NR50 and NR100) and ropivacaine (R50 and R100) intoxication. Isometric contraction data were obtained in basal condition (0.2 Hz), after increasing the frequency of stimulation to 1.0 Hz and after 5, 10 and 15 min of local anesthetic intoxication. Data were analyzed as relative changes of variation.

RESULTS: Developed tension was higher with R100 than B100 at D1 (4.3 ± 41.1 vs -57.9 ± 48.1). Resting tension was altered with B50 (-10.6 ± 23.8 vs -4.7 ± 5.0) and R50 (-14.0 ± 20.5 vs -0.5 ± 7.1) between D1 and D3. Maximum rate of tension development was lower with B100 (-56.6 ± 38.0) than R50 (-6.3 ± 37.9) and R100 (-1.9 ± 37.2) in D1. B50, B100 and NR100 modified the maximum rate of tension decline from D1 through D2. Time to peak tension was changed with NR50 between D1 and D2.

CONCLUSIONS: Racemic bupivacaine depressed myocardial contractile force more than non-racemic bupivacaine and ropivacaine. Non-racemic and racemic bupivacaine caused myocardial relaxation impairment more than ropivacaine.

Key words: Anesthetics, Local. Cardiotoxicity. Papillary Muscles. Rats.
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Introduction

Local anesthetics (LA) cardiotoxicity has been one of the greatest concerns among anesthesiologists since Albright’s reports on cardiac arrest following regional anesthesia. LA cardiotoxic manifestations include electrocardiographic (ECG) changes, hypotension and myocardial contractile impairment. Many mechanisms have been described as LA cardiotoxic effects like sodium channel blockade, mitochondrial dysfunction, sarcolemmal injuries and calcium handling impairment.

LA chemical properties, such as lipid solubility and isomerism, have also been related to LA cardiotoxic potential. Isomerism is the phenomenon in which certain compounds with the same molecular formula are different because of their atoms organization. In optical isomerism, R and S enantiomers are the mirror image from each other. Racemic bupivacaine is a solution composed by 50% R isomers and 50% S isomers. Non-racemic bupivacaine has R and S isomers in a ration different from 1:1. It is well known that bupivacaine R isomer has greater sodium channel affinity and induces ECG changes more frequently than S(-)bupivacaine. In addition, QRS time was increased with racemic bupivacaine compared to ropivacaine and S(-)bupivacaine.

LA isomers also have different effects on myocardial contractile function. Racemic bupivacaine was more potent than ropivacaine in reducing guinea pig isolated heart inotropism. Bupivacaine had greater negative inotropic effects than ropivacaine in isolated guinea pig heart, besides no differences between R and S isomers were noted. S(-)bupivacaine depressed rat papillary muscle relaxation more than racemic bupivacaine and ropivacaine.

Different LA enantiomeric preparations have been studied as a way to improve clinical outcomes without cardiotoxic effects. Adding 25% of the R isomer to S(-)-bupivacaine (S75/R25) promoted stronger muscle relaxation and motor nerve blockade than bupivacaine isomers alone. However, the effects of non-racemic bupivacaine (S75/R25) on myocardial contractile function remain understudied. Thus, the aim of this investigation was to compare the effects of non-racemic bupivacaine, racemic bupivacaine and ropivacaine at equimolar concentrations on the rat myocardial contractile function. The hypothesis was non-racemic bupivacaine had an intermediary cardiotoxic potential compared to racemic bupivacaine and ropivacaine.

Methods

This investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals, issued by the United States National Institutes of Health and was approved by the institutional Animal Care and use Committee (Protocol number 754/2009).

Sixty-day-old male Wistar rats weighing 280-300g were used. Forty two animals were randomly and equally distributed in six groups: intoxication with racemic bupivacaine 50 mM (B50), intoxication with racemic bupivacaine 100 mM (B100), intoxication with non-racemic bupivacaine 50 mM (NR50), intoxication with non-racemic bupivacaine 100 mM (NR100), intoxication with ropivacaine 50 mM (R50), and intoxication with ropivacaine 100 mM (R100).

LA were provided by Cristália Produtos Químicos e Farmacêuticos LTDA, Itapira-SP, Brazil. Cristália was not involved in the study design neither its results and conclusions.

Papillary muscle mechanical study

At the time of the study, rats were anesthetized with sodium pentobarbital 50 mg.kg-1 intraperitoneally and killed by decapitation. Their hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution (118.5 mM NaCl, 4.69 mM KCl, 2.5 mM CaCl2, 1.16 mM MgSO4, 1.18 mM KH2PO4, 24.88 mM NaCO3 and 5.5 mM glucose) at 28°C. Papillary muscles from the left ventricle were carefully dissected, mounted between two spring clips and placed vertically in a chamber containing 50 mL Krebs-Henseleit solution. The perfusate PO2 was maintained between 550 and 600 mmHg by passing 95% O2 and 5% CO2 through sintered glass discs located at the bottom of the chamber. Temperature was maintained at 28°C with a recirculating bath (Refrigerating/Heating -20°C to 150°C, PolyScience Division of Peson Industries, Niles, IL, USA). The lower spring clip was attached to a force transducer (Grass FT03 Force Displacement Transducer, GRASS Technologies, West Warwick, RI, USA), which passed through a mercury seal at the bottom of the chamber, avoiding friction between the transducer and the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm above which a micrometer stop (L.S. Starret Company, Athol, MA, USA) was mounted for the adjustment of muscle strength. The lever arm was made of magnesium with a ball-bearing fulcrum and a lever arm ratio of 4:1. A 5g preload was mounted at the lever to promote initial papillary muscle stretch. Preparations were stimulated 12 times/min (0.2 Hz) with a 5 ms square wave pulses through parallel platinum electrodes at voltages that were 10% greater than the minimum required to produce a maximal mechanical response.

After 60 min stabilizing period, during which preparations were allowed to shorten while carrying light loads, the 5g load was increased to 50g load, which allowed muscles to contract isometrically. After 15 min, papillary muscles were gradually stretched using the micrometer, until maximum tension development. Papillary muscle...
length relative to maximum tension development ($L_{\text{max}}$) was measured between spring clips with a cathetometer (Gartner, Gartner Scientific Corporation, Chicago, IL, USA). Papillary muscles were maintained in isometric contraction and mechanical data were initially obtained (basal condition). Frequency of stimulation was progressively increased to 60 times/min (1.0 Hz) and mechanical data were obtained from papillary muscles in isometric contraction at $L_{\text{max}}$ after 30 min (M0). Local anesthetic was added to the papillary muscle preparations and posterior mechanical data were obtained in isometric contraction at $L_{\text{max}}$ after 5, 10 and 15 min (M1, M2 and M3, respectively).

At the end of each experiment, papillary muscles between both spring clips were blotted, dried and weighed. Cross-sectional area (CSA) was calculated from muscle weigh (MW) and length at Lmax by assuming cylindrical uniformity and specific gravity of 1.0 (CSA = MW. $L_{\text{max}}^{-1}$).

**Functional study**

Mechanical parameters were measured in isometric contraction at $L_{\text{max}}$ and were registered in a computer system (AcqKnowledgeTM MP 100, Biopac Systems Inc., Santa Barbara, CA, USA). Isometric contraction curves (Figure 1) were analyzed to determine the mechanical parameters: developed tension (DT, g.mm$^{-2}$), resting tension (RT, g.mm$^{-2}$), maximum rate of tension development ($+dT/dt$, g.mm$^{-2}$.s$^{-1}$), maximum rate of tension decline ($-dT/dt$, g.mm$^{-2}$.s$^{-1}$) and time to peak tension (TPT, ms).

Data were obtained with a frequency of stimulation of 0.2 Hz (basal condition) and 1.0 Hz (M0) without local anesthetics and after 5 (M1), 10 (M2) and 15 min (M3) of LA addition. All mechanical data were normalized for the muscle CSA to compare the performance of different muscles.

LA effects were better evaluated using the percentage of variation (%) in relation to the preceding measure for all mechanical parameters calculated as follows: $D1 = (M1 - M0) \times M0^{-1} \times 100$; $D2 = (M2 - M1) \times M1^{-1} \times 100$ and $D3 = (M3 - M2) \times M2^{-1} \times 100$.

**Statistical analysis**

Values are shown as mean ± standard deviation for normal distribution and median ± interquartile-range for non-normal distribution. Mechanical data in basal conditions (0.2 Hz) and after increasing the frequency of stimulation to 1.0 Hz (M0) were compared using two-way analysis of variance (ANOVA) followed by Tukey test. LA effects were presented as relative change (%) from the previous measure (D1, D2 and D3) and were compared using the Kruskal-Wallis one way analysis of variance followed by Dunn’s test if $p<0.05$. The level of significance considered was 5% ($p<0.05$).

**Results**

Two papillary muscles stopped beating at M3 with racemic bupivacaine 50 mM and one papillary muscle stopped beating at M4 with racemic bupivacaine 100 mM; non-racemic bupivacaine 50 mM induced one papillary muscle arrest at M3. One papillary muscle stopped beating after adding ropivacaine 50 mM at M2. Data from papillary muscles that stopped beating anytime during the experiment were discarded. Data from 38 papillary muscles in isometric contraction were analyzed according to the group distribution as follows: B50 (n=5), B100 (n=6), NR50 (n=7), NR100 (n=7), R50 (n=6), and R100 (n=7). Mechanical data with frequency of stimulation of 0.2 Hz and 1.0 Hz were not different among groups (ANOVA, Tukey test).

Figure 2 shows DT oscillation with LA effect. DT variation was different between D1 and D3 in B100 (-57.9 ± 48.1 vs -2.3 ± 13.8, $p=0.04$) and between D1 and D2 in R50 (-6.6 ± 27.6 vs 4.4 ± 6.9, $p=0.03$).
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There were no differences among moments in B100, NR50, NR100 and R100. RT varied less with NR100 (-6.7 ± 15.4) in relation to B50 (-10.6 ± 23.8), B100 (-14.4 ± 10.3), NR50 (-7.5 ± 10.3) and R50 (-14.0 ± 20.5) in D1 (p=0.02). The same situation is observed with R100 (-8.5 ± 18.1) compared to B50 (-10.6 ± 23.8), B100 (-14.4 ± 10.3) and NR50 (-7.5 ± 10.3) in D1 (p=0.02). There were no differences among groups in D2 and D3.

\[ \text{+dT/dt} \] relative variation was different between D1 and D3 in B100 \((-56.6 ± 38.0 \text{ vs } -0.4 ± 21.4, p=0.007)\). There were no differences among moments in B50, NR50, NR100, R50 and R100. B100 \((-56.6 ± 38.0)\) caused a higher decrease in \[ +dT/dt \] than R50 \((-6.3 ± 37.9)\) and R100 \((-1.9 ± 37.2)\) in D1 (p=0.005). There were no differences among groups in D2 and D3.

There were no differences among moments in B50, NR50, NR100 and R100. B100 caused a higher oscillation in DT than R100 in D1 \((-57.9 ± 48.1 \text{ vs } 4.3 ± 41.1, p=0.02)\). There were no differences among groups in D2 and D3.

Figure 3 shows RT variation with LA. RT decrease was higher in D1 compared to D3 in B50 \((-10.6 ± 23.8 \text{ vs } -4.7 ± 5.0, p=0.03)\) and R50 \((-14.0 ± 20.5 \text{ vs } -0.5 ± 7.1, p=0.009)\).

\[ \text{FIGURE 2} \ - \text{Developed tension (DT) percentage of variation in different groups and moments. Values are median ± semi-range. B50: papillary muscles intoxicated with racemic bupivacaine 50 mM; B100: papillary muscles intoxicated with racemic bupivacaine 100 mM; NR50: papillary muscles intoxicated with non-racemic bupivacaine 50 mM; NR100: papillary muscles intoxicated with non-racemic bupivacaine 100 mM; R50: papillary muscles intoxicated with ropivacaine 50 mM; R100: papillary muscles intoxicated with ropivacaine 100 mM. D1: variation from M1 in relation to M0; D2: variation from M2 in relation to M1; D3: variation from M3 in relation to M2. * B100 vs R100 (p=0.02); # D1 vs D3 (p=0.04); § D1 vs D2 (p=0.03). Kruskal-Wallis and Dunn’s test.} \]

\[ \text{FIGURE 3} \ - \text{Resting tension (RT) percentage of variation in different groups and moments. Values are median ± semi-range. B50: papillary muscles intoxicated with racemic bupivacaine 50 mM; B100: papillary muscles intoxicated with racemic bupivacaine 100 mM; NR50: papillary muscles intoxicated with non-racemic bupivacaine 50 mM; NR100: papillary muscles intoxicated with non-racemic bupivacaine 100 mM; R50: papillary muscles intoxicated with ropivacaine 50 mM; R100: papillary muscles intoxicated with ropivacaine 100 mM. D1: variation from M1 in relation to M0; D2: variation from M2 in relation to M1; D3: variation from M3 in relation to M2. * B100 vs R100 (p=0.02); # D1 vs D3 (p=0.03); § D1 vs D2 (p=0.03). Kruskal-Wallis and Dunn’s test.} \]

\[ \text{FIGURE 4} \ - \text{Maximum rate of tension development (+dT/dt) percentage of variation in different groups and moments. Values are median ± semi-range. B50: papillary muscles intoxicated with racemic bupivacaine 50 mM; B100: papillary muscles intoxicated with racemic bupivacaine 100 mM; NR50: papillary muscles intoxicated with non-racemic bupivacaine 50 mM; NR100: papillary muscles intoxicated with non-racemic bupivacaine 100 mM; R50: papillary muscles intoxicated with ropivacaine 50 mM; R100: papillary muscles intoxicated with ropivacaine 100 mM. D1: variation from M1 in relation to M0; D2: variation from M2 in relation to M1; D3: variation from M3 in relation to M2. * B100 vs R100 and R100 (p=0.005); # D1 vs D3 (p=0.007). Kruskal-Wallis and Dunn’s test.} \]

\[ \text{FIGURE 5} \ - \text{+dT/dt variation with LA. D1 was different from D2 and D3 in B50 \((-33.5 ± 22.2 \text{ vs } -4.5 ± 39.1 \text{ and } -3.9 ± 23.5, p=0.03)\); B100 \((-56.3 ± 31.6 \text{ vs } -11.9 ± 107.5 \text{ and } -1.5 ± 11.8, p=0.003)\); NR50 \((-50.0 ± 45.5 \text{ vs } 1.9 ± 102.4 \text{ and } 4.7 ± 4.2, p=0.008)\). NR50, R50 and R100 did not cause \(-dT/dt\) oscillation among moments. \(-dT/dt\) variation was more important in B100 \((-56.3 ± 31.6)\) compared to R50 \((1.7 ± 56.4)\) and R100 \((0.0 ± 37.9)\) in D1 (p=0.001). R50 \((1.7 ± 56.4)\) was also lower than NR100 \((-50.0 ± 45.5)\) in D1 (p=0.001). There were no differences among groups in D2 and D3.} \]
Figure 6 shows the TPT oscillation among groups and moments with LA. D1 was different from D2 with NR50 (-6.5 ± 98.9 vs 4.0 ± 103.9, p=0.04). There were no differences among moments in B50, B100, NR100, R50 and R100. TPT oscillation was higher with R50 (7.0 ± 59.4) and R100 (8.0 ± 59.4) compared to B50 (0.0 ± 8.2), B100 (0.0 ± 8.2), NR50 (4.0 ± 103.9) and NR100 (2.9 ± 103.8) in D2 (p=0.009). There were no differences in TPT variation among groups in D1 and D3.

Discussion

Our results demonstrate that high concentrations racemic bupivacaine impaired myocardial contractile function more than non-racemic bupivacaine and ropivacaine. In addition, high doses of racemic and non-racemic bupivacaine and low doses of racemic bupivacaine caused papillary muscle relaxation impairment compared to ropivacaine.

There is a widespread number of experimental models and methods applied to evaluate the myocardial contractile function, but isolated papillary muscle preparation is a way to measure the ability of the cardiac muscle to develop force independent of influences that can modify mechanical myocardial performance in vivo, such as heart rate, preload and afterload.

The present study confirmed the myocardial contractile dysfunction caused by potent LA. Developed tension and maximum rate of tension development were both decreased more intensely with 100 mM racemic bupivacaine in relation to other LA in this study (Figures 2 and 4). Previous researches demonstrated racemic bupivacaine had major impact to cause developed tension decrease in relation to other LA. Maximum rate of tension decline was affected with both racemic bupivacaine concentrations and with 100 mM non-racemic bupivacaine (Figure 5). It is important to note that only 75% of S(-)bupivacaine had the same effect of pure levobupivacaine in the myocardial relaxation as previously reported in equimolar concentrations. Ropivacaine did not impair the myocardial function compared to racemic and non-racemic bupivacaine in this study. Preceding researchers reported equimolar concentrations of ropivacaine had the same negative inotropic effect of racemic bupivacaine and levobupivacaine.

Previous studies demonstrated negative inotropic effects with different LA. Divergent LA concentrations were described to induce myocardial contractile function impairment. While 10 mM racemic bupivacaine decreased DT values in rat ventricular muscle, other authors have found that 0.5 mM racemic bupivacaine caused myocardial contractile dysfunction. Besides, non-racemic bupivacaine had the maximal contractility impairment at 20 mM. Ropivacaine has
been reported to cause negative inotropic effects with 100 to 1,000 mM. Myocardial relaxation was also impaired by LA. Racemic bupivacaine, S(-)-bupivacaine and ropivacaine caused rat papillary muscle relaxation dysfunction. Differences among LA concentrations causing myocardial contractile function could be understood by divergent frequencies of stimulation used in the experiments. As LA blockade is called phasic, when decreasing frequency of stimulation LA concentration should be increased to obtain the same effect. In the present study, myocardial performance was impaired with LA concentrations 20 times less than previous researches, however, with papillary muscle frequency of stimulation 5 times superior. The same authors have reported that increasing the frequency of stimulation with the same LA concentration increases the LA negative inotropic effect, which returned to baseline after reducing the frequency of stimulation to initial conditions.

Experiments demonstrated the importance of isomerism to improve drugs safety. Myocardial performance impairment was increased with racemic bupivacaine and R(+) bupivacaine compared to S(-)-bupivacaine and non-racemic bupivacaine. Even though LA cardiotoxicity has been widely described and R(+) isomer cardiotoxic potential is frequently reported as more important than S(-), S(-)-bupivacaine had more consequences to myocardial relaxation than racemic bupivacaine and ropivacaine. Non-racemic bupivacaine with 75% S(-) bupivacaine also impaired cardiac muscle relaxation as reported in the present study.

Many mechanisms were related to myocardial dysfunction associated to LA isomerism. Cardiac sodium channel blockade was caused by both bupivacaine isomers leading to heart conduction impairment, rhythm changes and hemodynamic instability. Moreover, R(+)bupivacaine effect was more potent than S(-) isomer. LA was also implicated in sarcolemmal K\textsubscript{ATP} channel harm. Racemic bupivacaine blockade sarcolemmal K\textsubscript{ATP} channel is the same as threefold S(-)-bupivacaine and ropivacaine.

Calcium handling impairment was thoroughly studied to explain LA cardiotoxicity. Isolated myocardium developed tension and intracellular calcium concentration were equally reduced by racemic bupivacaine and ropivacaine with a potency ratio of 2:1. Racem bupivacaine and its isomers increased intracellular calcium concentration in isolated myocytes. Authors believed sarcoplasmic reticulum ryanodine efflux was favored by racemic bupivacaine and its isomers in the same manner. Besides sarcoplasmic reticulum calcium release, sarcoplasmic reticulum calcium reuptake was blockade and calcium sensitivity of the contractile system was increased with racemic bupivacaine and its isomers, displaying a stereoselectivity to S(-)-bupivacaine and leading to myocardial relaxation slowness. It was also demonstrated high ropivacaine concentrations caused L-type calcium channel blockade, leading to negative inotropic effects in canine ventricular muscle.

Previous studies described heart mitochondrial injuries caused by LA. Racemic bupivacaine induced more respiratory chain impairment than ropivacaine, lidocaine and etidocaine in cardiac mitochondria. Myocardial oxygen consumption was reduced in isolated rat heart intoxicated with S(-)-bupivacaine. Mitochondrial injuries, myocardial oxygen consumption decrease and negative inotropic effects were reported after bupivacaine and ropivacaine isolated heart intoxication.

Potency is another LA chemical property associated with cardiotoxicity. It could be a bias to this research because ropivacaine is less potent than racemic and non-racemic ropivacaine and all LA were studied in equimolar concentrations. One very important study evaluated the myocardial contractility impairment with LA and demonstrated ropivacaine did not depress the developed tension compared to equimolar concentrations of racemic bupivacaine and levobupivacaine. However, myocardial function evaluated in the isolated rat heart was equally depressed with ropivacaine and racemic bupivacaine. Ropivacaine concentration was 1.75 times higher than racemic bupivacaine. Dose-response relationships could also be appropriate to better discuss the LA cardiotoxic effects instead of evaluating two different concentrations. It was also considered a limitation to the present study.

**Conclusions**

High concentrations non-racemic bupivacaine, with 75% S(-)-bupivacaine and 25% R(+)bupivacaine, depressed the myocardial relaxation similarly to racemic bupivacaine at low and high concentrations. High concentrations of racemic bupivacaine impaired the myocardial contractile function more than non-racemic bupivacaine and ropivacaine. Equimolar ropivacaine concentrations did not lead to significantly myocardial function impairment.

**References**


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