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Original Contribution

Calcium chloride for the prevention of uterine atony during cesarean delivery: A pilot randomized controlled trial and pharmacokinetic study☆

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ABSTRACT

Study objective: To assess the feasibility, patient tolerance, pharmacokinetics, and potential effectiveness of a randomized controlled trial protocol investigating intravenous calcium chloride for the prevention of uterine atony during cesarean delivery.
Design: Double-blind, randomized controlled pilot trial with nested population pharmacokinetic analysis.
Setting: This study was performed at Lucile Packard Children's Hospital, from August 2018 to September 2019.
Patients: Forty patients with at least two risk factors for uterine atony at the time of cesarean delivery.
Interventions: One gram of intravenous calcium chloride (n = 20 patients) or a saline placebo control (n = 20 patients), in addition to standard care with oxytocin, upon umbilical cord clamping.
Measurements: The primary efficacy-related outcome was the presence of uterine atony defined as the use of a second-line uterotonic medication, surgical interventions for atony, or hemorrhage with blood loss >1000 mL. Blood loss, uterine tone numerical rating scores, serial venous blood calcium levels, hemodynamics, and potential side effects were also assessed.
Main results: The study protocol proved feasible. The incidence of atony was 20% in parturients who received calcium compared to 50% in the placebo group (relative risk 0.38, P = 0.07, 95% CI 0.15–1.07, NNT 3.3). Calcium recipients tolerated the drug infusion well, with no adverse events and an equal incidence of potential side effects in the calcium and placebo groups. Ionized calcium concentration rose significantly in all patients who received calcium infusion, from baseline 1.18 mmol/L to peak levels 1.50–1.60 mmol/L. One-compartment population pharmacokinetics established clearance of 0.93 (95% CI 0.63–1.52) L/min and volume of distribution 76 (95% CI 49–94) L.
Conclusions: In this pilot study, investigators found that intravenous calcium chloride was well-tolerated by the 20 patients assigned to receive the study drug and may be effective in prevention of uterine atony. A 1-g dose was sufficient to substantially increase calcium levels without any critically elevated lab values or concern for adverse side effects. These encouraging findings warrant further investigation of calcium as a novel agent to prevent uterine atony with an adequately powered clinical trial.
Clinical trial registry NCT03867383 https://clinicaltrials.gov/ct2/show/NCT03867383

1. Introduction

Postpartum hemorrhage (PPH) is the leading cause of maternal mortality worldwide [1]. Uterine atony, defined as failure of the uterus to adequately contract after placental delivery, causes at least 70–80% of PPH [2]. Current management of uterine atony involves prophylaxis with oxytocin and treatment with second-line uterotonics, including methylergonovine, carboprost, and misoprostol [3]. However, these medications all carry significant limitations of poor efficacy, adverse side effect profiles, expense, or contraindications to use [3]. For

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example, methylergonovine is contraindicated in patients with pre-eclampsia or hypertensive diseases, and carboprost, which may cost up to $1000 per dose, is contraindicated in patients with asthma. Misoprostol causes fever and rigors, and its efficacy for treating uterine atony has been called into question [4]. As such, additional prevention or treatment modalities for uterine atony are needed.

Calcium has an essential physiological role in uterine contractility. Uterine myometrial contractility depends upon an influx of calcium from intracellular stores in the sarcoplasmic reticulum and extracellular calcium [5]. Several *in vitro* studies have established that myometrial contraction amplitude diminishes in the setting of low or absent extracellular calcium [6–9]. Oxytocin's efficacy in inducing myometrial contraction diminishes significantly in the setting of low or absent extracellular calcium levels in *in vitro* studies with human uterine strips [10]. Clinical studies also show that serum calcium levels impact uterine contractility during spontaneous labor and postpartum hemorrhage [11,12]. Although the safety and lack of hemodynamic consequences of intravenous calcium infusion in parturients have been assessed in one study [13], we are unaware of any clinical studies designed to evaluate the potential role of intravenous calcium in preventing or treating uterine atony or PPH.

This pilot study was designed to assess feasibility, patient tolerance, and preliminary assessment of efficacy to determine whether intravenous calcium chloride warrants future investigation as an agent for the prevention of uterine atony. We hypothesized that the study protocol would be feasible, well-tolerated, and show a signal of efficacy. The primary efficacy-related hypothesis is that providing exogenous calcium to generate supraphysiologic ionized calcium levels will augment uterine contraction and decrease the incidence of atony in parturients. Secondary aims included assessment of potential side effects of calcium infusion, including hemodynamic changes, discomfort at the site of the intravenous catheter, nausea, and vomiting. Finally, population pharmacokinetics were assessed as a secondary aim to guide dosing in future studies.

2. Methods

After obtaining Stanford University Institutional Review Board approval (Protocol 43076, April 2017), submission to ClinicalTrials.gov (NCT03867383), and written informed consent, obstetric patients undergoing cesarean delivery at Lucile Packard Children's Hospital, Stanford, California, were enrolled in this randomized, double-blinded, placebo-controlled study. Inclusion criteria were patients 18–45 years of age, undergoing cesarean delivery, who had at least two of the following established risk factors for uterine atony: intrapartum delivery, oxytocin infusion for 4 h or longer, magnesium infusion, chorioamnionitis, multiple gestation, polyhydramnios, and prior history of postpartum hemorrhage [3]. Two risk factors were chosen to further enrich the study population as at-risk for uterine atony and postpartum hemorrhage. Exclusion criteria included renal dysfunction with serum creatinine >1.0 mg/dL, maternal treatment with digoxin, maternal treatment with a calcium channel blocker, and maternal history of cardiac condition including arrhythmia, congenital cardiac disease, or coronary disease. Patients with placenta accreta spectrum were also excluded from participation.

Patients admitted to the labor and delivery ward were provided a brief informational sheet regarding the study. The clinical staff subsequently approached patients who met inclusion criteria for the study for written informed consent if the obstetrician agreed that this discussion would not interfere with case urgency. For example, in laboring patients who required intrapartum cesarean delivery, the patient was approached for informed consent conditional on the obstetrician agreeing that maternal and fetal status were stable enough for a slight delay to obtain final consent. Not all patients who met study inclusion criteria were approached; study participants were approached when the clinical service allowed. As part of consent, participants were asked if they would permit the team to draw 3 blood specimens for ionized calcium assessment and nested pharmacokinetic analysis; however, those who declined phlebotomy were still allowed study participation. Patients were informed about possible side effects, including intravenous line discomfort, during the consent process and asked to inform the anesthesiologist if they experienced this at any point during the delivery course. The anesthesiologist was instructed to assume all patients were receiving calcium chloride, and to immediately discontinue the infusion if any concerns developed regarding medication extravasation, arrhythmia, hemodynamic disturbance, or severe nausea or vomiting.

Patients were sequentially assigned to one of two computer-generated random groups (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA) using a sealed, opaque envelope system. Patients either received 1-g intravenous calcium chloride diluted to a total volume of 60 mL with normal saline or 60 mL saline placebo in addition to our institution's standard oxytocin regimen (2 U bolus and 7.5 U/h infusion for 4 h). All participants including the obstetrician, supervising and resident anesthesiologists, patient, and study investigator collecting data were blinded to the study solution received. An anesthesiologist with no further involvement in patient care prepared the study solution in a different operating theater.

A one-gram dose of calcium chloride was chosen for several reasons: First, one-gram of calcium chloride is the widely-available dose with substantial drug safety data. Second, when Farber and colleagues studied the hemodynamic effects of calcium chloride in parturients, a 400 mg dose only increased ionized calcium concentration to 1.25–1.35 mmol/L, which falls within the normal physiologic range of 1.20–1.40 mmol/L [13]. As such, a one-gram dose was chosen for this pilot protocol to test the hypothesis that a supraphysiologic ionized calcium level would augment uterine contractility. Pharmacokinetic modeling was planned to facilitate dose refinement, if needed.

Upon entering the operating room, standard noninvasive monitors were applied: pulse oximetry, electrocardiography (ECG), heart rate (HR), and baseline non-invasive blood pressure (NIBP). Mean arterial blood pressure (MAP) was measured with an automated, non-invasive sphygmomanometer (Dinamap, Critikon Inc., Tampa, FL, USA) at 1-min intervals until fetal delivery and hysterotomy closure and then at 2.5-min intervals at the discretion of the clinical anesthesiologist.

All patients received neuraxial anesthesia for the cesarean procedure. Laboring patients with an epidural catheter *in situ* received preservative free 2% lidocaine with epinephrine in 5 mL increments to achieve a T4 level to pinprick (plus epidural fentanyl 100 μg and morphine 3 mg after delivery), whereas patients without *in situ* blocks received spinal anesthesia with 1.6 mL hyperbaric 0.75% bupivacaine with intrathecal fentanyl 15 μg and morphine 150 μg.

All patients had an 18-gauge peripheral intravenous cannula in place at the time of cesarean delivery. Lactated Ringers solution was administered at the discretion of the clinical anesthesiologist with general guidelines not to exceed 3 L during the cesarean delivery. Total administered fluid volume was recorded. Colloid infusion with 6% hydroxyethyl starch in 0.9% sodium chloride or 5% albumin was also recorded. Patients received prophylactic phenylephrine infusion (starting at 0.5 μg/kg/min) and phenylephrine (50–100 μg) or ephedrine (5–10 mg) bolus doses as needed at the discretion of the clinical anesthesiologist to maintain MAP within 10% of baseline. The total phenylephrine infusion dose received as well as total bolus doses of vasopressors was left to the discretion of the clinical anesthesiologist and recorded.

Following fetal delivery and umbilical cord clamping, patients received 2 U oxytocin bolus, and oxytocin infusion was initiated at a rate of 7.5 U/h. At this same time point, the 60-mL study drug infusion was administered over 10 min using an Alaris syringe infusion pump (BD Alaris syringe module, Beckton, Dickinson and Company, Franklin Lakes, NJ, USA) through microbore infusion tubing connected to the intravenous injection port most proximal to the patient. Per institutional protocol, adequacy of uterine tone (yes or no) was formally assessed at 3,

6, and 10 min after delivery by the operating obstetrician. If tone was deemed inadequate at 3 min or 6 min, a 2 U bolus of oxytocin was administered, and the oxytocin infusion rate was doubled. If at 10 min, tone was still deemed inadequate, a second-line uterotonic agent of intramuscular methylergonovine 200μg, intramuscular carboprost 250μg, or buccal misoprostol 600μg was chosen at the discretion of the obstetrician and clinical anesthesiologist and recorded. At 10-min, the obstetrician also graded the uterine tone formally on the 0–100 scale as specified above. After leaving the operating theater, all patients received our institution's standard protocol of oxytocin 7.5 U/h for 4 h.

2.1. Feasibility, acceptability, patient tolerance, and preliminary safety

Feasibility was defined per the National Institute for Health Research definition as "willingness of participants to be randomized, willingness of clinicians to recruit participants, number of eligible patients, and characteristics of proposed outcome measures" and well as whether the "recruitment, treatment, and follow up assessments all run smoothly". As such, it was assessed by the following: number of protocol deviations, amount of missing or incorrectly obtained data, and ability to enroll the desired number of patients [14]. Patient acceptance of the protocol was assessed as the number of patients who declined participation, withdrew informed consent at any point, or declined to provide blood samples. Patient tolerance of the intervention was assessed through a detailed assessment of any potential drug side effects or adverse effects. Hemodynamic variables analyzed included heart rate and mean arterial pressure at baseline, at fetal delivery, and at 5-min intervals for the next 30 min. Additionally, vasopressor boluses and infusions and total volume crystalloid and colloid administered were recorded. The clinical anesthesiologist filled out a checklist prior to exiting the operating theater at case conclusion answering whether the patient experienced nausea, vomiting, intravenous line discomfort, changes in heart rate or blood pressure, any arrhythmia, or other possible side effect (with a free-text field) during or following the study drug infusion.

2.2. Primary outcome

The primary outcome was incidence of uterine atony, a binary composite outcome defined as any of the following: second-line uterotonic requirement, blood loss >1000 mL, placement of a Bakri balloon, B-lynch suture, or O'Leary sutures by the obstetrician, uterine artery embolization or hysterectomy. For this pilot study, any *P* value less than 0.2 was defined *a priori* as indicating potential for efficacy, and thus warranting a future, larger study.

2.3. Secondary outcomes

Several secondary outcomes related to uterine atony and hemorrhage were assessed. At the completion of study drug infusion (10 min after umbilical cord clamp), the operating obstetric attending was asked to assess and grade the uterine tone based on the palpation of the fundus using a verbal numerical scale score from 0 to 100 (0 = completely atonic, 100 = fully contracted). Blood loss was estimated by visual examination of the graduated suction jars, surgical sponges, drapes, table, and floor. The difference in preoperative and postoperative day 1 hematocrit was calculated. The total volume of intravenous fluid given from start to conclusion of the surgical procedure was recorded. Transfusion and number of units transfused during the duration of the hospital stay were recorded. Total units of oxytocin bolused and maximum oxytocin infusion rate were recorded, as were all second line uterotonics. If any uterotonics were administered after leaving the operating room, these were included in our primary outcome and also separately recorded as "delayed uterotonic use" to account for possible delayed onset of uterine atony or PPH.

2.4. Statistical analysis

A sample size of 40 parturients was selected for this pilot study as most likely feasible to perform in approximately one year or less at our institution. Internal quality improvement data at our institution revealed that in patients with more than one atony risk factor, incidence of EBL >1000 mL or second line uterotonic use was 40–60%. If calcium were to halve the rate of uterine atony, we estimated that a sample size of 20 subjects in the calcium and 20 subjects in the placebo group should allow sufficient data to show a difference between groups with 75% power and a two-sided type I error probability of 0.2.

For analysis of the primary outcome, the relative risk (RR), and its 95% confidence interval were calculated according to Altman [15]. The number needed to treat (NNT) was defined and calculated using the terminology suggested by Altman [16]. The 95% confidence interval for NNT was calculated according to Daly [17], and *P*-value was calculated according to Sheskin [18]. Bootstrap analysis for sample size calculation for future studies was performed using R programming and 10,000 replicates of the pilot dataset.

Demographic and baseline characteristics are reported as mean ± standard deviation (SD) or median and interquartile range [IQR] as appropriate for continuous variables. Categorical variables are presented as percent. The mean differences and their 95% confidence intervals (CI) are presented for outcomes of interest. Univariate comparisons between groups were performed using Chi-square analysis or the Kruskal–Wallis test, as appropriate for categorical or continuous variables, respectively. Statistical analyses were performed using STATA version 14.0 (StataCorp, CollegeStation, TX, USA).

2.5. Pharmacokinetic analysis

For patients who consented to phlebotomy, blood specimens were collected into a non-heparinized syringe at three different time intervals: once in the operating room prior to fetal delivery for a baseline value, once within 1–30 min of completing study drug infusion, and once 30 min to 3 h after completing study drug infusion. To allow for painless sample collection for patients with a surgical lumbar neuraxial block, a tourniquet was placed at the patient's lower calf, and a 21-gauge winged blood collection needle was utilized to collect a 3 mL venous specimen from the foot or saphenous vein. The ionized calcium level and pH were immediately determined with an i-STAT 1 system (i-STAT Corp., Princeton, NJ, USA).

Calcium pharmacokinetics were analyzed with NONMEM (Nonlinear Mixed-Effects Modeling; Globomax, Ellicott City, MD) using PLT Tools (PLT Soft, San Francisco, CA). We evaluated calcium kinetics using a 1-compartment model with administration into the central compartment as the sparseness of pilot data was not anticipated to support 2- or 3-compartment models. We used an exponential model for interindividual variability on the volume of distribution and clearance and an additive model of interindividual variability on the baseline calcium concentration. We used an additive plus proportional model for residual intraindividual variability. We tested for the effect of pH using the likelihood ratio test, with a decrease in log-likelihood of 3.84 considered statistically significant (χ^2 for $P = 0.05$ with 1 degree of freedom).

The model parameters were estimated by NONMEM using the first order conditional estimation approach. Confidence intervals for each parameter were assessed using log likelihood profiling and bootstrap analysis. The loglikelihood profile calculated the −2 log likelihood by fixing the value of the parameter at different estimates and re-estimating the other model parameters. Parameters that are known with certainty are expected to have a narrow range before the −2 log likelihood increases by more than 3.84 (chi-square distribution for $P = 0.05$, 1 degree of freedom). The full data set is provided (see Supplemental Digital Content 1).

3. Results

Forty patients were recruited for this study between August 2018 and September 2019. All patients completed the study and were included in the analysis (Fig. 1). There were no significant differences in any of the demographic or obstetric variables assessed (Table 1). The trial was concluded upon successful enrollment of 40 patients as planned.

3.1. Feasibility, acceptability, and tolerability

Forty patients were recruited over the study period without dedicated funding or research staff. Of the 66 patients assessed for study inclusion, only 7 declined to participate (Fig. 1). Six patients were excluded due to failure to meet inclusion or exclusion criteria, and 13 cases were ultimately too urgent. No patients withdrew consent. Twenty-four of 31 patients asked were willing to provide serial venous blood specimens for pharmacokinetic modeling. Nine patients were not asked to submit phlebotomy specimens as they delivered during nighttime or weekend hours when not enough anesthesia staff were available for the additional task. Baseline ionized calcium concentration, as well as two additional samples, were successfully collected from all 24 participating patients. All patients who participated in the study appropriately met inclusion and exclusion criteria.

There were no adverse events. One saline placebo infusion was paused for significant hypertension and tachycardia that were noted 3 min after infusion initiation (Fig. 1). No calcium chloride infusions were paused or discontinued for potential severe side effects. The incidence of side effects including intravenous line discomfort, flushing, nausea, vomiting, bradycardia, tachycardia, hypertension and hypotension was 30% in both groups ($P \geq 0.99$, Table 2).

3.2. Primary outcome

In the calcium chloride group, 20% of patients experienced the primary outcome of uterine atony, compared to 50% in the saline placebo group (RR 0.38, $p = 0.07$, 95% CI 0.14–1.02, Table 3). As such, the NNT with intravenous calcium to prevent one case of uterine atony among this cohort of women is 3.3 (95% CI 1.7–51.6).

The breakdown of which primary outcome criteria each patient met can be seen in Supplement 3. Two patients in the calcium group who met the primary outcome of uterine atony had a delayed requirement for uterotonic administration after leaving the operating room compared to none of the patients in the control group ($P = 0.49$). The only patient that required a hysterectomy, due to unremitting uterine atony with hemorrhage, received a placebo infusion.

Based upon a bootstrap of 10,000 replicates using these pilot study results, a study with 44 patients per assignment group would have 80% power to achieve $p < 0.05$, and a study of 64 individuals per assignment group would have 80% power to achieve $p < 0.01$.

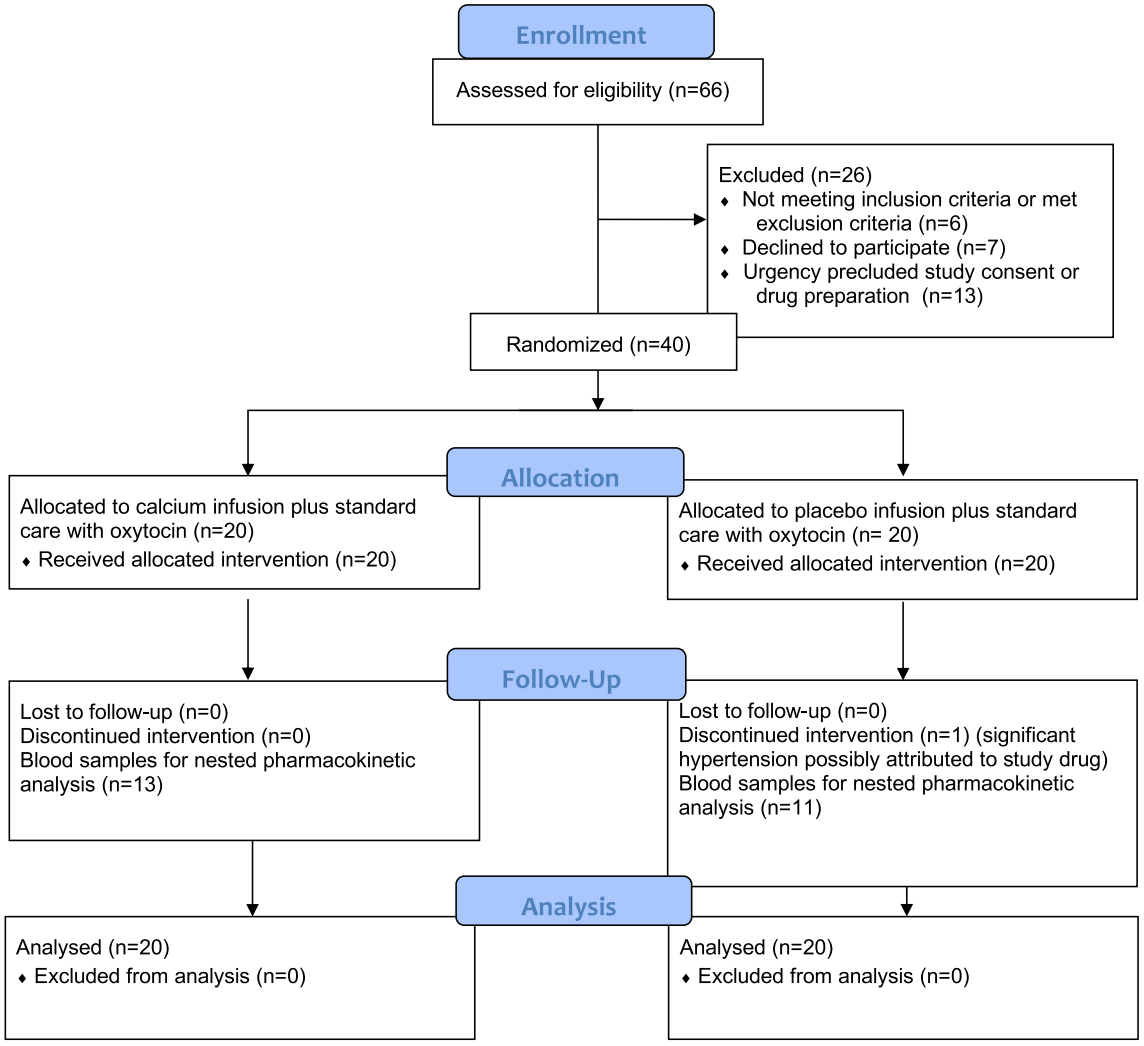

Fig. 1. Consort flow diagram.

Table 1
Patient characteristics.

	Calcium Chloride ($n = 20$)	Placebo ($n = 20$)	P value
Age *(years)*	34.5 (6.6)	33.5 (3.7)	0.54
Height *(cm)*	160.9 (8.3)	161.6 (6.8)	0.76
Weight *(kg)*	81.4 (16.2)	78.2 (13.4)	0.51
Body Mass Index *(kg m^{-2})*	31 (6)	30 (6)	0.55
Race/Ethnicity			0.87
Asian	4 (20%)	6 (30%)	
Black	0 (0%)	1 (5%)	
Hispanic/ Latina	4 (20%)	4 (20%)	
White (non-Hispanic)	10 (50%)	8 (40%)	
Other	2 (10%)	1 (5%)	
Obstetric characteristics			
Gestational age *(weeks)*	38.4 [36.8–39.3]	39.0 [35.7–39.7]	0.44
Gravidity	1.5 [1–3.5]	2 [1–2.5]	0.80
Parity	0 [0–1]	0 [0–1]	0.70
Prior cesarean delivery			>0.99
0	16 (80%)	17 (85%)	
1	3 (15%)	3 (15%)	
2	1 (5%)	0 (0%)	
Risk factors for atony or hemorrhage			
Laboring	17 (85%)	14 (70%)	0.45
Oxytocin infusion>4 h	15 (75%)	13 (65%)	0.49
Failed operative delivery	0 (0%)	0 (0%)	
Magnesium infusion	5 (25%)	2 (10%)	0.41
Chorioamnionitis	2 (10%)	4 (20%)	0.66
Multiple gestation	2 (10%)	5 (25%)	0.41
Polyhydramnios	0 (0%)	1 (5%)	>0.99
Premature preterm rupture of membranes	2 (10%)	1 (5%)	>0.99
Preterm (<34 weeks GA)	3 (15%)	3 (15%)	>0.99
Prior postpartum hemorrhage	2 (10%)	3 (15%)	>0.99

Values as mean (standard deviation), median [interquartile range] and number (percentage).

Table 2
Tolerability: assessment of potential drug side effects experienced by study participants.

	Calcium Chloride ($n = 20$)	Placebo ($n = 20$)	P value
Any potential side effects	6 (30%)	6 (30%)	>0.99
Burning or discomfort in intravenous site	1 (5%)	0 (0%)	>0.99
Hypertension	0 (0%)	2 (10%)	0.49
Sinus tachycardia, sinus bradycardia, or other arrhythmia	3 (15%)	3 (15%)	>0.99
Nausea or vomiting	5 (25%)	2 (10%)	0.41
Other	0 (0%)	1 (5%)	1.0
Maximal increase in heart rate from baseline (bpm)	15.4 [8.8]	14.2 [7.7]	0.71
Maximal decrease in heart rate from baseline (bpm)	−19.1 [13.1]	−16.7 [14.4]	0.63
Maximal increase in mean arterial blood pressure (mmHg)	15.9 [12.4]	13.2 [10.5]	0.60
Maximal decrease in mean arterial blood pressure (mmHg)	−33.8 [14.8]	−32.0 [21.3]	0.76

Values are shown as number of patients (percentage of patients) and median value [interquartile range] P value for categorical values was calculated using a Fisher's exact test given sparseness of data. The P value for hemodynamic variables was calculated using a Wilcoxon rank sum test given the non-normal distribution of values.

3.3. Secondary outcomes

Secondary outcomes are shown in Table 3. Secondary outcomes that showed trends for improvement in the calcium compared to the placebo group which did not achieve statistical significance included EBL, uterine tone score as determined by the operating obstetrician at 10 min after cord clamping, and colloid requirement. Patients who received calcium chloride received less crystalloid than controls (Table 3). Hemodynamics, change in hematocrit from preoperative to postoperative day one values, and transfusion requirement did not differ between groups. One patient in each group required a blood transfusion; one placebo recipient for atonic postpartum hemorrhage, and one calcium recipient for a surgical left gonadal artery laceration causing retroperitoneal bleeding.

3.4. Calcium concentrations and pharmacokinetics

Twenty-four patients consented to serial phlebotomy for pharmacokinetic analysis (Fig. 2). Thirteen patients received calcium and 11 received placebo saline infusion. The baseline ionized calcium concentration was 1.18 (95% CI 1.16–1.19) mmol/L and did not differ between groups ($P = 0.82$). Ionized calcium rose to a peak value of 1.5–1.6 mmol/L in the treatment group. The clearance was 0.93 (95% CI 0.63–1.52) L/min. The volume of distribution was 76 L (95% CI 49–91 L). The median prediction error was 0% and the median absolute prediction error was 3%. The model was not improved by adjustment for pH (Fig. 2).

4. Discussion

In this pilot study, a randomized controlled trial study protocol investigating co-administration of 1-g intravenous calcium chloride with oxytocin during cesarean delivery was feasible, acceptable to patients, well-tolerated, and potentially effective. The preliminary efficacy data show that calcium warrants investigation as a novel agent for the prevention of uterine atony during high-risk cesarean delivery. The NNT with calcium to prevent one case of uterine atony in this small pilot study was estimated at just over 3 patients. There were no adverse

Table 3
Primary and secondary outcomes related to uterine atony and blood loss.

	Calcium Chloride ($n = 20$)	Placebo ($n = 20$)	P value
Uterine atony *(n)*	4 (20%)	10 (50%)	0.07
Estimated blood loss *(mL)*	750 [600–800]	850 [650–1000]	0.15
Obstetrician's assessment of uterine tone *(0–100)*	89 [80–90]	80 [75–89]	0.13
Change in hematocrit from preoperative to postoperative day 1 values (%)	7.7 (4.4)	6.7 (2.6)	0.41
Oxytocin			
Total bolus dose (units)	2 [2–2]	2 [2–4]	0.14
Maximal infusion rate (units/h)	7.5 [7.5–15]	15 [7.5–22.5]	0.41
Fluids and transfusion			
Crystalloid (mL)	1200 [1000–2000]	1750 [1500–2000]	0.03
Colloid (mL)	0 [0–0] range [0–500]	0 [0–0] range [0–1000]	0.08
Red blood cells (units)	0 [0–0]	0 [0–0]	0.30

Values as number (percentage), mean (standard deviation), or median [interquartile range]. Full range is also displayed and labeled as such for colloid volume. The primary outcome of uterine atony or hemorrhage was defined as requirement for a second line uterotonic, mechanical surgical intervention for atony, or estimated blood loss greater than one liter.

events, no hemodynamic differences, and no increase in side effects compared to the placebo, though this will need continued assessments in larger trials. One-compartment population pharmacokinetics were also established and demonstrated a rapid, supraphysiologic peak in ionized calcium concentration. These results strengthen the hypothesis that calcium may be a safe, efficacious adjuvant for preventing uterine atony during cesarean delivery.

There is biological plausibility for calcium as a prevention or treatment modality for uterine atony. *In vitro* studies have demonstrated that an intracellular rise in calcium ion levels is necessary for uterine myometrial contraction, and that influx from extracellular calcium stores provides at least some of this calcium [5]. For example, human *in vitro* studies have shown that, in the absence of extracellular calcium, the contractile response of myometrium to oxytocin is decreased compared to physiologic levels of extracellular calcium [10]. Multiple other *in vitro* studies have demonstrated a consistent decrease in myometrial contraction force and/or frequency in hypo-calcemic conditions [6–8]. *In vivo* animal models show that calcium chelation impedes uterine contractions [19]. Human studies have found that calcium levels rise during the third trimester of pregnancy, and women in spontaneous labor have higher serum calcium concentrations than those at a similar gestational age who are not laboring [12]. Further proof of principle can be extrapolated from the successful use of calcium channel blockers as tocolytic agents to halt preterm contractions [20].

One might then wonder why calcium is not already used for prevention or treatment of uterine atony. Calcium chloride has long been used to reverse the uterine relaxant effects of magnesium infusion in cases of uterine atony and is FDA-approved for reversal of magnesium toxicity in parturients [21]. However, several *in vitro* studies have shown that at some level, hypercalcemia may in fact prove detrimental to uterine contractility due to a membrane hyperpolarization effect [6,10]. The difficulty in extrapolating that *in vitro* data to clinical practice is in determining how calcium in a saline solution compares to calcium in the human bloodstream. Total serum calcium levels are divided into ionized (active) and unionized (protein bound) fractions, and this is difficult if not impossible to replicate in an organ tissue bath. For example, Talati and colleagues reported that oxytocin-naïve myometrial strips had superior contractile response to oxytocin in a normocalcemic bath than hypo- or hyper-calcemic baths [9]. However, the salt solutions they utilized contained calcium concentrations which closely mirror *total* blood calcium concentrations and not the biologically-active *ionized* calcium concentration in the human body. As such, one might interpret their experimental conditions to represent normocalcemia, hypercalcemia, and extreme hypercalcemia rather than hypocalcemia, normocalcemia, and hypercalcemia when applied to *in vivo* human physiology. Clinical studies are essential to determine the optimal serum calcium concentration for uterine contractility.

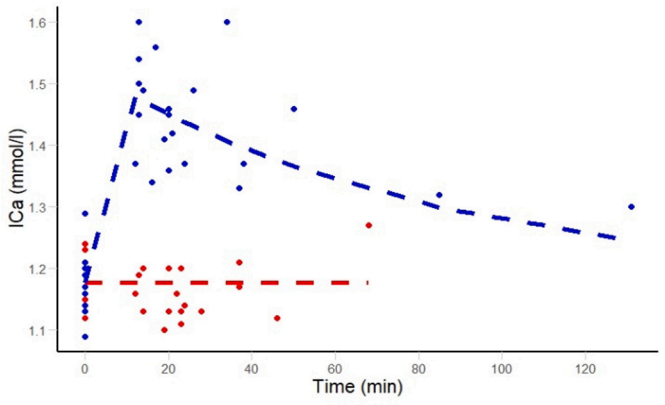

Fig. 2. Pharmacokinetics of intravenous calcium chloride infusion in parturients.

In the only published human clinical study investigating the role of calcium at the time of delivery, Farber and colleagues investigated the hemodynamic effects of administration of 0, 200 or 400 mg calcium chloride with a 5 U oxytocin bolus at the time of elective cesarean delivery [13]. The authors found no differences in the uterine tone or EBL among groups; however, these measures were secondary outcomes and the overall incidence of uterine atony was low as anticipated during elective cesarean delivery. Furthermore, the serum concentration of ionized calcium were still within the normo-calcemic range for all three intervention groups. Our study addressed these limitations by utilizing a higher dose of calcium chloride, examining a patient cohort at high risk for uterine atony, and studying uterine atony as a primary efficacy-related outcome.

The results of this pilot study should be interpreted with caution, as there are several limitations to our design. Most importantly, positive pilot studies require confirmation in larger studies. The possibility remains that calcium would prove ineffective in larger studies, or that the effect size would prove less impressive. As such, no definitive recommendations can be made regarding the use of calcium for the prevention of uterine atony at this time.

The outcome metrics utilized are additional study limitations. Our primary outcome measure was a composite binary outcome defined as the incidence of uterine atony. The composite outcome was used over rare singular outcomes such as death, transfusion, and intensive care unit admission to encompass most clinical indicators of hemorrhage. We acknowledge that some of the outcomes used in the study such as the decision to administer second-line uterotonics are of a subjective nature. In addition, multiple obstetricians assessed uterine tone, which decreases consistency but increases the generalizability of this metric. However, randomization was employed to account for the subjective influence in these outcomes. The study population chosen was women at

high risk for uterine atony during cesarean delivery, and applicability to lower risk populations will need to be studied. Similarly, this study queried the use of calcium as a uterine atony preventive measure, and applicability as a treatment modality also warrants independent investigation.

The patients who participated in our pilot study did not experience any adverse effects or differences in side effects compared to the control group. However, intravenous calcium chloride has the potential to cause adverse events including severe hemodynamic shifts, bradycardia, and even rare cardiac arrest if administered rapidly. It may also cause peripheral intravenous line discomfort and skin necrosis if extravasated [21]. In our study, calcium infusion was administered slowly per package insert recommendations, at infusion rate 100 mg/min and in dilute saline solution, and was very well-tolerated [21]. The concerns for extravasation were low as all study subjects were awake, sensate in the upper extremity of infusion, and had already received multiple medications and intravenous fluids through that intravenous cannula at the start of study drug infusion. Extreme caution should be utilized if infusing calcium in patients received general anesthesia or below the level of a surgical neuraxial block.

In conclusion, an infusion of dilute calcium chloride in addition to standard oxytocin warrants definitive study as a uterine atony preventive modality in parturients at high risk for uterine atony. The population pharmacokinetic profile and absence of significant side effects justify a one-gram dosing strategy. Our findings, if replicated in an adequately-powered trial, would suggest that calcium chloride, an inexpensive and shelf-stable medication, may be efficacious for prevention of uterine atony.

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Details of author contributions

Jessica Ansari: This author's roles included concenptualization, methodology, project administration (patient enrollment), data curation, formal analysis, and writing-original draft.

Neil Kalariya: This author's roles included project administration (enrollment), formal analysis, and writing-review.

Brendan Carvalho: This author's roles included supervision, conceptualization, and writing-review.

Pamela Flood: This author's roles included formal analysis, supervision, and writing-review.

Nan Guo: This author 's role included formal analysis and writing-review.

Edward Riley: This author's roles included supervision, conceptualization, and writing-review.

Data availability

Supplementary data that the data will be provided to the readers on request.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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