

In Vitro Comparative Effect of Carbetocin and Oxytocin in Pregnant Human Myometrium with and without Oxytocin Pretreatment

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ABSTRACT

Background: The purpose of this study was to compare *in vitro* contractile effects of oxytocin and carbetocin on human term pregnant myometrium with and without oxytocin pretreatment.

Methods: This laboratory investigation was conducted on myometrial samples from women undergoing elective cesarean deliveries. The samples were dissected into four strips and suspended in individual organ bath chambers containing physiologic salt solution. After equilibration, they were pretreated with oxytocin 10^{-5} M (experimental group) or physiologic salt solution (control group) for 2 h and then subjected to dose-response testing with increasing concentrations of oxytocin or carbetocin (10^{-10} to 10^{-5} M). The amplitude, frequency, motility index (amplitude \times frequency), and area under the curve of contractions were recorded and analyzed during the equilibration and dose-response periods. Comparisons were made between oxytocin-induced and carbetocin-induced contractions in control and oxytocin-pretreated groups. Motility index was the primary outcome measure.

Results: Sixty-three experiments were performed (carbetocin, $n = 31$; oxytocin, $n = 32$) on samples from 18 women. The motility index of contractions ($\sqrt{\text{g. contractions}/10 \text{ min}}$) produced by oxytocin was significantly higher than carbetocin in both control (regression-estimated difference, 0.857; 95% CI, 0.290 to 1.425; $P = 0.003$) and oxytocin-pretreated (0.813; 0.328 to 1.299; $P = 0.001$) groups. The motility index was significantly lower in oxytocin-pretreated groups than their respective controls for both oxytocin (-1.040 ; -1.998 to -0.082 ; $P = 0.03$) and carbetocin (-0.996 ; -1.392 to -0.560 ; $P < 0.001$).

Conclusions: *In vitro* contractions produced by oxytocin are superior to carbetocin in human myometrium with or without oxytocin pretreatment. Oxytocin pretreatment results in attenuation of contractions induced by both oxytocin and carbetocin. (ANESTHESIOLOGY 2016; 124:378-86)

OXYTOCIN remains the first-line uterotonic agent for the prevention and treatment of postpartum hemorrhage (PPH) secondary to uterine atony. Despite its routine use worldwide, oxytocin has limitations that include hemodynamic side effects and need for infusion dosing due to its short half-life.¹⁻³ Furthermore, it has reduced effect in women who have undergone labor augmentation.^{4,5} This has led to a search for newer agents for the prevention of PPH with greater efficacy and fewer side effects.

Carbetocin (1-deamino-1-carba-2-tyrosine(*O*-methyl)-oxytocin), a long-acting synthetic analog of oxytocin, was developed as an alternative to oxytocin and has been used in clinical practice for the last 2 decades. The Society of Obstetricians and Gynecologists of Canada recently updated their practice guideline to specifically recommend carbetocin over oxytocin infusion for the prevention of PPH during elective cesarean deliveries (CDs).⁶

What We Already Know about This Topic

- Despite routine use of oxytocin to prevent and treat postpartum hemorrhage secondary to uterine atony, it has limitations, including a short half-life
- Carbetocin is a long-acting synthetic analog of oxytocin that has been used in clinical practice
- Data comparing the abilities of carbetocin and oxytocin to prevent and treat postpartum hemorrhage are inconsistent, and *in vitro* myometrial contraction data are limited

What This Article Tells Us That Is New

- Oxytocin produced stronger contractions of term pregnant human myometrium *in vitro* than did carbetocin over the entire range of equimolar concentrations studied
- Oxytocin pretreatment of term pregnant human myometrium *in vitro* attenuated contractions produced by both oxytocin and carbetocin

This article received the first place award in the Gertie Marx Research Competition at the 44th Annual Meeting of the Society for Obstetric Anesthesia and Perinatology, Monterey, California, May 2-5, 2012, and also in the Richard Knill Competition at the Canadian Anesthesiology Society Meeting, Quebec City, Quebec, Canada, June 15-18, 2012. Dr. Cole contributed to patient recruitment, experimentation, and as author of first draft; Dr. Carvalho contributed to conception and design of the study and critical manuscript revisions; Ms. Erik-Soussi and Dr. Ramachandran contributed to patient recruitment, experimentation, and manuscript revisions; and Dr. Balki contributed to conception and design of the study, and writing and revision of the manuscript.

Submitted for publication December 2, 2014. Accepted for publication September 29, 2015. From the Department of Anesthesia and Pain Management (N.M.C., J.C.A.C., M.E.-S., N.R., M.B.) and Department of Obstetrics and Gynecology (J.C.A.C., M.B.), Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

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One of the potential advantages of carbetocin over oxytocin is its longer half-life (4 to 10 times that of oxytocin), allowing for single bolus dosing rather than the continuous infusion or multiple doses of oxytocin in the postpartum period.^{7,8} Despite limited data, it appears to have a similar side effect profile to that of oxytocin.⁹ The data on the comparative effects of carbetocin *versus* oxytocin for the prevention and treatment of PPH are inconsistent in the existing literature.^{6,10–14} The limited evidence based on *in vitro* myometrial contractility studies in rats and humans suggests that carbetocin is less potent than oxytocin, thus requiring dose adjustments for clinical efficacy.^{15–17}

Oxytocin preexposure has been shown to cause desensitization of oxytocin receptors *in vitro* in cultured human myocytes¹⁸ and to attenuate oxytocin-induced myometrial contractions in pregnant rats¹⁹ and humans.^{20,21} This phenomenon is likely responsible for the high incidence of uterine atony and PPH in women with oxytocin-augmented labors.^{5,22,23} None of the existing clinical or *in vitro* studies have yet examined the effect of oxytocin preexposure on contractile efficacy of carbetocin.

The purpose of this study was to compare the effects of oxytocin and carbetocin in human term pregnant myometrium *in vitro*, with and without oxytocin pretreatment. We hypothesized that oxytocin would induce stronger contractions than carbetocin and that oxytocin pretreatment would attenuate the myometrial contractility induced by both drugs.

Materials and Methods

After approval by the Research Ethics Board, Mount Sinai Hospital, Toronto, Ontario, Canada (Research Ethics Board No.: 11-0257-E, dated December 6, 2011), this prospective laboratory study was conducted at Mount Sinai Hospital and the Samuel Lunenfeld Research Institute in Toronto, Ontario, Canada, from December 30, 2011 to September 3, 2013 (ClinicalTrials.gov registry NCT01689298; principal investigator: M.B., Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada). The study included nonlaboring singleton pregnant women at 37 to 41 weeks gestational age undergoing elective primary or first repeat CD under spinal anesthesia. Written informed consent was obtained from all women enrolled in the study. Exclusion criteria were laboring women, those with previous multiple CDs or other myometrial surgeries, CD under general anesthesia, placental abnormalities, and other risk factors for PPH.

Anesthetic Technique, Tissue Collection, and Preparation

The attending anesthesiologist administered spinal anesthesia using 0.75% hyperbaric bupivacaine 1.8 ml with fentanyl 10 µg and epimorph 100 µg, as per routine in our hospital with standard monitoring. The obstetrician performed the CD *via* Pfannenstiel incision. After delivery of the fetus and placenta, a small sliver of myometrium (approximately 2 cm × 1 cm × 0.5 cm; weight 0.02 g) was excised from the upper

margin of the lower segment transverse uterine incision, just before the administration of oxytocin. The uterus was then closed in a routine manner. The collected specimen was immediately placed in 3-*N*-morpholino propanesulfonic acid buffer solution (145 mM NaCl, 4.7 mM KCl, 1.5 mM CaCl₂, 1.17 mM MgSO₄·7H₂O, 1.2 mM NaH₂PO₄·H₂O, 3.0 mM 3-*N*-morpholino propanesulfonic acid solution, 5.0 mM glucose, and 2.0 mM pyruvate) with pH 7.4. The endometrium and serosa were discarded if seen during dissection. The myometrial sample was then divided longitudinally into four strips of equal area, 10 mm × 2 mm × 2 mm each, parallel to the direction of the muscle fibers.

Isometric Tension Recordings of Contractility

As previously described,^{20,21} for the contractility analysis, the myometrial strips were mounted individually in four organ bath chambers (Radnoti four unit tissue-organ bath system, model 159920; Harvard Apparatus Canada, Canada) filled with physiological salt solution (PSS) (112 mM NaCl, 25 mM NaHCO₃, 1 mM KH₂PO₄, 5 mM KCl, 1.2 mM MgSO₄·7H₂O, 11.5 mM glucose, and 2.5 mM CaCl₂) at 37°C and pH 7.4, to mimic physiological conditions. The organ bath solution was aerated continuously with a mixture of 95% O₂ and 5% CO₂ (carbogen). An initial resting tension of 1 g was applied to each strip. The myometrial strips were allowed to equilibrate until 25 min of continuous, spontaneous rhythmic contractions developed (usually within 2 h of mounting). If the spontaneous contractions did not start immediately, the PSS was changed by flushing every 10 to 15 min until spontaneous contractions were observed. After equilibration, the myometrial strips were stimulated with 120 mM KCl to provide both viability verification of the strips and a reference maximal contraction for analysis. The KCl solution was drained from the organ baths, and any residual solution was removed by washing three times with PSS.

Two of the four myometrial strips were pretreated with oxytocin 10⁻⁵ M (experimental group) and the other two were bathed in PSS (control group) for 2 h as a proxy for the laboring and nonlaboring uterus, respectively. This method is based on the tested model of our earlier study, which showed a significant attenuation of oxytocin-induced myometrial contractions after pretreatment with oxytocin 10⁻⁵ M for 2 h.²⁰ After the pretreatment period, the tissues were flushed three times to wash off all oxytocin in the bath. Thereafter, two strips, one from the experimental (oxytocin pretreated) and one from the control group, were subjected to dose–response testing with increasing concentrations of carbetocin in a pattern of 1 log molar increase every 10 min, from 10⁻¹⁰ to 10⁻⁵ M. The remaining two strips were similarly tested with increasing concentrations of oxytocin from 10⁻¹⁰ to 10⁻⁵ M (fig. 1). Myometrial contractions were continuously recorded using an isometric force transducer connected to a data acquisition system with AcqKnowledge® 3.9.0 software, MP 100 (Biopac System Inc., USA). At the

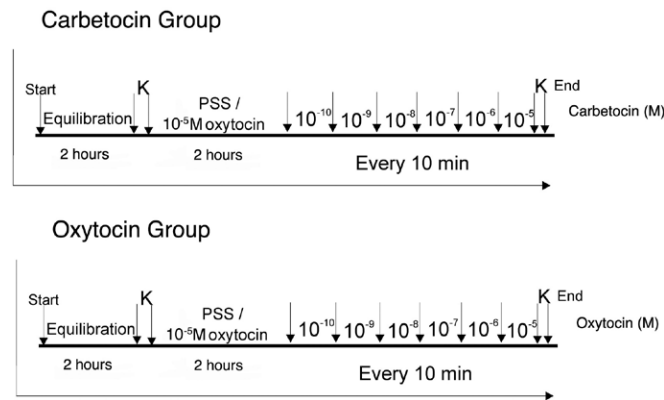


Fig. 1. Experimental design. Myometrial strips were bathed in physiologic salt solution (control groups) or oxytocin 10^{-5} M (experimental groups) for 2 h and then subjected to a dose-response testing with increasing concentrations of carbetocin or oxytocin in a pattern of 1 log molar increase every 10 min from 10^{-10} to 10^{-5} M. K = potassium; PSS = physiologic salt solution.

end of the experiment, a final stimulation with 120 mM KCl confirmed the viability of the myometrial strips (fig. 1).

The amplitude (g) and frequency (number of contractions in 10 min) of contractions were recorded for 1,500 s during the equilibration period and for 600 s during each step of the dose-response period. The motility index (amplitude \times frequency; g.contractions/10 min) and the area under the curve (integral force; g.seconds) were calculated to determine the uterine activity and the strength of contractions, respectively.²⁰ The contractile parameters were compared among the groups.

The primary outcome was the motility index of myometrial contractions induced by oxytocin or carbetocin. Secondary outcomes included all other contractile parameters.

Statistical Analysis

The data were analyzed with linear regression models (maximum likelihood method for parameter estimations) and adjusted for repeated measures per sample (*i.e.*, measurements at multiple concentrations) through a compound symmetry covariance structure. A compound symmetry covariance structure assumes that all observations on a given subject are equally correlated with each other. Generalized estimating equations were generated including the type of drug and patient group (control and experimental) during the dose-response period. Generalized estimating equation models allow for an unequal number of observations on each subject and reweights observations through a covariance structure to obtain a balanced regression model in which each subject has the same statistical weight, regardless of the number of observations. The outcome parameters were square-root transformed to adjust for their skewed distribution.^{20,21} The models determine the effect of increasing concentration stratified by drug and were adjusted using various covariates such as patient characteristics (age, body mass index, and gestational age), baseline tone and contraction parameters (amplitude, frequency, area under the curve, and motility index) during equilibration, maximum amplitude after KCl

given at the beginning and end of the dose-response testing, and dry weight of sample. The values were expressed as predicted mean and standard error. The half maximal effective concentration (EC₅₀) was estimated based on pharmacodynamic modeling (four-parameter dose-response curve) and compared across various groups. Statistical analysis was carried out using SAS statistical software version 9.2 (The SAS Institute, USA), and the statistician was blinded to various study groups. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Based on our previous studies, a sample size of 9 to 12 patients per drug group was considered sufficient for this study.^{20,21}

Results

Fifty-six women were approached for participation in the study and 20 of them consented, yielding 20 myometrial samples. Each myometrial sample was divided into four strips for a total of 80 strips. Of these 80 experiments, 17 were excluded for failure to contract and human/apparatus error, resulting in 63 successful experiments with similar distribution of strips across the four study groups. The study flow chart with patient recruitment and sample distribution is shown in figure 2.

The clinical characteristics of the participants are shown in table 1. The mean (SD) age of the patients was 36.1 (2.8) yr, body mass index was 28.7 (4.9) kg/m², and gestational age at delivery was 39.1 (range, 37.4 to 40.9) weeks. None of the participants experienced any complications including PPH up to 24 h postpartum.

The baseline values of contractile parameters during equilibration in all treatment groups, along with mean sample weights, are shown in table 2. Table 3 shows the contractile parameters induced by increasing concentrations of oxytocin and carbetocin in both control and experimental groups. After adjustment for all covariates, in the control groups, the predicted mean (standard error) motility index of contractions produced by oxytocin (3.01 [0.36] $\sqrt{\text{g.contractions/10 min}}$)

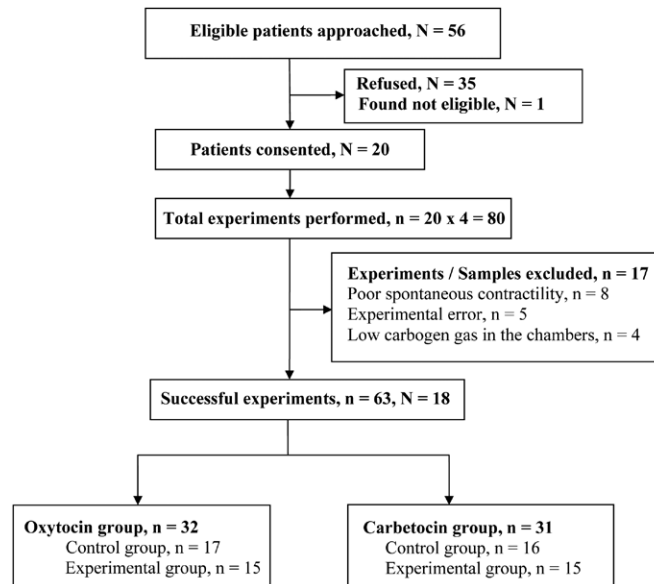


Fig. 2. Flowsheet demonstrating patient recruitment and subsequent allocation of myometrial strips to the study groups. Control group = not pretreated with oxytocin; experimental group = oxytocin pretreated; N = number of patients; n = number of myometrial samples.

Table 1. Patient Characteristics

Characteristics	N = 18
Maternal age (yr)*	36.1 (2.8)
BMI (kg/m ²)*	28.7 (4.9)
Gestational age (weeks)†	39.1 (37.4–40.9)
Gravida	
1	5 (27%)
2	9 (50%)
≥ 3 (range, 3–6)	4 (23%)
Para	
0	4 (22%)
1	10 (56%)
2	4 (22%)
Primary CD	6 (33%)
Indication for CD	
Breech	5 (27%)
Repeat	11 (61%)
Fetal congenital anomalies	1 (6%)
Genital herpes	1 (6%)
Need for additional uterotonic agents	0 (0%)
PPH within 24 h	0 (0%)

N is the number of patients (data obtained from these patients were used for the final analysis, patients with excluded samples or experiments are not included in this table).

* Values presented are mean (SD), † mean (range), or N (%).

BMI = body mass index; CD = cesarean delivery; PPH = postpartum hemorrhage.

across all concentrations from 10^{-10} to 10^{-5} M was found to be superior to that of carbetocin ($2.16 [0.14] \sqrt{\text{g.contractions/10 min}}$) (regression-estimated difference, $0.857 [95\% \text{ CI}, 0.290 \text{ to } 1.425] \sqrt{\text{g.contractions/10 min}}$; $P = 0.003$). Similarly, oxytocin-pretreated experimental strips produced stronger motility index of contractions with oxytocin ($1.97 [0.20] \sqrt{\text{g.contractions/10 min}}$) compared with

carbetocin ($1.16 [0.22] \sqrt{\text{g.contractions/10 min}}$) during the dose-response period ($0.813 [0.328 \text{ to } 1.299] \sqrt{\text{g.contractions/10 min}}$; $P = 0.001$).

Oxytocin pretreatment resulted in a significant reduction in motility index of contractions in the oxytocin group ($-1.040 [-1.998 \text{ to } -0.082] \sqrt{\text{g.contractions/10 min}}$; $P = 0.03$) as well as in the carbetocin group ($-0.996 [-1.392 \text{ to } -0.560] \sqrt{\text{g.contractions/10 min}}$; $P < 0.001$) (table 3) compared with their respective controls.

The estimated EC₅₀, based on pharmacodynamic modeling, showed a similar trend in the findings although the differences between the groups were not significant. The EC₅₀ (95% CI) for oxytocin was $10^{-7.3}$ M ($10^{-7.7}$ to $10^{-6.8}$ M) in control and $10^{-6.5}$ M ($10^{-7.1}$ to $10^{-5.9}$ M) in experimental group, whereas that for carbetocin was $10^{-8.9}$ M ($10^{-13.0}$ to $10^{-4.7}$ M) for control and $10^{-7.0}$ M ($10^{-11.1}$ to $10^{-3.0}$ M) for experimental group.

The dose-response curves of motility index for both oxytocin and carbetocin demonstrated a statistically significant gradient at concentrations 10^{-8} , 10^{-9} , and 10^{-10} M. The concentration in the motility index model has a chi-square value of 13.4 (degrees of freedom = 5) and $P = 0.02$. The square-root transformation makes curves appear flatter than they actually are.

The primary regression models for this study treated both study drug and study group as the two independent bivariable variables rather than a single variable with four distinct study groups. The pairwise inferences (described in the Results section) are derived from the contrasts of these two separate variables. Considering the parameterization approach, a P value adjustment was not necessary. However, recognizing that a different approach would have required adjustment, we reran the model using a four group design with

Table 2. Baseline Data during Equilibration

Covariates	Oxytocin Control (n = 17)	Oxytocin Experimental (n = 15)	Carbetocin Control (n = 16)	Carbetocin Experimental (n = 15)
Basal tone (g)	0.99 (0.44)	0.91 (0.46)	0.66 (0.38)	0.77 (0.38)
Amplitude (g)	1.83 (0.56)	1.82 (0.61)	1.49 (0.64)	1.80 (0.99)
Frequency (contractions/10 min)	6.54 (4.72)	6.55 (4.57)	7.22 (3.98)	6.72 (2.87)
Motility index (grams.contractions/10 min)	12.53 (11.54)	12.25 (10.94)	11.26 (10.00)	12.66 (11.45)
Area under the curve (grams.seconds)	2,738.88 (832.86)	2,737.20 (907.91)	2,240.62 (956.53)	2,700.19 (1,478.73)
ΔT (s)	1,500.69 (0.52)	1,500.50 (0.33)	1,500.56 (0.4)	1,500.56 (0.46)
KCl				
Max amplitude 1 (g)	4.94 (2.95)	6.02 (2.83)	5.65 (2.46)	5.77 (3.67)
Max amplitude 2 (g)	5.44 (2.91)	5.01 (2.74)	5.78 (2.51)	4.94 (3.00)
Sample weight (g)	0.02 (0.00)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)

Values are expressed as mean (SD). The data represent contractile parameters measured during the equilibration period before the administration of any drug. Max amplitude 1 = maximum amplitude after KCl administered before the preexposure period; Max amplitude 2 = maximum amplitude after KCl administered at the end of dose-response; n = number of experiments; ΔT = duration for equilibration period.

Table 3. Contractile Parameters in Control and Experimental Drug Groups

Parameters	Experimental	Control	Experimental vs. Control	P Value
Amplitude (g)				
Oxytocin	1.34 (0.03)	1.49 (0.06)	−0.150 (−0.304 to 0.004)	0.06
Carbetocin	1.22 (0.03)	1.31 (0.02)	−0.188 (−0.289 to −0.088)	< 0.001
Frequency (contractions/10 min)				
Oxytocin	1.47 (0.10)	1.87 (0.18)	−0.407 (−0.874 to 0.061)	0.09
Carbetocin	1.08 (0.12)	1.59 (0.07)	−0.516 (−0.755 to −0.278)	< 0.001
Motility index (grams.contractions/10 min)				
Oxytocin	1.97 (0.20)	3.01 (0.36)	−1.040 (−1.998 to −0.082)	0.03
Carbetocin	1.16 (0.22)	2.16 (0.14)	−0.996 (−1.392 to −0.560)	< 0.001
Area under the curve (grams.seconds)				
Oxytocin	31.9 (0.8)	35.5 (1.6)	−3.587 (−7.465 to 0.291)	0.07
Carbetocin	27.1 (0.9)	31.6 (0.7)	−4.532 (−6.967 to −2.097)	< 0.001

All values are expressed in square-root units. Values are expressed as estimated mean (standard error) and estimated difference (95% CIs). Values are presented as summation of measurements over the range of concentrations from 10^{-10} to 10^{-5} M during the dose-response period.

Dunnett–Hsu adjustment and found the same results. The box plots in figure 3 show the motility index of carbetocin-induced and oxytocin-induced contractions in oxytocin-pretreated and control groups.

Discussion

The results of our *in vitro* study show that in term pregnant human myometrium with no prior *in vivo* exposure to exogenous oxytocin, oxytocin produces stronger contractions than carbetocin over the entire studied range of equimolar concentrations and that oxytocin pretreatment attenuates the contractions produced by both drugs.

In vitro studies comparing carbetocin and oxytocin are scarce in the literature, with only three studies published so far: two in rat^{15,16} and one in human myometrial samples.¹⁷ Atke and Vilhardt¹⁵ demonstrated in nonpregnant rat myometrium that the uterotonic activity of carbetocin was approximately 30 times smaller than that of oxytocin. Engström *et al.*¹⁶ compared carbetocin and oxytocin over a dose-response range of 10^{-9} to 10^{-6} M in nonpregnant rat myometrium and found the maximum contractile effect

of carbetocin was approximately 50% lower than that of oxytocin. However, these studies in nonpregnant myometrium do not account for pregnancy-associated changes in oxytocin signaling or receptor modulation. Norström *et al.*¹⁷ demonstrated the need for a fivefold higher concentration of carbetocin than oxytocin (10×10^{-10} M vs. 2×10^{-10} M, respectively) to produce defined contractions in human term pregnant myometrium; however, the study did not quantitatively measure the strength of these contractions. Of note, the sample size was small in all three studies (four to six cases each) and the concentration of treatment drugs varied across studies, making it difficult to quantify their effects on contractility. Moreover, the effect of oxytocin-induced desensitization on contractions was not examined.

Our findings are in agreement with these previous studies, showing that oxytocin produces stronger contractions than carbetocin at similar concentrations. Furthermore, our study design and larger sample size allowed us to compare the effects of carbetocin and oxytocin in pregnant human myometrium over a wide range of similar concentrations using

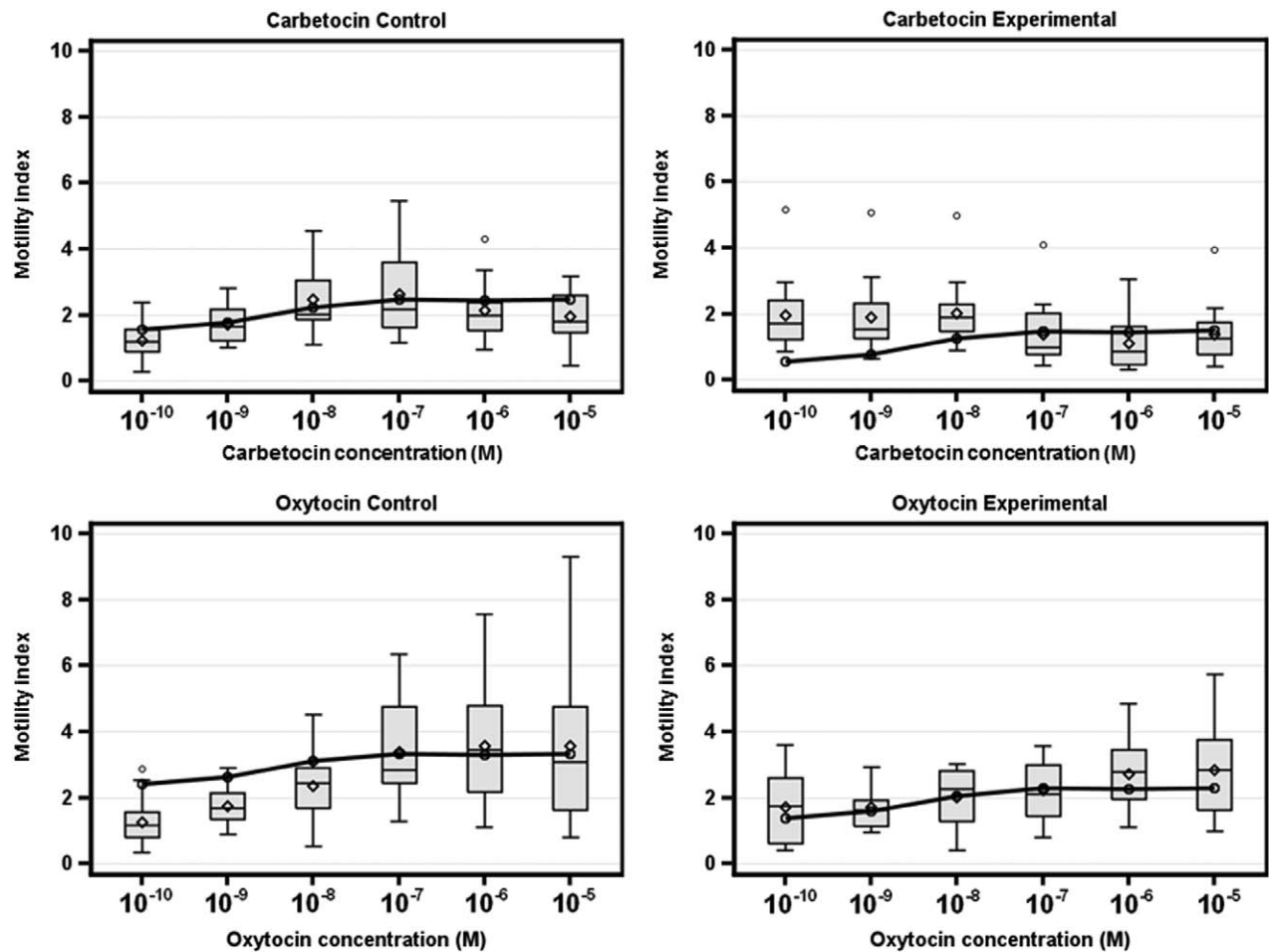


Fig. 3. Dose-response curves of motility index ($\sqrt{\text{g.contractions/10 min}}$) in control and experimental groups for carbetocin and oxytocin. The curves are from regression models, whereas the box plots are from the actual data.

multiple contractile parameters and evaluate the effect of oxytocin pretreatment on the uterotonic activity of both drugs.

Existing clinical evidence supports the efficacy of carbetocin for the prevention of PPH in CD, but research supporting this evidence is limited and requires further validation.^{10,14} In 2009, the Society of Obstetricians and Gynecologists of Canada revised their guideline for the prevention and treatment of PPH in elective CD, recommending carbetocin 100 μg given as an IV bolus over 1 min rather than continuous oxytocin infusion.⁶ More recently in 2012, the *Cochrane Database of Systematic Reviews*¹⁰ studied the 11 existing clinical trials comparing carbetocin to conventional uterotonics (oxytocin or syntometrine) or placebo for prevention of PPH. Out of these trials, four studies compared carbetocin to oxytocin at CD, demonstrating that carbetocin was associated with a decreased need for additional uterotonics (relative risk [RR], 0.62; 95% CI, 0.44 to 0.88), reduced need for uterine massage (RR, 0.54; 95% CI, 0.37 to 0.79), and reduced risk of PPH (RR, 0.55; 95% CI, 0.31 to 0.95), but no significant difference in the average estimated blood loss.^{7,11–13} As noted by the authors, this review was limited by several potential sources of bias, a small number of included

studies, and heterogeneity of study populations across the trials. Furthermore, nonuniform oxytocin dosing was compared with a standard dose of carbetocin administered intravenously or intramuscularly in these studies.¹⁰

Since the publication of the 2012 *Cochrane Review*,¹⁰ three additional clinical trials have been published comparing carbetocin and oxytocin.^{24–26} Two of these studies showed a decreased need for additional uterotonic drugs with carbetocin *versus* oxytocin at CD, similar to the Cochrane study.^{24,25} The third study, however, found no difference between the two drugs in composite variable endpoint of blood loss above 1,500 ml, transfusion, hemoglobin reduction of 4 g/dl or more, or operative intervention.²⁶ As in prior trials, heterogeneity among these studies and their study populations as well as overall lack of randomization make the results difficult to interpret conclusively.

Although the literature comparing carbetocin to oxytocin is modest, an apparent contradiction is emerging between existing *in vivo* and *in vitro* evidence. Clinical studies suggest carbetocin may be either equally or more effective than oxytocin at elective CD,^{7,11,13,24–26} whereas laboratory studies, including the current study, have shown that carbetocin

appears to be less potent than oxytocin at similar concentrations. This paradox could be related to the use of nonequivalent doses of these drugs in clinical practice. Although the relative potencies of these drugs, when administered at delivery, have not been described so far, there is evidence that carbetocin 100 µg produces similar contractions to oxytocin 10 µg (equivalent to 5 U).²⁷

An alternative explanation for the discrepancy between *in vitro* and *in vivo* studies of carbetocin versus oxytocin might be related to the pharmacokinetics of carbetocin. Its increased lipophilicity,¹⁵ tissue distribution, and resistance to peptidase degradation contribute to a prolonged elimination half-life *in vivo*.⁸ The extended action of carbetocin may, in turn, decrease the need for additional uterotonics, thereby reducing the risk of uterine atony that accompanies the use of additional uterotonics.

Oxytocin receptor desensitization after oxytocin preexposure is an important phenomenon that has been well documented in the literature both *in vivo* and *in vitro* in human myometrium.^{18,28,29} The mechanism of desensitization is not fully understood, but it is known to involve receptor down-regulation as well as a decrease in oxytocin receptor mRNA expression and oxytocin-binding sites.^{18,30} The end result after prolonged exposure to oxytocin may be the diminished tissue responsiveness to additional oxytocin and poor myometrial contractility.^{18,20} This was demonstrated in the contractility studies by Balki *et al.*,²⁰ which found attenuation of oxytocin-induced contractions after pretreatment with oxytocin 10^{-5} M for at least 2 h. This phenomenon has been supported by poor uterine contractility and increased blood loss at delivery after administration of high doses or prolonged exposure to oxytocin during labor.^{4,21,22,31}

The effect of oxytocin preexposure on carbetocin-induced contractions was largely unknown until recently. Our *in vitro* study suggests that, similar to oxytocin, the attenuation of myometrial contractions after oxytocin preexposure is also seen with carbetocin. Because carbetocin is a partial oxytocin receptor agonist/antagonist^{8,16} with similar binding affinity as oxytocin,¹⁶ oxytocin receptor desensitization may be the underlying mechanism for these observations with clinical implications that may be extrapolated. If carbetocin-induced contractility is attenuated by oxytocin preexposure in the clinical setting, similar concentrations of carbetocin may be less effective to prevent PPH in women with prolonged or oxytocin-augmented labor compared with women undergoing elective CD, and these women may require dose adjustments. However, the lack of monotonic response with carbetocin in laboring women and the high rate of arrhythmias with doses higher than 120 µg questions the utility of this drug in laboring women.³²

There are some limitations to this study. Although we found significant differences in motility index between control and oxytocin-pretreated groups, such differences were not found to be significant when compared through EC₅₀, despite a similar trend in the findings. Hence, we suggest that

our observations should be interpreted with caution. Furthermore, experiments performed *in vitro* may not account for multiple clinical variables affecting myometrial contractility and blood loss. However, the absence of these external variables allows for a clear comparison of carbetocin and oxytocin in human myometrium while minimizing confounders. Direct translation of the concentrations used in this study to clinical doses may not be possible without further studies. We chose these concentrations based on standard concentrations used in most dose-response studies in the literature and our previous research findings.^{18,20,21} Oxytocin plasma levels have been determined at delivery, but a wide range has been measured during labor, between 10^{-12} and 10^{-8} M.^{33–36} In elective CD, Yamaguchi *et al.*³⁷ observed plasma levels of 1.16×10^{-9} M, 4.07×10^{-10} M, and 6.15×10^{-9} M after oxytocin infusions of 10 U/30 min (0.33 U/min), 10 U/3 min:45 s (2.67 U/min), and 80 U/30 min (2.67 U/min), respectively. They suggested that plasma oxytocin concentrations may not accurately correlate with uterine activity, with local uterine concentrations possibly correlating more directly. Serum levels of carbetocin during labor and delivery have yet to be studied. Finally, similar to oxytocin, biomolecular studies may be required with carbetocin to understand the underlying mechanisms of our results and to confirm whether the attenuation of carbetocin-induced myometrial contractions after oxytocin preexposure is related to the oxytocin desensitization phenomenon.

In summary, we have compared the contractile effects of carbetocin and oxytocin in human pregnant myometrium *in vitro* and demonstrated that oxytocin is superior to carbetocin in producing contractions at similar concentrations. We have further demonstrated a reduction in contractility induced by oxytocin after oxytocin preexposure, with an even greater reduction in carbetocin-induced contractility after oxytocin preexposure, likely as a function of the oxytocin desensitization phenomenon. It is important to note that the half-life, protein binding, and effect on the receptor are significant clinical parameters that modulate each drug's indications. Additional studies are warranted for clinical correlation with these findings.

Acknowledgments

The authors thank Stephen Lye, Ph.D., and Lee Adamson, Ph.D., Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada, for their guidance and collaborative support during the experiments. The authors also thank Cedric Manlhiot, Ph.D., Department of Pediatrics, Labatt Family Heart Centre and Hospital for Sick Children, Toronto, Ontario, Canada, for lending his statistical expertise to the data analysis.

This study was supported by Merit Awards from the Department of Anesthesia and Pain Management, University of Toronto, Toronto, Ontario, Canada.

Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available from Dr. Balki: mrinalini.balki@uhn.ca. Raw data available from Dr. Balki: mrinalini.balki@uhn.ca.

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Address correspondence to Dr. Balki: Department of Anesthesia and Pain Management, Mount Sinai Hospital, Room 19–104, 600 University Avenue, Toronto, Ontario, Canada M5G 1X5. mrinalini.balki@uhn.ca. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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