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ORIGINAL ARTICLE

The effects of 2-chloroprocaine on coagulation and fibrinolysis in the parturient: an in vitro study

Bhavani Shankar Kodali · Monica Sa Rego · A. Murat Kaynar · Richard D. Urman

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Abstract
Purpose Amide local anesthetics are known to inhibit coagulation. 2-chloroprocaine is the only ester agent used in obstetric anesthesia. It is used during obstetric emergencies, and also to supplement inadequate epidural block produced by amide local anesthetics. There is no study to date that has evaluated the effect of ester local anesthetics on blood coagulation and fibrinolysis in the parturient.
Methods In this study, we obtained blood samples from healthy, term-parturients and mixed them with varying amounts of 2-chloroprocaine for final concentrations ranging from 0.26 to 7.8 mM. Thromboelastograph® was used to study the coagulation profile of these samples.
Results Chloroprocaine impaired coagulation in a dose dependent manner, with increased R and K, and decreased MA and α. The difference, when compared to saline controls, reached statistical significance at a dose of 7.8 mM. An additional significant finding was that 2-chloroprocaine also enhanced fibrinolysis.
Conclusions Amide local anesthetics are known to impair coagulation, but 2-chloroprocaine produced significant fibrinolysis in addition to decreasing coagulation. This is the first study to date to demonstrate fibrinolytic properties of an ester local anesthetic. Further study evaluations are required to determine the cause of the variation in fibrinolysis. There is also a need to address the mechanism of increased fibrinolysis observed with 2-chroloprocaine.

Keywords Local anesthetics · 2-chloroprocaine · Coagulation · Fibrinolysis · Pregnancy

Introduction

Amide local anesthetics are, by far, the most commonly used local anesthetic agents in obstetric anesthesia practice. However, occasionally ester local anesthetic 2-chloroprocaine is used during obstetric emergencies and also to supplement inadequate epidural block produced by amide local anesthetics. 2-chloroprocaine is efficacious in these circumstances due to its higher agent concentration and greater diffusability.

There has been a debate regarding whether the amide group of local anesthetics contributes to the failure of prophylactic epidural blood patches (EBP) through the inhibitory effects on coagulation [1]. The effects of amide local anesthetics on coagulation have been studied using various ex vivo techniques, including thromboelastography® (TEG), which evaluates blood coagulation and fibrinolysis within the same assay [1–5]. The goal of most of these studies was to investigate any existing correlation between amide local anesthetics and potential risks of failed prophylactic epidural blood patches (EBP) for the prevention and treatment of "post dural puncture headache" or systemic effects of epidural local anesthetics. These studies used blood samples from both pregnant and non-pregnant volunteers [1, 3, 4].

Amide local anesthetics exert anticoagulant effects by stabilizing membranes of platelets, inhibiting Ca^{++} and

B. S. Kodali and A. M. Kaynar contributed equally to the paper.

B. S. Kodali · M. Sa Rego · R. D. Urman (✉)
Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115, USA
e-mail: urmanr@gmail.com

A. M. Kaynar
Department of Critical Care Medicine and Anesthesiology, University of Pittsburgh, Pittsburgh, PA, USA

α-granule release, inhibiting activation-dependent change in the conformation of the GPIIb/IIIa complex, protein kinase C, platelet aggregation and clot stability [6–13]. There is no study that has evaluated the effects of ester local anesthetics on blood coagulation and fibrinolysis in the parturient. In our current study, we used TEG to determine the effect of 2-chloroprocaine on coagulation in blood samples obtained from healthy term-parturients.

Methods

The study protocol was approved by the institutional review board. Healthy pregnant women between 37 and 39 weeks of gestation admitted for uncomplicated deliveries were randomly selected for the study. Patients with pre-existing or gestational hypertension (DBP > 90 mmHg), preeclampsia, diabetes mellitus, coagulation abnormalities, using medications known to alter platelet activity or coagulation were excluded from the study. Five ml of blood was drawn into buffered citrated tubes at the time of intravenous catheter placement. The blood samples were analyzed within 15–20 min of collection. Two pre-calibrated Haemoscope® dual channel TEG analyzers (model 5000, Niles, IL) were used for this study. One ml of citrated blood was added to each of the 4 tubes containing 35 μL Celite. Of this activated blood, 330 μL was added to each of the pre-warmed cups of TEG analyzers containing 20 μL of 0.2 M calcium chloride. Normal saline (30 μL) was added to channel 1 as a control. Varying amounts of normal saline were added to 3 % 2-chloroprocaine to create four serial concentrations of 2-chloroprocaine. Thirty μL of the study solutions were added to the other three cups of TEG analyzers containing blood to achieve effective final concentrations of 2-chloroprocaine of 0.26, 1.3, 2.6 and 7.8 mM. The highest concentration (7.8 mM) was equivalent to an agent:blood ratio of 1:11. TEG variables (R, K, α, MA, and fibrinolysis) were analyzed using one-way analysis of variance (ANOVA) for repeated measures with Bonferroni's post hoc test. A value of $p < 0.05$ was considered significant R time (mm, or min = mm/2) is defined as the period of time the blood was placed in the TEG until the initial fibrin formation; K time is measured (mm, or min = mm/2) from R until the level of clot firmness reaches 20 mm (divergence of the lines from 2 to 20 mm); alpha angle is formed by the slope of the TEG tracing at 'R' from the horizontal line. Like 'K', it also denotes the speed at which solid clots form; MA is defined as the maximum amplitude (mm) and is the measurement of maximum strength of the developed clot, which depends on fibrin and platelets; LY30 and LY60 measure percent lysis at 30 and 60 min after MA is reached. Measurements are based on the reading of the area under the TEG tracing from the time MA is measured until 30 or 60 min after the MA. Therefore, when LY30 or LY60 values are high, the fibrinolytic activity is high [14, 15].

Results

Blood samples were obtained from 10 pregnant women: mean age was 27 (SD 3.5), and mean gestation age 38.1 (SD 0.9). The effects of 2-chloroprocaine on coagulation are presented in Table 1. Statistical analysis of repeated measures revealed statistically significant increases in *R* (time to clot formation), *K* (rate of clot strengthening), and decreases in alpha angle (fibrinogen activity) and *MA* (clot strength) at 7.8 mM of 2-chloroprocaine suggesting decreased coagulation as compared to the control group (Fig. 1). Another notable finding of this analysis was that 2-chloroprocaine at this concentration caused statistically significant lysis of formed clot ($p < 0.05$), as shown in Fig. 2.

Discussion

Thromboelastograph® provides an arena to evaluate comprehensively both the coagulation cascade and fibrinolysis in one single test. Furthermore, TEG is probably the only means available to determine the existence of the hypercoagulable state [16, 17].

Amide local anesthetics such as lidocaine and bupivacaine are known inhibitors of platelet aggregation and release of granules [6–13]. The effects of bupivacaine on coagulation are reflected on TEG as a dose dependent decrease in MA, a measure of clot formation and strength [18]. Lidocaine has also been reported to significantly decrease blood coagulation and enhance fibrinolysis in blood samples from healthy volunteers at 9.2, 18.5 and 36.9 mM [5, 18]. In contrast, a recent study using whole blood samples from healthy parturients did not show any fibrinolytic changes using amide local anesthetics (bupivacaine, l-bupivacaine, ropivacaine and lidocaine); however, ester local anesthetics were not studied [4]. In our study, the highest concentration used for 2-chloroprocaine was 7.8 mM, and this concentration not only decreased coagulation, but also resulted in significant fibrinolysis in whole blood samples of healthy term gestation parturients. This is the first study to date demonstrating that an ester local anesthetic can result in fibrinolysis. We chose to examine lower concentrations of 2-chloroprocaine in our study because our pre-study trials of higher concentration of 2-chloroprocaine (>30 mM based on amide local anesthetic studies) showed substantial lysis [5, 18].

The mechanism for the abnormal fibrinolysis in our in vitro study with 2-chloroprocaine is unclear, but it may

Table 1 Effects of 2-chloroprocaine on TEG profile

Substance	Concentration	*R* (mm)	*K* (mm)	MA (mm)	α (°)	Lysis 30 (%)
NS (Control)	0.9 % NS	6.175 ± 1.31	2.15 ± 0.47	70.32 ± 3.86	75.87 ± 3.34	1.15 ± 1.27
2-Chloroprocaine	0.26 mM	7.1 ± 1.3	2.4 ± 0.82	68.6 ± 6.34	75.1 ± 4.95	1.7 ± 2.16
2-Chloroprocaine	1.3 mM	5.9 ± 1.29	1.8 ± 0.27	71.7 ± 3.05	78.6 ± 1.98	0.2 ± 0.27
2-Chloroprocaine	2.6 mM	6.4 ± 2.63	2.2 ± 0.57	68.3 ± 3.45	76.6 ± 5.21	7.9 ± 14.08
2-Chloroprocaine	7.8 mM	15.9 ± 3.69*	4.8 ± 0.75*	53 ± 8.68*	62.6 ± 2.94*	16.6 ± 12.25*

All values are mean ± SD ($n = 10$), and all the data were measured in millimeters from a chart run at 2 mm/min

NS normal saline, *R* reaction time, *K* coagulation time, *MA* maximum amplitude; α angle, *Lys30* Lysis 30, percent decrement in the amplitude 30 min after the MA is achieved, an index of fibrinolysis

* $p < 0.05$ was considered statistically significant using analysis of variance for repeated measures with Bonferroni post hoc analysis for 2-chloroprocaine-treated blood versus saline controls

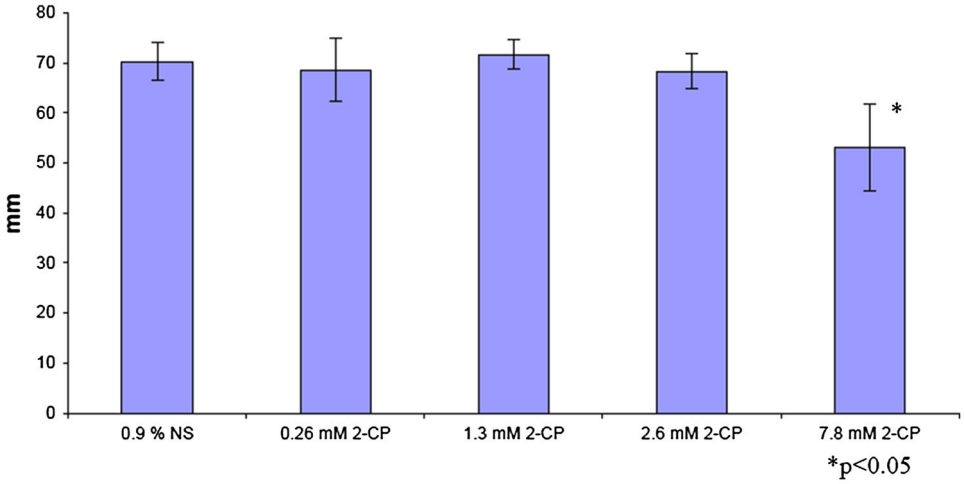

Fig. 1 Effects of 2-chloroprocaine on MA. Statistical analysis of repeated measures revealed significant increases in *R* (time to clot formation), *K* (rate of clot strengthening), and decreases in *alpha angle* (fibrinogen activity) and MA (clot strength) at 7.8 mM of 2-chloroprocaine, suggesting decreased coagulation as compared to the control group

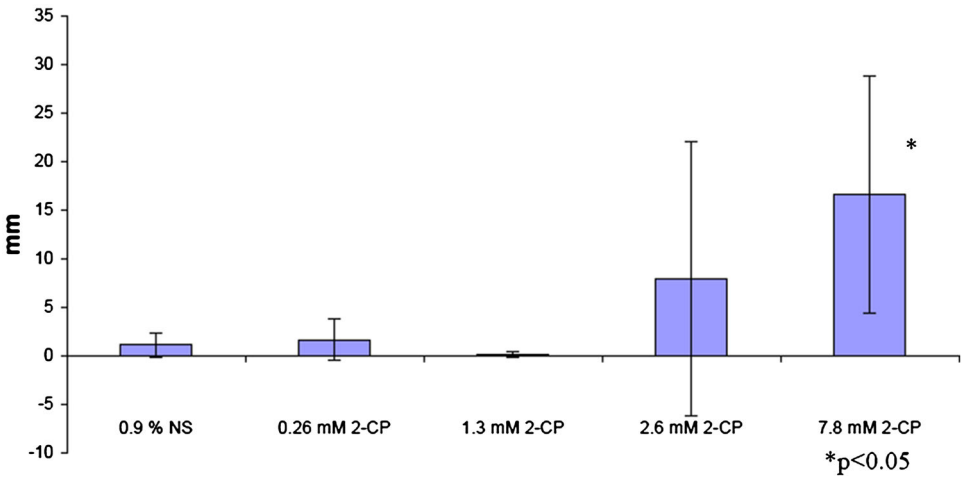

Fig. 2 Effects of 2-chloroprocaine on Lys30. 7.8 mM of 2-Chloroprocaine caused significant lysis of formed clot

be independent from the plasminogen pathway [19]. Tissue plasminogen activator (t-PA) is the primary activator converting plasminogen to plasmin for the cleavage of fibrin fibers. This system requires the presence of vascular endothelium; the source for t-PA and our in vitro set-up excludes this possibility. It is also unlikely that alterations in coagulation can be attributed to hemodilution. In our present study, all of the samples underwent a standard dilution with a study solution or normal saline control (30 μL in a final volume of 380 μL, ~7.8 %); thus, hemodilution could not have affected coagulation or fibrinolysis in 2-chloroprocaine group more than the saline

controls. In fact, hypercoagulability has been demonstrated by TEG after mild (15 %) to moderate (25 %) in vivo and in vitro hemodilution with normal saline [20–23].

Our study finding that 2-chloroprocaine can decrease coagulation and enhance fibrinolysis at 7.8 mM as compared to previously studied amide local anesthetics at higher concentrations (lidocaine 18.5 mM) is rather novel. This is despite 2-chloroprocaine being less potent than lidocaine or bupivacaine [5, 18]. This raises important pharmacological differences between esters and amides in terms of their effect on coagulation. It is unknown if this difference is a result of different chemical composition of these agents or due to metabolic by-products. 2-chloroprocaine is known to be metabolized rapidly by plasma pseudo cholinesterases and at a slower rate by esterases present in the cerebrospinal fluid. Therefore, it is conceivable that metabolites with longer half-lives, *β-diethylaminoethanol* and *2-chloro-4-aminobenzoic acid*, may play a role in the fibrinolytic activity of 2-chloroprocaine [24, 25]. Especially 2-chloro-4-aminobenzoic acid, which is structurally similar to para-amino benzoic acid (PABA), may possibly act as an inhibitor to coagulation factors IXa and Xa [26–28]. These studies also suggest that PABA-related compounds may have special affinity for clot substrates. In this study, a variability in the extent of fibrinolysis (high standard deviation) was observed with increasing 2-chloroprocaine concentrations. It is unknown if this is due to variance in the penetration of 2-chloroprocaine into the formed clot in the TEG cup or variations in the degradation of 2-chloroprocaine in the blood sample by the enzymes. Further study evaluations are required to determine the cause of the variation in fibrinolysis. There is also a need to address the mechanism of increased fibrinolysis observed with 2-chloroprocaine.

It is difficult to interpret the clinical significance or consequences of these observed effects of 2-chloroprocaine on blood coagulation in the laboratory, particularly its role in fibrinolysis. The possibility that 2-chloroprocaine is a potential fibrinolytic may represent a significant finding given that injury to epidural vascular plexus is not that uncommon during neuroaxial procedures. This is particularly alarming as the initial concentration of 2-chloroprocaine would be much higher in the epidural space, following epidural injection of 20–30 ml solution, than the concentration ranges evaluated in our study. However, we hypothesize that several factors may decrease the negative consequences of 2-chloroprocaine. The rapid absorption of 2-chloroprocaine from the epidural space and its subsequent metabolism in the blood decreases the final drug availability in the epidural space to produce significant fibrinolysis. This is further facilitated by the lower affinity of this drug to fat as compared to amide local anesthetics, such as bupivacaine [29].

It is possible that 2-chloroprocaine in the epidural space may contribute to failure of prophylactic EBP against post dural puncture headache as it is suggested with other amide local anesthetics [5, 18]. In this study, we chose concentrations of 2-chloroprocaine to mimic the possible admixtures, which may take place in the epidural space. The main limitation of the current as well as the other studies involving the amide group of agents is the difficulty of measuring the local anesthetic concentrations and volumes in the epidural space. Other than local absorption, blood may dilute epidural 2-chloroprocaine to lower concentrations [26–28]. On rare occasions, cerebrospinal fluid from an unintended arachnoid tear may also dilute the anesthetic. Another important limitation of this study is the inability to determine how well blood and 2-chloroprocaine mix in the epidural space. Mixing in the TEG cuvette is nearly complete; it is probably not complete in the in vivo setting. However, despite these uncertainties, it can be stated that it is usually rare in clinical practice to perform prophylactic EBP following the use of 2-chloroprocaine, when compared to more often used amide local anesthetic agents. An outcome study of this scenario is also a very unlikely possibility.

The in vitro findings of this study should, however, instill some caution in our clinical practice. It will be difficult to prove if 2-chloroprocaine is associated with greater incidence of epidural bleeding following epidural vascular trauma during neuroaxial procedures. Therefore, one could exercise caution and risk–benefit considerations while using this agent in patients with borderline coagulation abnormalities, while performing multiple attempts at neuroaxial placement, or in the case of obvious vascular trauma during a neuroaxial procedure.

In summary, our study presents evidence for the first time that 2-chloroprocaine could inhibit coagulation and promote fibrinolysis in blood samples obtained from healthy parturients. The findings are in contrast with previous work using amide local anesthetics. Fortunately, ester local anesthetics are not used too frequently in clinical practice involving neuraxial anesthesia. Further studies are needed to elucidate the role of ester anesthetics such as 2-chloroprocaine and the mechanisms involved in potentially inducing coagulopathy during neuroaxial anesthesia.

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