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Lipid Therapy with Two Agents in Ropivacaine-Induced Toxicity: Experimental Study in Swine

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Summary: Bonfim MR, Melo MS, Dreyer E, Borsoi LFA, Oliveira TG, Udelsmann A – Lipid Therapy with Two Agents in Ropivacaine-Induced Toxicity: Experimental Study in Swine.

Background and objective: Compare hemodynamic changes after ropivacaine-induced toxicity followed by treatment with two lipid emulsions in swine.

Methods: Large White pigs were anesthetized with thiopental, followed by intubation, and kept on mechanical ventilation. Hemodynamic variables at rest were recorded with invasive pressure monitoring and pulmonary artery catheterization. After 30 minutes, 7 $mg.kg^{-1}$ ropivacaine were injected intravenously and new hemodynamic measurements were performed within one minute. The animals were then randomly allocated into three groups and received: 4 $mL.kg^{-1}$ saline solution, or 4 $mL.kg^{-1}$ lipid emulsion with long-chain triglycerides, or 4 $mL.kg^{-1}$ lipid emulsion with long- and medium-chain triglycerides. Hemodynamic changes were reevaluated at 5, 10, 15, 20 and 30 minutes.

Results: Ropivacaine-induced toxicity mainly caused a drop in blood pressure and cardiac index without significant changes in vascular resistance. Therapy with lipid emulsions restored blood pressure primarily through increased vascular resistance, as cardiac index showed no significant improvement. Lipid emulsion with medium-chain triglycerides caused a greater increase in vascular resistance, particularly pulmonary.

Conclusion: In groups receiving lipid emulsions, hemodynamic results were better than in control group. There were no differences in systemic arterial pressure and cardiac index between animals receiving lipid emulsion with long-chain triglycerides and mixed long- and medium-chain triglycerides.

Keywords: Anesthetics, Local/ropivacaine; Accidents; Fat Emulsions, Intravenous; Swine.

INTRODUCTION

Local anesthetics (LA) are agents widely used in medical practice. Although there have been many advances in recent years on drugs and methods of administration, adverse effects and complications still occur and, although rare, it may compromise the patient's prognosis. In a 2006 estimate, the incidence of these events was 7.5 – 20:10.000 peripheral nerve blocks and 4:10.000 epidural anesthesia [1]. In case of accidental intravenous injection of high doses, the most feared effects are on central nervous system and cardiovascular system. The first precede the cardiovascular condition, ranging from a metallic taste to tingling that may progress to convulsions and coma, and the latter are characterized by decreased cardiac contractility, loss of vasomotor tone, cardiovascular collapse, arrhythmias difficult to reverse, and even asystole [2]. Several factors may influence the severity of LA systemic toxicity, including the patient's individual risk factors, concurrent medication, location and block technique, as well as the agent used, its dosage and volume [3]. Bupivacaine is still the most widely used LA in locoregional blocks. In 1979, Albright called attention to this agent toxicity in an *Anesthesiology* editorial [4]. Since then, efforts were made initially to find less toxic drugs. In 1996, ropivacaine was released, an agent with less lipid solubility and marketed only with its levorotatory isomer, which gives it a significantly better safety profile [5]. Although the results have been encouraging, accidents are still happening and may even manifest late after the blockade [6]. The next step was to find a specific agent to treat injuries caused by inadvertent intravascular injection of large doses of LA. In 1998, Weinberg et al. demonstrated in a preliminary study that lipid emulsions (LE), used since 1961 in parenteral nutrition, were able to mitigate the cardiotoxicity of bupivacaine in rats [7] and in 2003 they confirmed this finding in dogs [8]. Since then, the use of LE has gained international approval as an antidote in the clinical setting, reaching a recommendation from Anesthesiology societies of various countries [3,9,10]. Indeed, lipid therapy has shown to be more

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effective than conventional therapies in cases of resuscitation post-intoxication [11], but some limits have been identified and, in case of intoxication accompanied by significant hypoxia, these emulsions seem to compromise the normal cardiac function recovery [10]. The available LE for parenteral nutrition may have long-chain triglycerides or a mixture of long- and medium-chain triglycerides in its composition, and others may contain olive oil and fish oil. Mazoit et al. observed in vitro that LE with long-chain triglycerides have greater binding ability to local anesthetics [12] and, therefore, could be more effective as an antidote in cases of toxicity.

OBJECTIVE

The aim of this study was to evaluate the hemodynamic changes in pigs subjected to ropicavacaine-induced toxicity and treated with two types of LE: one with long-chain triglycerides and another with 50% medium- and 50% long-chain triglycerides.

METHOD

The study protocol was approved by the Animal Ethics Research Committee of the Instituto de Biologia da Universidade Estadual de Campinas. Thirty Large White pigs, weighing between 20 and 25 kg, were fasted from solid food overnight but free access to water. On the study morning, the animals received 10 $mg.kg^{-1}$ intramuscular ketamine, followed by weighing, and an estimate of the body surface using the formula [13] SC (m^2) = (9 x weight in $grams^{2/3}$) x 10^{-4}. The result was entered into the monitor system AS/3 Engstrom® for subsequent calculation of hemodynamic indices. Subsequently, venipuncture was made in the ear and anesthesia induced with sodium thiopental 2.5% at a dose of 25 $mg.kg^{-1}$. The animals were intubated and maintained under controlled mechanical ventilation with partial rebreathing circuit, tidal volume of 15 $mL.kg^{-1}$, and respiratory rate adjusted to obtain an $ETCO_2$ of 32-34 mm Hg. A mixture of air and 100% O_2 was used to keep hemoglobin O_2 saturation above 97%, measured by pulse oximetry positioned on the tongue. An electrocardioscope in lead II was installed for heart rhythm monitoring. Anesthesia was maintained with 1% end-tidal isoflurane concentration. Local anesthesia was performed with 5 mL of lidocaine 1% without vasoconstrictor in the inner portion of posterior left leg for dissection of artery and femoral veins for catheterization and continuous measurement of blood pressure, introduction of Swan-Ganz catheter 7F placed in the pulmonary artery branch by checking the morphological aspect of the curve obtained. The following variables were measured: mean blood pressure (mBP), heart rate (HR), central venous pressure (CVP), cardiac index (CI), mean pulmonary artery pressure (mPAP), pulmonary capillary wedge pressure (PCWP), systemic vascular resistance index (SVRI) and pulmonary vascular resistance index (PVRI). After 30 minutes of stabilization, hemodynamic measurements were performed at rest (M_0), followed by intravenous administration of ropivacaine 1% (7 $mg.kg^{-1}$) in 30 seconds. One minute later, new hemodynamic measurements (M_1) were performed. The animals were then randomly allocated into three groups. After M_1 and at 1 minute, CTRL group (control) received 4 $mL.kg^{-1}$ of 0.9% saline solution; group LCT received 4 $mL.kg^{-1}$ of lipid emulsion with long-chain triglycerides; and group MCT received 4 $mL.kg^{-1}$ of lipid emulsion with 50% medium- and 50% long-chain triglycerides. Hemodynamic measurements were performed again at 5, 10, 15, 20 and 30 minutes after intoxication (M_5 to M_{30} respectively). To compare numerical variables between groups at baseline, the Kruskal-Wallis test was used. To compare longitudinal measurements between groups and times, we used analysis of variance (ANOVA) for repeated measures followed by multiple comparison using Tukey's test for groups at all times, and the contrast profile test to analyze the evolution between assessments in each group. The level of significance for statistical tests was 5%.

RESULTS

Table I shows the mean and standard deviations (SD), weight, and body surface (BS) in both groups. There were no significant differences between groups.

Table I – Weight and Body Surface

Group	Weight ± SD (kg)	BS ± SD (m^2)
CTRL	21.60 ± 1.45	0.65 ± 0.03
LCT	22.00 ± 1.20	0.66 ± 0.02
MCT	22.00 ± 1.27	0.66 ± 0.03

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

Figure 1 shows that after intoxication there was a significant decrease in mean arterial pressure in the three groups: in CTRL blood pressure returned to values similar to M_0 from M_{10}; in LCT and MCT these values became greater than M_0 at M_{10} and M_5, respectively, and remained that way ($p < 0.001$). The values were lower in CTRL than in LCT and MCT up to M_{30} and there was no difference between LCT and MCT ($p < 0.001$).

As shown in Figure 2, there was a decrease in heart rate after intoxication in the three groups: in CTRL heart rate remained below resting values up to M_{30}; in LCT and MCT it returned to values similar to M_0 from M_5 ($p = 0.02$.) There were no differences between LCT and MCT, and CTRL values were lower than LCT at M_{10} and MCT at M_5 and M_{10} ($p < 0.001$).

Figure 3 shows increased central venous pressure after intoxication in the three groups, not returning to values similar to those at rest in none of the groups. In CTRL, M_{20} was higher than M_{30}; in LTC, it was also higher at $M_1<M_5>M_{10}>M_{15}$; and in MCT it was $M_1<M_5>M_{10}>M_{15}>M_{20}$ ($p < 0.001$). In CTRL, it was lower than in MCT at M_5 a M_{15} and in LCT lower than in MCT at M_{15} e M_{30} ($p < 0.001$).

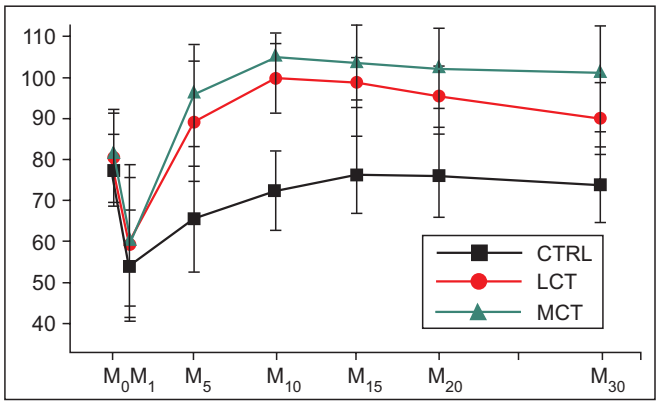

Figure 1 Mean Arterial Pressure (mm Hg).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

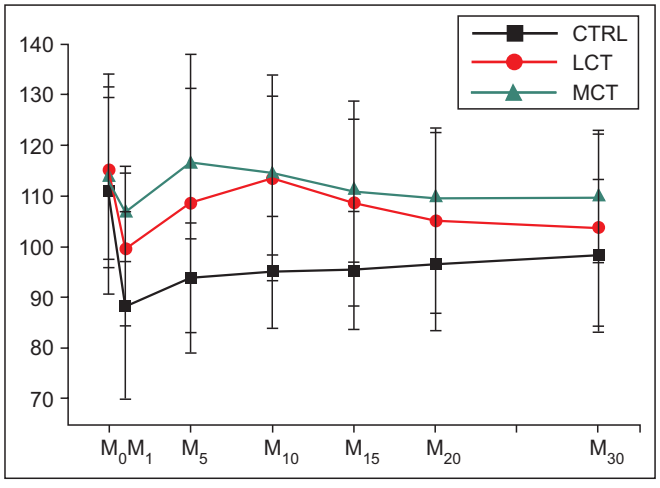

Figure 2 Heart Rate (bpm).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

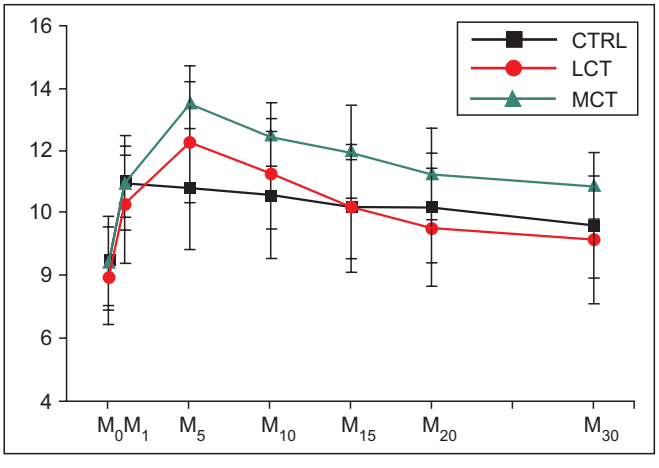

Figure 3 Central Venous Pressure (cm H_2O).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

As shown in Figure 4, there was a decrease in cardiac index after intoxication in the three groups, and they have not returned to levels similar to M_0. In CTRL, the values increased at M_5, M_{10}, M_{20} and M_{30}; in LTC, only at M_5; and in MCT, at M_{30} ($p < 0.001$). There was no difference between groups ($p = 0.38$).

Figure 5 shows that there was an increase in mean pulmonary artery pressure in all three groups immediately after intoxication and values did not return to similar ones at rest. In CTRL, the values from M_1 did not differ; in LCT, $M_5 > M_1$ and $M_{15} < M_{20} < M_{30;}$ in MCT, $M_5 > M_1$ e $M_{15} < M_{20}$ ($p < 0,001$). CTRL values were lower than in LCT from M_5 to M_{20} and lower than in MCT from M_5 to M_{30}, and LCT values were lower than in MCT at M_{20} and M_{30} ($p < 0.001$).

As shown in Figure 6, there was increased pulmonary capillary wedge pressure in the three groups, and values at M_0 were lower to all other measures. In CTRL, $M_5 > M_{10} > M_{15}$

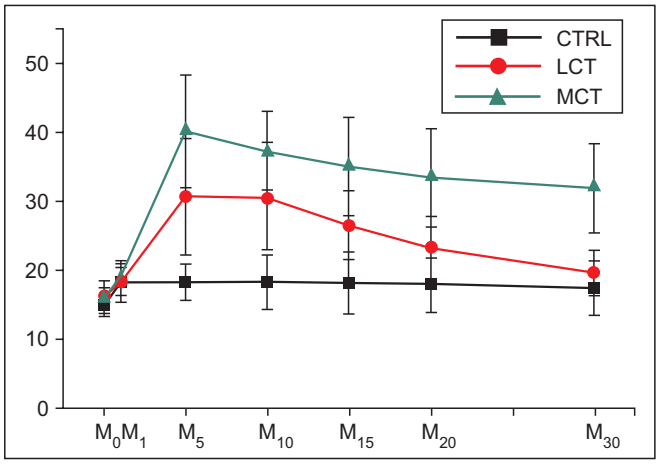

Figure 4 Cardiac Index ($L.min^{-1}.m^{-2}$).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

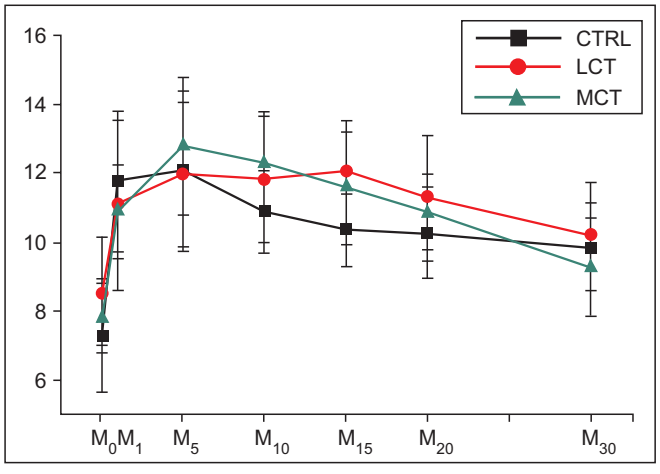

Figure 5 Mean Pulmonary Artery Pressure (mm Hg).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

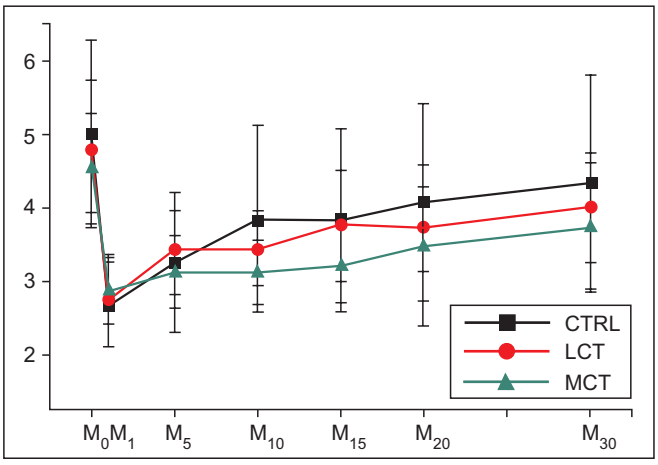

Figure 6 Pulmonary Capillary Pressure (mm Hg).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

e $M_{20}>M_{30}$; in LCT, $M_{20}>M_{30}$; and in MCT, $M_1<M_5$, $M_{10}>M_{15}$ and $M_{20}>M_{30}$ ($p = 0.035$). There were no differences between groups ($p = 0.45$).

As shown in Figure 7, CTRL values of systemic vascular resistance index at M_{10}, M_{15} and M_{20} were higher to the one the rest, in additional to $M_{15}>M_{20}>M_{30}$; in LCT, from M_5, all values were higher than the one at M_0 and $M_1<M_5<M_{10}>M_{15}$ and $M_{20}>M_{30}$; in MCT, also from M_5, all values were higher than the one at M_0 and $M_1<M_5<M_{10}$ ($p < 0.001$). In CTRL, results were lower than that in LCT and MCT from M_5 to M_{30}; and MCT had a lower value than LTC at M_5 ($p < 0.001$).

Figure 8 shows that, with intoxication, there was an increase in pulmonary vascular resistance at M_1 in the three groups. In CTRL, values at M_1 and M_5 were higher than at M_0; in LCT, all values were higher than the ones at M_0 and $M_1<M5$, $M_{10}>M_{15}$, and $M_{20}>M_{30}$; in MCT, all values were also higher than the ones at M_0 and $M_1<M_5$ ($p < 0.001$). CTRL showed results lower than LCT from M_5 to M_{20} and lower than MCT from M_5 to $M_{30;}$ in addition, LCT had lower results than MCT at M_5, M_{15}, M_{20} and M_{30} ($p < 0.001$).

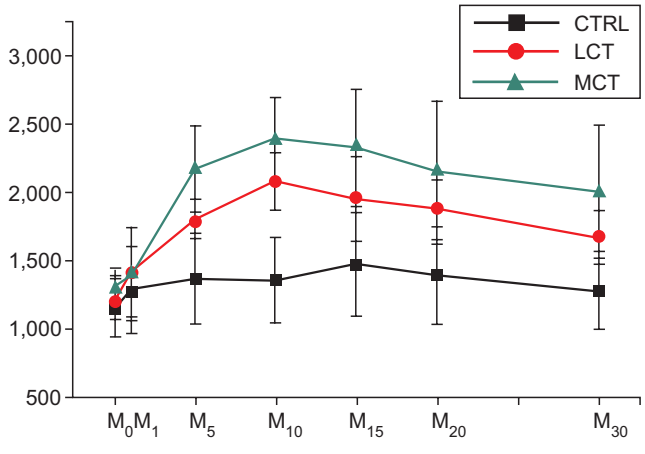

Figure 7 Systemic Vascular Resistance Index (dinas.s^{-1}.cm^{-5}.m^{-2}).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

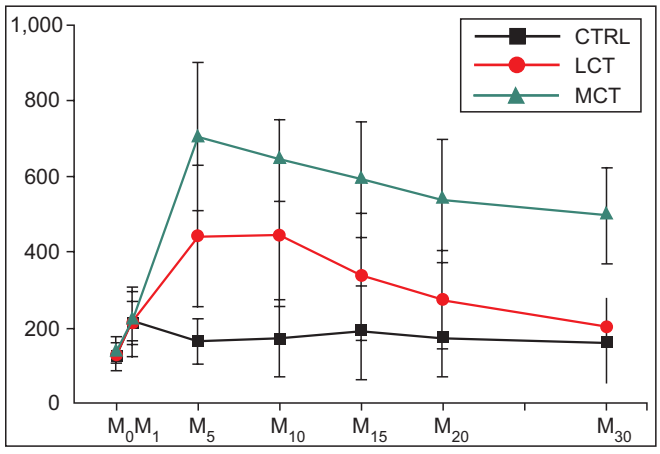

Figure 8 Pulmonary Vascular Resistance Index (dinas.s^{-1}.cm^{-5}.m^{-2}).

CTRL: Control; LCT: lipid emulsion with long-chain triglycerides; MCT: lipid emulsion with long- and medium-chain triglycerides.

DISCUSSION

Locoregional anesthesia has gone through major advances in recent years. New drugs have been synthesized, and new ways to localize nerve trunks is placed in evidence and are already part of the clinical practice in order to improve procedure quality and decrease the necessary dosage in blockades. Such care does not completely prevent the occurrence of adverse effects and complications; thus, a more specific therapy for cases of large dose intravascular injections was investigated, such as lipid emulsions. Three mechanisms have been proposed to explain the effects of these agents. The first suggest the pharmacokinetic balance with the expansion stage of plasma lipid, reducing the plasma levels of lipophilic drug free fractions and therefore the toxicity. This effect is achieved by chelating LA molecules and is known as "lipid sink" in Anglo-Saxon literature [14]. The second is based on the notion that local anesthetics are known to inhibit carnitine acetyltransferase, which is essential for transporting fatty acids into mitochondria [15], and LE could overcome this inhibition and the metabolic and toxic effect by "mass effect" alone or some other yet unknown mechanism. The third mechanism is based on the fact that fatty acids are known to increase the calcium levels of cardiac myocytes and thus may activate a direct inotropic route [14,16]. The hypothesis that lipid emulsions with *in vitro* long-chain triglycerides would be more effective [15] motivated this study to evaluate the hemodynamic effects of ropivacaine-induced toxicity in swine. In our experiments, we observed no significant differences in blood pressure using LE with long-chain triglycerides and mixed with long- and medium-chain. Improvement in hemodynamic deterioration after intravenous injection was achieved mainly by increasing systemic and pulmonary vascular resistance in pigs treated with lipid emulsion, a result similar to that found by Stojiljkovic et al. [17], who studied the hemodynamic changes of lipid infusion in humans, but different from those found by Kearney et al. [18]. In this study, increased resistance was seen mainly

in the pulmonary circulation in which LE with medium-chain triglycerides was significantly superior to the emulsion with only long-chain triglycerides. There was no improvement in cardiac index, a result similar to that of Litonius et al. [19] in a study of pigs with bupivacaine- and mepivacaine-induced toxicity, but different from that found by Stehr et al., who reported a positive inotropic effect in a study of isolated rat hearts [20].

In this study, lipid emulsions improved blood pressure after ropivacaine-induced toxicity, but emulsions with long-chain triglycerides and mixed with long- and medium-chain triglycerides had no significantly different outcomes. Still, much remains to be investigate about the use of lipid emulsions, but judging by the lack of adverse effects to date [21], expectations are encouraging and even recommended for accidents in case of obstetric anesthesia [22].

REFERENCES

1. Corcoran W, Butterworth J, Weller RS et al. – Local anesthetic-induced cardiac toxicity: a survey of contemporary practice strategies among academic anesthesiology departments. Anesth Analg, 2006;103:1322-1326.
2. Leone S, Cianni SD, Casati A, Fanelli G – Pharmacology, toxicology, and clinical use of new long acting long anesthetics, ropivacaine and levopubivacaine. Acta Biomed, 2008;79:92-105.
3. Neal JM, Weinberg GL, Bernards CM et al. – ASRA practice advisory on local anesthetic systemic toxicity. Reg Anesth Pain Med, 2010;35:152-61.
4. Albright GA – Cardiac arrest following regional anestesia with etidocaine or bupivacaine. Anesthesiology, 1979;51:285-287.
5. Negri P, Ivani G, Tirri T, del Pianno AC – New local anesthetics for pediatric anestesia. Curr Opin Anaesthesiol, 2005;18:289-292.
6. Sakai T, Manabe W, Kamitani T, Takeyama E, Nakano S – Ropivacaine induced late-onset systemic toxicity after transversus abdominis plane block under general anesthesia: successful reversal with 20% lipid emulsion. Masui, 2010;59:1502-1505.
7. Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ – Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. Anesthesiology, 1998;88:1071-1075.
8. Weinberg GL, Ripper R, Feinstein DL, Hoffman W – Lipid emulsion rescues dogs from bupivacaine-induced cardiac toxicity. Reg Anesth Pain Med, 2003;28:198-202.
9. Manavi MV – Lipid infusion as a treatment for local anesthetic toxicity: a literature review. AANA J, 2010;78:69-78.
10. Weinberg GL – Limits to lipid in the literature and lab: what we know, what we don't know. Anest Analg, 2009;108:1062-1064.
11. Weinberg GL, Di Gregorio G, Ripper R et al. – Resuscitation with lipid versus epinephrine in a rat model of bupivacaine overdose. Anesthesiology, 2008;108:907-913.
12. Mazoit JX, Le Guen R, Beloeil H, Benhamou D – Binding of long-lasting local anesthetics to lipid emulsions. Anesthesiology0 2009;110:380-386.
13. Holt JP, Rhode EA, Kines H – Ventricular volumes and body weight in mammals. Am J Physiol, 1968;212:704-715.
14. Cave G, Harvey M – Intravenous lipid emulsion as antidote beyond local anesthetic toxicity: a systematic review. Acad Emerg Med, 2009;16:815-824.
15. Shen X, Wang F, Xu S et al. – A cardiolipina é o alvo da cardiotoxicidade dos anestésicos locais? Rev Bras Anestesiol, 2010;60:445-454.
16. Aya AGM, Ripart J, Sebbane MA, de La Coussaye JE – Les émulsions lipidiques dans le traitement de la toxicité systémique des anesthésiques locaux: efficacité et limites. Ann Fr Anesth Reanim, 2010;29:464-469.
17. Stojiljkovic MP, Zhang D, Lopes HF et al. – Hemodynamic effects of lipids in humans. Am J Physiol Regul Integr Comp Physiol, 2001;280:1674-1679.
18. Kearney MT, Chowienczyk PJ, Brett SE et al. – Acute haemodynamic effects of lipolysis-induced increase of free fatty acids in healthy men. Clin Sci, 2002;102:495-500.
19. Litonius ES, Niiya T, Neuvonen PJ et al. – Intravenous lipid emulsion only minimally influences bupivacaine and mepivacaine distribution in plasma and does not enhance recovery from intoxication in pigs. Anesth Analg, 2011; [Epub ahead of print]
20. Stehr SN, Ziegeler JC, Pexa A et al. – The effects of lipid infusion on myocardial function and bioenergetics in l-bupivacaine toxicity in the isolated rat heart. 2007;104:186-192.
21. Picard J – Limit emulsion to treat drug overdose: past, present and future (editorial). Anaesthesia, 2009;64:119-121.
22. Bern S, Weinberg G – Local anesthetic toxicity and lipid resuscitation in pregnancy. Curr Opin Anesthesiol, 2011.24:262-267.