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Is Cardiolipin the Target of Local Anesthetic Cardiotoxicity?

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Summuary: Shen X, Wang F, Xu S, Qiang Y, Liu Y, Yuan H, Zhao Q, Feng S, Guo X, Xu J, Yang J – Is Cardiolipin the Target of Local Anesthetic Cardiotoxicity?

Background and objectives: Local anesthetics are used broadly to prevent or reverse acute pain and treat symptoms of chronic pain. Local anesthetic-induced cardiotoxic reaction has been considered the accidental event without currently effective therapeutic drugs except for recently reported intralipid infusion whose possible mechanism of action is not well known.

Contents: Cardiolipin, an anionic phospholipid, plays a key role in determining mitochondrial respiratory reaction, fatty acid metabolism and cellular apoptosis. Mitochondrial energy metabolism dysfunction is suggested as associated with local anesthetic cardiotoxicity, from an in vitro study report that the local anesthetic cardiotoxicity may be due to the strong electrostatic interaction of local anesthetics and cardiolipin in the mitochondria membrane, although there is a lack for experimental evidence. Herein we hypothesized that local anesthetic-cardiolipin interactions were the major determinant of local anesthetic-associated cardiotoxic reaction, established by means of theoretic and structural biological methods. This interacting model would give an insight on the underlying mechanism of local anesthetic cardiotoxicity and provide clues for further in depth research on designing preventive drugs for such inadvertent accidence in routine clinical practice.

Conclusions: The interaction between local anesthetic and mitochondrial cardiolipin may be the underlying mechanism for cardiotoxicity affecting its energy metabolism and electrostatic status.

Keywords: ANESTHETICS, Local; CARDIOLIPINS; COMPLICATIONS, Arrhythmias; cardiac, heart arrest, induced.

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INTRODUCTION

Local anesthetics are one of the major components of modern Anesthesiology. Local anesthetic-associated cardiotoxicity is, however, a thorny concern which causes atrioventricular block and asystole threatening the patients' life. While several reports gave a promising view regarding the successful post-accidental resuscitation of local anesthetic cardiotoxicity with intralipid [1-6], critical debates still exist for this controversial issue [7-12]. This may be related to unclear mechanisms of action for local anesthetics on cardiac myocytes.

Mitochondrial respiratory chain is the center of energy metabolism in cellular activities by synthesizing adenosine triphosphate (ATP) through oxidative phosphorylation, that can be changed or blocked or even destroyed in numerous pathological conditions. Cumulating evidence indicates that local anesthetics have diverse effects on oxidative phosphorylation in mitochondria largely depending on their chemical structures. Although such strong association between local anesthetic and mitochondria was documented, the precise role for local anesthetics in cardiac mitochondrial oxidative phosphorylation is yet unknown. Given that local anesthetic cardiotoxicity is a key part of their systemic toxicity, it is necessary to explore the potential mechanisms of local anesthetics on mitochondria metabolism in cardiac myocytes.

Cardiolipin, a unique tetra-acyl phospholipid firstly isolated from beef heart in the early 1940s is found almost exclusively in the inner mitochondrial membrane where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism [25]. Alterations in the content and/or structure of cardiolipin have been reported in several

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tissues in a variety of pathological settings, such as Barth syndrome, ischemia and reperfusion, aging, thyroid status, heart failure, neurodegenerative disease, dietary 18:2 deficiency, chronic ethanol consumption, and diabetes (detailed contents, see review 26). Önyüksel and colleagues showed that bupivacaine, but not lidocaine, interacts avidly and selectively with biomimetic small unilamellar liposomes containing cardiolipin and disrupts their integrity, which suggested that this interaction underlies in part the bupivacaine-induced cardiotoxicity [27]. Cardiolipin functions as a key mediator of mitochondrial metabolism [25]. Considering some previous reports on successful resuscitation after inadvertent intravenous administration of local anesthetics by means of lipid emulsion, we proposed, to this end, that cardiolipin could take an essential part in local anesthetic-induced cardiotoxicity.

We hereby hypothesized that the interaction between cardiolipin and local anesthetics is the underlying mechanism of local anesthetic cardiotoxicity considering a developed computational model.

Local anesthetic cardiotoxicity

The main clinical manifestation of systemic toxic reactions to local anesthetics follows the increasing in blood levels of local anesthetics [28-30]. Initially, symptoms from central nervous system (CNS) excitation are described including a metallic taste in the mouth, a ringing in the ears, or a circumoral tingling. Progressively, motor twitching in the periphery is followed by grand mal seizures, coma and respiratory arrest. Terminally, cardiac arrhythmia, hypotension, cardiovascular collapse and asystole occur.

Cardiotoxicity is the focus of local anesthetic-associated systemic toxic reactions mainly because the difficulty in achieving success in resuscitation after such an inadvertent accidence. Given the ethical concern, it is nearly impossible to perform clinical studies to clarify the precise association between cardiotoxicity and local anesthetics. Therefore, case reports and case series are the major clinical literatures in assessing the prognosis and frequency of such events. Multiple case reports of cardiac arrest and electrical standstill appeared after local anesthetic toxicity, and a large number were associated with difficult resuscitation, especially in the obstetrical population [31,32]. To these patients, even a cardiopulmonary bypass was used as a therapeutic choice [33]. Recently, several case reports documented successful resuscitation after lipid emulsion infusion [1,4,5], whereas its actual role in reducing the mortality has been intensely discussed [7-12]. However, it is still not an optimistic issue for patients and medical caregivers when using local anesthetics, especially the lipophilic amides, in case of the accidental intravenous injection or large dose of them reaching the toxic levels.

In reality, it is difficult to assess the frequency of local anesthetic-induced cardiotoxcity due to the lack of large-scale and strong evidence-based studies. A theoretic method created by Hanley and Lippman-Hand could estimate the risk of an accidental event that has not occurred in a prospective series [34], in which the upper limit of the predicted probability with a one-sided 95% confidence interval (95% CI) is three to n, where n is the number of observations without an adverse event. For example, if the n = 60, then the maximum risk of cardiotoxicity after systemic toxic reaction would be five per cent.

In consideration of the poor prognosis of local anesthetic cardiotoxicity combining the difficult prediction of the emergence frequency, further studies on the interaction mechanisms between local anesthetics and cardiotoxic reaction are need. Furthermore, theoretic and computational methods may be the better ways when clinical studies are restricted.

Cardiolipin and mitochondrion

Cardiolipin plays a crucial role in the mitochondrial membrane-stabilization, formation of the protein supercomplexes and functioning of respiratory chain. Cardiolipin is required for the electron transfer process in complex I and III of mitochondrial respiratory chain and structural organization of the complexes III and IV into a supercomplex. It stabilizes respiratory chain supercomplexes as well as the individual complexes, and is required to prevent formation of the resting state of cytochrome c oxidase in the membrane. Cardiolipin functions as an effector of activity of a mitochondrial cytochrome p-450 [35], and may contribute to the efficiency of oxidative phosphorylation both by decreasing the distance through which cytochrome c must travel between complexes III and IV as well as placing ADP/ATP carrier (AAC) in an environment that promotes its optimal activity [36]. In addition, the novel mitochondria-vacuole signaling pathway is mediated by the synthesis of cardiolipin [37]. The mitochondrial creatine kinase, a water-soluble octamer-dimer enzyme bound to the cytoplasmic side of the inner mitochondrial membrane, exerts functions via binding to cardiolipin as its receptor [38]. Taken these information together, cardiolipin has been regarded as the heart of mitochondrial metabolism for the importance of cardiolipin content and composition in various common diseases, such as diabetes and heart failure [25,39,40]. This role determined that detailed analyses on cardiolipin are needed for patients suffering from mitochondrial disease with unknown origin, since cardiolipin abnormalities might be their underlying cause.

Local anesthetics and mitochondrion

In in vitro isolated mitochondria, local anesthetics have diverse effects on oxidative phosphorylation depending on their chemical structures, including inhibiting electron transport [13,14] and F(1) ATPase [15-17], affecting transport of ions such as Ca^{2+} [18-21], and causing uncoupling [22,23]. Terada and colleagues found that the action of bupivacaine on mitochondria was mainly through accelerating the state-four respiration and activating the ATPase without the dissipation of the proton electrochemical potential where lacking hydrophobic anions, i.e., a decoupling action but not uncoupling was brought about [24]. The property of lipid solubility [41] but not the stereospecific effects [42] of lo-

cal anesthetics determines their effects on mitochondrial bioenergetics. Ropivacaine is less potent than bupivacaine on mitochondrial bioenergetics due to its lower lipid solubility in isolated rat heart mitochondria and in saponin-permeabilized ventricular fibers, and this cardiac energy metabolism impairment of bupivacaine can be enhanced with chronic hypoxia [43].

In cell culture, local anesthetics can reach mitochondria and reversibly decrease, or even collapse, their transmembrane potential [44]. The disruption of Ca^{2+} homeostasis in vivo has been suggested contributing to the bupivacaine toxicity [45,46]. Besides, mitochondrial dysfunction results in ATP depletion [47] and in turn is expected to have a major impact on intracellular Ca^{2+} homeostasis [48]. In addition, active oxidative metabolism is a key determinant in bupivacaine toxicity, and bupivacaine myotoxicity is a relevant model of mitochondrial dysfunction involving the permeability transition pore (PTP), a cyclosporin A-sensitive inner membrane channel that plays a key role in many forms of cell death, and Ca^{2+} dysregulation, and that it represents a promising system to test new PTP inhibitors that may prove relevant in spontaneous myopathies where mitochondria have long been suspected to play a role [49]. Long-acting local anesthetics induce marked negative inotropic and lusitropic effects on cardiomyocytes and such effect was mainly because of the impairment of calcium handling [50]. To the regulation of Ca^{2+} homeostasis, different local anesthetics possess different functions; bupivacaine, levobupivacaine, and ropivacaine can induce a deleterious effect in mitochondrial energy, though levobupivacaine disturbs Ca^{2+} homeostasis in the greatest degree [51].

Additionally, mitochondrial apoptotic pathway was considered as an essential part in leading to local anesthetic-induced cardiotoxicity. Mitochondrial swelling and oxidation of membrane protein thiol groups were associated with the activation of PTP, which was inhibited by the local anesthetic [52]. DNA fragmentation and DNA "ladder" formation, a typical feature for apoptosis, were induced by dibucaine with half-maximal concentration of 100 μM, and these effects were completely prevented by the unspecific caspase inhibitor z-Val-Ala-Asp-(OMe)-fluoromethylketone, thereby implicating caspase activation in the process [53]. Lidocaine-induced apoptosis was also associated with the mitochondrial caspase pathways [54]. Whether there are any interacting relationship among local anesthetics, caspase activation, apoptosis and cardiac toxic reaction still need further studies.

Recent reports [1-6,55] upon successful resuscitation with lipid emulsion after local anesthetic-induced cardiac collapse proposed that lipid metabolism might take a crucial part in explaining the mechanisms of the successfully resuscitated heart. Fatty acid metabolism was shown to predispose the isolated heart to bupivacaine toxicity, which confirmed that the local anesthetic exerts specific effects on lipid processes in cardiomyocytes [56]. Given the mitochondrion is the major compartment of lipid metabolism in living cells, and the so-called activation of fatty acids that is the obligatory step in fatty acid metabolism occurs partly in the outer mitochondrial membrane (for long-chain fatty acids) or in the mitochondrial matrix (for medium-chain fatty acids), the abnormal metabolism of fatty acids in heart mitochondria after local anesthetics overdosage should be explored in depth.

Local anesthetics and cardiolipin

Cardiolipin as described above has been recognized as a key mediator of the mitochondrial oxidative phosphorylation [35-38], phospholipid metabolism [57-59], and cellular apoptosis [60,61]. The in vitro study found that bupivacaine interacts selectively with biomimetic small unilamellar liposomes containing cardiolipin and disrupts the liposome integrity, suggesting that the bupivacaine-cardiolipin interaction might be the underlying mechanism of bupivacaine-induced cardiotoxicity [27]. In addition to this implication, further investigations proved indirectly that (i) anionic, pegylated liposomes (polyethylene glycol (PEG) attached, at one end of the polymer chain, to the surface of liposomes) exhibit high binding for bupivacaine [1], (ii) liposomes containing cardiolipin possess property of membrane fluidity [62], (iii) local anesthetics accelerate the water permeability by destabilizing the membrane structure and this effect was governed by the hydrophobicity of the anesthetics [63], and (iv) a minimal Ca^{2+} concentration is required for the fusion of large (0.1 μm) unilamellar cardiolipin/phosphatidylcholine (1:1) vesicles monitored by the mixing of the aqueous contents [64]. Currently, local anesthetics are used as model drug compounds when investigating the mechanism of drug release from oil suspensions in vitro [65], whereas the cardiotoxicity of local anesthetics evoked by interacting with mitochondrial cardiolipin or not is yet to be known. Besides, whether such theoretic interaction between the local anesthetic and cardiolipin played an expected role in local anesthetic-induced cardiac toxic reaction, it has to be explored in depth with experiments and theoretic models.

Hypothesis – the interacting model of cardiolipin and local anesthetics

Interacting model established by using computational theories became one of the pivotal means of theoretic biology. Cardiolipin-related interaction models have been developed such as the investigation of cardiolipin and its effect on the structure of lipid bilayers with molecular dynamics computer simulations [66], the formation of the cytochrome c-cardiolipin complex by ionic strength [67], mathematical model of pattern formation of molecular species of mitochondrial cardiolipin [58], and cardiolipin's electrostatic locking role in the Bax-alpha1 targeting sequence interact with mitochondrial membranes [68]. While numerous models of cardiolipin has been developed, the interacting model of the local anesthetic with cardiolipin has so far not been established especially when discussing the negative effect of local anesthetics on cardiac activity.

Muhonen *et al.* studied the interactions between local anesthetics and lipid dispersions using liposome electrokinetic capillary chromatography (LEKC), and found that all tested anesthetics (bupivacaine, lidocaine and prilocaine) that once

attached to negatively charged cardiolipin molecules in the liposomes by electrostatic forces could not be released from the intralipid particles anymore [69]. Even so, the precise interacting relation between local anesthetics and cardiolipin is still not known. Methods used in computational biology, such as molecular docking and packing, quantitative structure-activity relationships (QSAR), Monte Carlo simulated annealing approach, structural bioinformatics, pharmacophore modeling, and signal peptide prediction, among others, can effectively provide bioinformation and insights into corresponding molecule-molecule interactions. Herein we hypothesized that the interacting model between the local anesthetic and mitochondrial cardiolipin suggests a key role in determining the cardiac toxic reaction of local anesthetics, and such model can be developed with aforementioned methods to predict the cardiotoxicity of the local anesthetic.

CONCLUDING REMARKS AND CLINICAL IMPLICATIONS

Not merely the inadvertent injection of local anesthetics into blood vessels, but the sustained and prolonged duration of local anesthesia [70] can result in toxic reaction. How to reduce or prevent the local anesthetic-induced cardiotoxicity is an essential issue for clinical professionals when they used local anesthetics routinely. Nonetheless, under the condition of effectively preventive strategic guidelines, the reliability and safety of drugs to reverse the cardiotoxic reaction are very important in the event of an accident. It is necessary a detailed and clear understanding of the underlying mechanisms of local anesthetic cardiotoxicity.

In view of recently reported successful cases resuscitated with intralipid administration [1-6]; the facts related to a mitochondrial dysfunction during local anesthetic cardiotoxicity [13-24,44-51], and the key role of cardiolipin in mitochondrial functioning [35-40], we therefore propose that the interaction between the local anesthetic and cardiolipin determines the toxic effect of local anesthetics on cardiomyocytes by interfering with the mitochondrial energy metabolism. This interaction model can be produced with methods of theoretic and structural biology which gives insights of the potential interacting relation and clues for designing interventional drugs for such inadvertent events.

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