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REPORTS OF ORIGINAL INVESTIGATIONS

Effect of magnesium sulfate on oxytocin-induced contractility in human myometrium: an *in vitro* study

Effets du sulfate de magnésium sur la contractilité du myomètre humain induite par l'ocytocine: une étude *in vitro*

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Abstract

Purpose *The purpose of this study was to determine the contractile patterns induced by oxytocin in myometrium exposed to magnesium sulfate ($MgSO_4$). We hypothesized that $MgSO_4$ pretreatment would reduce oxytocin-induced myometrial contractions in both oxytocin-naïve and oxytocin-desensitized myometrium.*

Methods *In this prospective in vitro study, myometrial samples were obtained from 26 women undergoing elective Cesarean deliveries. Samples were divided into six groups. Four groups were apportioned to no pretreatment (control group), oxytocin 10^{-5} M pretreatment (desensitization group), $MgSO_4$ 3.5 mM pretreatment, and $MgSO_4$ 3.5 mM + oxytocin 10^{-5}M pretreatment. This was followed by dose-response testing to oxytocin 10^{-10} to 10^{-5}M in all four groups. Two additional groups included $MgSO_4$ 3.5 mM pretreatment and $MgSO_4$ 3.5 mM + oxytocin 10^{-5} M pretreatment, followed by dose-response testing to oxytocin along with $MgSO_4$ 3.5 mM. The outcomes were motility index (MI), as defined by the amplitude (g) × frequency of myometrial contractions (c) over ten minutes, and area under the curve (AUC).*

Results *In oxytocin-naïve myometrium, the mean (standard error [SE]) MI was not affected by $MgSO_4$ pretreatment [3.31 (0.20) $\sqrt{g \cdot c/10}$ min] as compared with control ($P = 0.88$), even when $MgSO_4$ was continued during dose-response testing [2.50 (0.19) $\sqrt{g \cdot c/10}$ min; $P = 0.41$]. In the oxytocin-desensitized model, mean (SE) MI was not affected by $MgSO_4$ pretreatment [2.60 (0.21) $\sqrt{g \cdot c/10}$ min; $P = 0.68$], but when $MgSO_4$ was continued during the dose-response period, the MI was significantly reduced compared with control [1.89 (0.13) $\sqrt{g \cdot c/10}$ min; $P < 0.001$]. The results for AUC were similar to MI, except for a significant reduction in oxytocin-naïve myometrium when $MgSO_4$ was continued during dose-response testing ($P = 0.02$).*

Conclusion *Magnesium sulfate pretreatment does not impair oxytocin-induced myometrial contractility in oxytocin-naïve or desensitized myometrium unless it is continued during oxytocin dose-response testing. These results suggest that its tocolytic effect is likely dependent on an extracellular mechanism. The study was registered with ClinicalTrials.gov, number NCT02647268.*

Résumé

Objectif *L'objectif de cette étude était de déterminer les schémas de contractilité induits par l'ocytocine dans un myomètre exposé à du sulfate de magnésium ($MgSO_4$). Nous avons émis l'hypothèse qu'un prétraitement à base de $MgSO_4$ réduirait les contractions myométriales induites par l'ocytocine tant dans un myomètre n'ayant jamais été exposé à l'ocytocine que dans un myomètre désensibilisé à cet agent.*

Méthode *Dans cette étude prospective in vitro, des échantillons myométriques ont été obtenus auprès de 26 femmes subissant un accouchement par césarienne non urgent. Les échantillons ont été divisés en six groupes. Quatre groupes ont été répartis comme suit : aucun*

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prétraitement (groupe témoin), prétraitement de 10^{-5} M d'ocytocine (groupe désensibilisation), prétraitement de 3,5 mM $MgSO_4$, et prétraitement de 3,5 mM $MgSO_4$ + 10^{-5} M ocytocine. Ce prétraitement a été suivi par un test de dose-réponse à l'ocytocine à une dose progressant de 10^{-10} à 10^{-5} M dans les quatre groupes. Deux groupes supplémentaires ont reçu soit un prétraitement de 3,5 mM $MgSO_4$ ou un prétraitement de 3,5 mM $MgSO_4$ + 10^{-5} M ocytocine, suivi d'un test de dose-réponse à l'ocytocine avec 3,5 mM $MgSO_4$. Les résultats recherchés étaient l'indice de motilité (IM) tel que défini par l'amplitude (g) x la fréquence des contractions myométriales (c) sur une période de dix minutes, et la surface sous la courbe (SSC).

Résultats *Dans les myomètres naïfs à l'ocytocine, l'IM moyen (erreur-type) n'a pas été affecté par le prétraitement au $MgSO_4$ [3,31 (0,20) $\sqrt{g \cdot c/10}$ min] par rapport au groupe témoin (P = 0,88) et ce, même lorsque le $MgSO_4$ a été maintenu pendant le test de dose-réponse [2,50 (0,19) $\sqrt{g \cdot c/10}$ min; P = 0,41]. Dans le modèle désensibilisé à l'ocytocine, l'IM moyen (erreur-type) n'a pas été affecté par le prétraitement au $MgSO_4$ [2,60 (0,21) $\sqrt{g \cdot c/10}$ min; P = 0,68], mais lorsqu'on a poursuivi l'administration de $MgSO_4$ pendant la période de test de dose-réponse, l'IM était significativement réduit par rapport au groupe témoin [1,89 (0,13) $\sqrt{g \cdot c/10}$ min; P < 0,001]. Les résultats pour la SSC étaient semblables à l'IM, hormis une réduction significative observée dans le myomètre naïf à l'ocytocine lorsque l'administration de $MgSO_4$ était maintenue pendant le test de dose-réponse (P = 0,02).*

Conclusion *Le prétraitement au sulfate de magnésium n'entrave pas la contractilité myométriale induite par l'ocytocine dans le myomètre naïf ou désensibilisé à l'ocytocine, à moins que le traitement ne se poursuive pendant la période de test de dose-réponse à l'ocytocine. Ces résultats suggèrent que son effet tocolytique est probablement dépendant d'un mécanisme extracellulaire. Cette étude a été enregistrée au ClinicalTrials.gov, numéro NCT02647268.*

Magnesium sulfate ($MgSO_4$) is increasingly used in the high-risk pregnant population. It is well known to have a relaxant effect on uterine muscle and, as such, has been used as a tocolytic agent in preterm labour.[1] It has a high probability of delaying delivery by 48 hr[2]; however, it may increase infant mortality with the high doses used for tocolysis.[3] Magnesium sulfate has also been shown to have neuroprotective effects in preterm fetuses at delivery,[4,5] with well-recognized evidence that it reduces the risk of death, cerebral palsy, and gross motor dysfunction without major maternal or fetal side effects up to two years of age.[6] A major use of $MgSO_4$ is in the prevention and treatment of seizures in preeclampsia and eclampsia. It has been shown to halve the risk of eclamptic seizures in preeclampsia compared with placebo.[7] It is thus recommended as a first-line treatment for eclampsia, as seizure prophylaxis in severe preeclampsia, and in non-severe preeclampsia with specific symptoms characteristic of HELLP syndrome (severe hypertension, low platelet levels, and elevated liver enzymes) or headaches and visual symptoms.[8]

Severe preeclampsia and eclampsia make substantial contributions to maternal morbidity and mortality.[9] An association between preeclampsia and postpartum hemorrhage (PPH) has been suggested, with the risk of PPH being at least 1.5 times greater in preeclamptic than in normotensive patients.[9] Szal *et al.*[10] showed that women with preeclampsia had a higher rate of PPH than normotensive women, the rate of PPH being highest in preeclamptic women treated with $MgSO_4$ compared with those who were not. By contrast, Graham *et al.*[11] found no increase in blood loss at Cesarean delivery (CD) in women with preeclampsia receiving $MgSO_4$.

The interaction between $MgSO_4$ and oxytocin, as related to myometrial contractility and PPH, is not fully understood. Oxytocin causes myometrial contraction via the oxytocin receptor (OTR); however, pre-exposure to oxytocin has been shown to result in OTR desensitization[12,13] in both a time- and concentration-dependent manner.[14] The resultant need for a higher oxytocin dose to cause adequate uterine contraction *in vivo* has also been shown in labouring women who received oxytocin for labour augmentation.[15] Furthermore, there are studies suggesting that use of $MgSO_4$ may lead to increased requirements for oxytocin[16,17] or that $MgSO_4$ may increase PPH in preeclamptic patients.[17] Nevertheless, further studies are warranted to assess these potential interactions.

The purpose of this study was to investigate the *in vitro* effects of $MgSO_4$ on the contractility of pregnant human myometrium. We hypothesized that $MgSO_4$ would reduce oxytocin-induced contractions in both oxytocin-naïve and oxytocin-desensitized myometrium.

Methods

After approval by the Research Ethics Board, Mount Sinai Hospital, Toronto, ON, Canada (REB 15-0217-A; September 25, 2015), we conducted this prospective *in vitro* study in non-labouring term pregnant women undergoing elective CD. Written informed consent was obtained from each patient enrolled. Inclusion criteria were women with a gestational age of 37-41 weeks undergoing

elective primary or first repeat CD under spinal anesthesia. Exclusion criteria were patient refusal, need for general anesthesia, more than one previous uterine surgery/CD, bleeding disorders, and the presence of any other risk factors for PPH, such as abnormal placentation, multiple gestation, macrosomia, polyhydramnios, or a history of PPH. Patients with medical and pregnancy-related conditions, such as diabetes, preeclampsia, and essential hypertension, were also excluded as well as those receiving medications that could affect uterine contractility, such as labetalol and magnesium sulfate.

Myometrial strip isolation and preparation

All CDs were performed under spinal anesthesia. During the procedure, the obstetrician excised a small sliver of myometrium, approximately 20 mm × 10 mm × 5 mm, from the upper incisional surface of the lower uterine segment, after the delivery of the fetus and placenta but before the administration of oxytocin. The collected specimen was immediately placed in 3-(N-morpholino)propanesulfonic acid (MOPS) buffer solution (145 mM NaCl, 4.7 mM KCl, 1.5 mM $CaCl_2$, 1.17 mM $MgSO_4{\cdot}7H_2O$, 1.2 mM $NaH_2PO_4{\cdot}H_2O$, 3.0 mM MOPS, 5.0 mM glucose, 2.0 mM pyruvate) and transferred to the laboratory for experimentation. This process of myometrial biopsy has been widely used by both our research group and others and is not associated with any known risks.[18-20]

Reagents

Study solutions were prepared by serial dilutions in sterile double-distilled water. All salts and reagents used in the preparation of the MOPS, physiological salt solution (PSS), and oxytocin (lyophilized powder) were obtained from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada). The MOPS buffer solution (145 mM NaCl, 4.7 mM KCl, 1.5 mM $CaCl_2$, 1.17 mM $MgSO_4{\cdot}7H_2O$, 1.2 mM $NaH_2PO_4{\cdot}H_2O$, 3.0 mM MOPS, 5.0 mM glucose, and 2.0 mM pyruvate) and PSS (112 mM NaCl, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 5 mM KCl, 1.2 mM $MgSO_4{\cdot}7H_2O$, 11.5 mM glucose, and 2.5 mM $CaCl_2$) were prepared in advance and stored at 4°C.[14] A fresh solution of 3.5 mM $MgSO_4$ was prepared prior to the start of each experiment by combining 112 mM NaCl, 25 mM $NaHCO_3$, 1 mM KH_2PO_4, 5 mM KCl, 3.5 mM $MgSO_4{\cdot}7H_20$, 11.5 mM glucose, and 2.5 mM $CaCl_2$ in double-distilled water.

Contractility analysis

Myometrial specimens were divided into six strips with the same muscle fibre direction, each 10 mm x 2 mm x 2 mm in dimension. Each strip was then mounted in a single tissue-organ bath chamber (Radnoti® 8-Chamber Tissue-Organ Bath System, model 159920; Harvard Apparatus Canada, Saint-Laurent, QC, Canada) filled with PSS at 37°C and pH 7.4 to mimic physiological conditions. An initial resting tension of 1 g was applied to each strip. The organ bath solution was aerated continuously with a mixture of 95% O_2 and 5% CO_2.[18-20] The myometrial strips were then allowed to equilibrate in PSS until spontaneous rhythmic contractions developed. After equilibration, the strips were stimulated with KCl 120 mM to provide both viability verification and a contraction to be used as the reference for experimental myometrial responses. After washing and re-equilibration, the strips were pretreated (PT) with either $MgSO_4$ 3.5 mM (Mg) and/or oxytocin 10^{-5} M (Oxy), followed by dose-response (DR) testing to oxytocin 10^{-10} to 10^{-5} M with/without $MgSO_4$ 3.5 mM. The samples were allocated to one of six treatment groups: 1) No PT/Oxy DR (control); 2) Oxy PT/Oxy DR (desensitization); 3) Mg PT/Oxy DR; 4) Mg PT/Mg + Oxy DR; 5) Mg + Oxy PT/Oxy DR; 6) Mg + Oxy PT/Mg + Oxy DR. At the end of the experiment, a final stimulation with KCl 120 mM confirmed the viability of the strips during the experimental period (Fig. 1). For each patient, a pre-set list of the six groups was created before experimentation and allocated to the six tissue-organ bath chambers in a sequential manner. An additional two tissue-organ baths were reserved for samples that failed to demonstrate spontaneous contractions. The study groups were sequentially rotated between the organ baths to ensure similar allocation of each study group to each organ bath and to control for any unidentified variation in the organ bath conditions. A tracking sheet of all patients and experiments was maintained, and all electronic tracings were labelled with the study group name.

The myometrial contractions of each sample were continuously recorded using an isometric force transducer integrated into the tissue-organ bath (Radnoti® 8-Chamber Tissue-Organ Bath System, model 159920; Harvard Apparatus Canada, Saint-Laurent, QC, Canada) and connected to a data acquisition system equipped with Acq*Knowledge*® 3.9.0 software, MP 100 (Biopac System Inc., Goleta, CA, USA). In each group, the amplitude (g) and frequency (per ten minutes) of contractions (c), motility index (MI; amplitude x frequency; g·c/10 min), and area under the curve (AUC; g·s) of oxytocin-induced contractions were recorded during the equilibration as well as during each step of the dose-response period. Contractions were recorded for 25 min during equilibration and for ten minutes during each stage of dose-response testing. Data from samples that failed to contract or those with technical errors were excluded from our analyses.

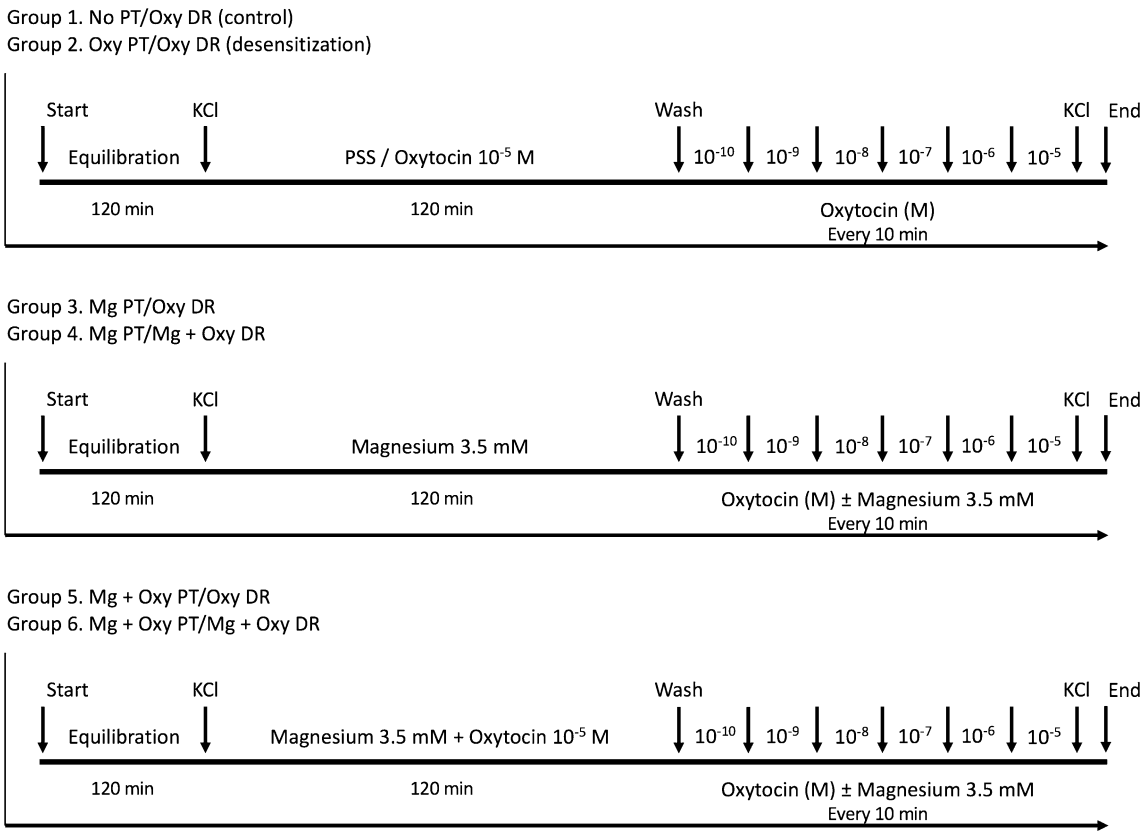

Fig. 1 Experimental design. Myometrial samples were divided into one of the six groups and then subjected to increasing concentrations of oxytocin 10^{-10} to 10^{-5} M with or without $MgSO_4$ 3.5 mM. DR = dose-response; KCl = potassium chloride; PSS = physiological salt solution; PT = pretreatment

The primary outcome was the MI, derived from the amplitude and frequency, to reflect the uterine activity over a ten-minute time frame.[21] The secondary outcomes were amplitude, frequency, and their area under the curve (AUC) responses. These contractile parameters, measured during the dose-response period, were compared across all six study groups. The demographic details of each patient were recorded (age, body mass index, gestational age, gravidity, parity, indication for CD, medical or obstetric conditions, and current medication) as well as clinical parameters such as the need for additional uterotonics and PPH within 24 hr of CD.

Statistical analysis

Previous studies have shown that the effect size between any two treatment groups, where a significant difference was apparent in MI, can be expected to be 0.7-1.4 $\sqrt{g \cdot c/10}$ min, with a standard error (SE) of 0.25-0.35 $\sqrt{g \cdot c/10}$ min.[14,22] Using these assumptions and powering the analysis for a beta of 80% and an alpha of 0.05, the sample size required was calculated as 28 experiments per treatment group.

Numerical contraction data were analyzed for each myometrial strip with increasing oxytocin concentrations during the dose-response period. All statistical analyses were performed after square-root transformation of the contractile parameters measured during the dose-response period to adjust for their skewed distribution. Amplitude, frequency, AUC, and MI were analyzed with linear regression models (maximum likelihood method for estimating parameters) adjusted for repeated measures per sample using a compound symmetry covariance structure. Models for each contractile parameter were adjusted for baseline tone, MI, amplitude, frequency and AUC during equilibration, weight of sample, and maximum amplitude after KCl administration before the pretreatment period.

Multiple pairwise comparisons between control and various study groups were computed with full covariate adjustment (15 comparisons for each outcome) using the Tukey-Kramer procedure. The values were expressed as predicted mean (SE) or estimated differences (95% confidence interval [CI]) between groups. Interpretation was based on adjusted P values. A $P < 0.05$ (two-sided) was considered statistically significant. Statistical analysis was carried out using GraphPad Prism 5 (GraphPad

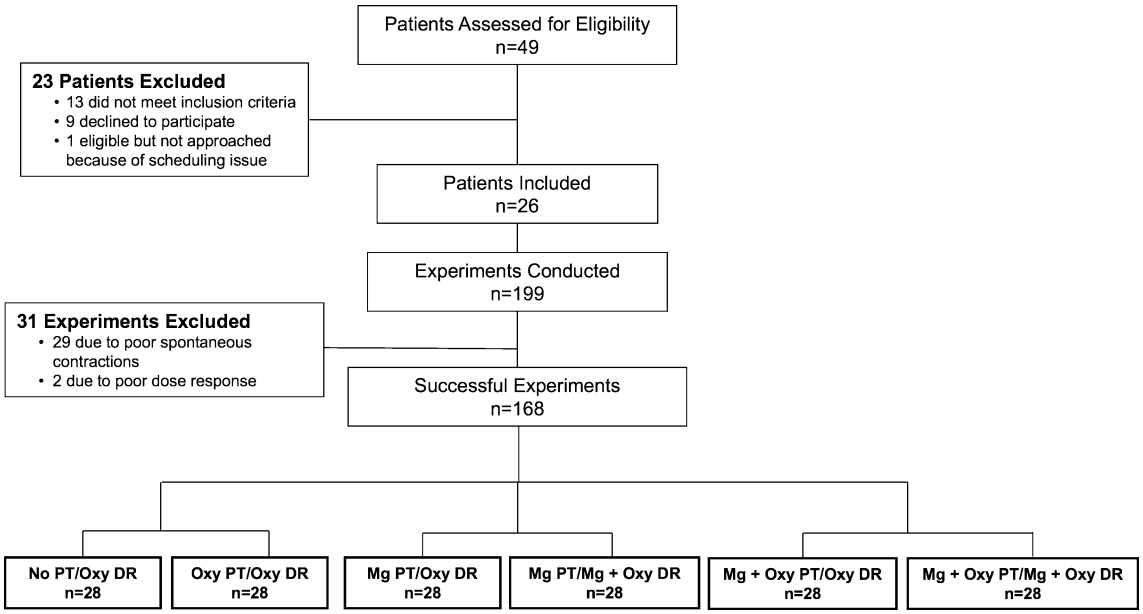

Fig. 2 Study flow sheet with patient recruitment and myometrial samples divided into various groups. DR = dose-response; PT = pretreatment

Table 1 Contractile parameters during the dose-response period

Study Group	MI	AUC	Amp	Freq
No PT/Oxy DR (Control)	3.01 (0.19)	40.15 (0.77)	1.64 (0.03)	1.70 (0.09)
Oxy PT/Oxy DR (Desensitization)	2.09 (0.15)	34.35 (1.01)	1.40 (0.04)	1.40 (0.07)
Mg PT/Oxy DR	3.31 (0.20)	40.58 (1.04)	1.66 (0.04)	1.85 (0.08)
Mg PT/Mg + Oxy DR	2.50 (0.19)	35.55 (1.14)	1.45 (0.05)	1.57 (0.08)
Mg + Oxy PT/Oxy DR	2.60 (0.21)	36.52 (0.87)	1.49 (0.04)	1.70 (0.12)
Mg + Oxy PT/Mg + Oxy DR	1.89 (0.13)	34.62 (0.86)	1.41 (0.04)	1.25 (0.06)

Values are expressed as mean (standard error). These are presented as the summation of the measurements captured during the entire dose-response period. AUC = area under the curve ($\sqrt{}$g·s); Amp, amplitude ($\sqrt{}$g); DR = dose-response; EST = estimated difference; Freq = frequency ($\sqrt{}$contractions/10 min); Mg = $MgSO_4$; MI = motility index ($\sqrt{}$g·contractions/10 min); Oxy = oxytocin; PT = pretreatment; SE = standard error

Software; San Diego, CA, USA) and SAS® statistical software v9.2 (The SAS Institute, Cary NC, USA).

Results

Patients were recruited from January-April 2016. Forty-nine women were pre-screened for participation in the study; 36 of those were deemed eligible, and 26 consented to participate. This yielded 199 experiments, of which 168 were successful and retained for analysis (Fig. 2). The mean (standard deviation [SD]) age of participants was 33.3 (4.1) yr, body mass index was 28.5 (3.8) kg·m^{-2}, and gestational age was 38.7 (0.8) weeks. Ten (38%) of the women underwent primary CD, while 16 (62%) underwent first repeat CD. None of the women required additional uterotonics or had PPH within 24 hr of the CD.

Overall comparison between various study groups

The mean (SE) contractility parameters across groups are shown in Table 1. Dose-response testing with oxytocin in the $MgSO_4$ pretreatment (Mg PT/Oxy DR) and control (No PT/Oxy DR) groups provided the highest MI of contractions. Oxytocin pretreatment (Oxy PT/Oxy DR) significantly attenuated the mean (SE) oxytocin-induced MI as compared with the control group [2.09 (0.15) *vs* 3.01 (0.19) $\sqrt{}$g·c/10 min, respectively; estimated difference, −0.92 $\sqrt{}$g·c/10 min; 95% CI, −1.43 to −0.42; $P = 0.004$]. The exception was when, compared with the Mg PT/Oxy DR group, the control group displayed significantly higher AUC than all other treatment groups. The full complement of pairwise comparisons is shown in Table 2. The dose-response curves for the contractile parameters for each group are shown in Fig. 3.

Table 2 Pairwise comparisons of study group contractile parameters

Reference Group	Study Group	MI		AUC		Amp		Freq	
		EST (95% CI)	*P value	EST (95% CI)	*P value	EST (95% CI)	*P value	EST (95% CI)	*P value
Control	Oxy PT/Oxy DR	−0.92 (−1.43 to −0.42)	0.004	−5.81 (−8.42 to −3.19)	<0.001	−0.24 (−0.34 to −0.13)	<0.001	−0.30 (−0.55 to −0.06)	0.14
Control	Mg PT/Oxy DR	0.29 (−0.23 to 0.81)	0.88	0.43 (−1.98 to 2.84)	1.00	0.02 (−0.08 to 0.12)	1.00	0.15 (−0.09 to 0.38)	0.83
Control	Mg PT/Mg + Oxy DR	−0.52 (−1.06 to 0.02)	0.41	−4.61 (−7.48 to −1.73)	0.02	−0.19 (−0.31 to −0.07)	0.02	−0.13 (−0.38 to 0.11)	0.90
Control	Mg + Oxy PT/Oxy DR	−0.42 (−0.97 to 0.14)	0.68	−3.64 (−5.73 to −1.54)	0.009	−0.15 (−0.23 to −0.06)	0.009	−0.01 (−0.29 to 0.28)	1.00
Control	Mg + Oxy PT/Mg + Oxy DR	−1.13 (−1.58 to −0.67)	<0.001	−5.53 (−7.95 to −3.12)	<0.001	−0.23 (−0.32 to −0.13)	<0.001	−0.45 (−0.67 to −0.23)	0.001
Oxy PT/Oxy DR	Mg PT/Oxy DR	1.22 (0.68 to 1.75)	<0.001	6.23 (3.25 to 9.22)	0.001	0.25 (0.13 to 0.38)	0.001	0.45 (0.22 to 0.68)	0.002
Oxy PT/Oxy DR	Mg PT/Mg + Oxy DR	0.40 (−0.04 to 0.85)	0.48	1.20 (−1.56 to 3.96)	0.96	0.05 (−0.07 to 0.16)	0.96	0.17 (−0.02 to 0.37)	0.50
Oxy PT/Oxy DR	Mg + Oxy PT/Oxy DR	0.51 (−0.03 to 1.05)	0.44	2.17 (−0.73 to 5.07)	0.68	0.09 (−0.03 to 0.21)	0.71	0.30 (0.00 to 0.59)	0.36
Oxy PT/Oxy DR	Mg + Oxy PT/Mg + Oxy DR	−0.20 (−0.54 to 0.13)	0.85	0.27 (−2.13 to 2.67)	1.00	0.01 (−0.09 to 0.11)	1.00	−0.15 (−0.31 to 0.02)	0.50
Mg PT/Oxy DR	Mg PT/Mg + Oxy DR	−0.81 (−1.39 to −0.24)	0.06	−5.03 (−8.23 to −1.83)	0.03	−0.21 (−0.34 to −0.07)	0.03	−0.28 (−0.52 to −0.04)	0.20
Mg PT/Oxy DR	Mg + Oxy PT/Oxy DR	−0.71 (−1.25 to −0.17)	0.11	−4.06 (−6.60 to −1.52)	0.02	−0.17 (−0.27 to −0.06)	0.02	−0.15 (−0.42 to 0.12)	0.88
Mg PT/Oxy DR	Mg + Oxy PT/Mg + Oxy DR	−1.42 (−1.92 to −0.92)	<0.001	−5.96 (−8.72 to −3.20)	<0.001	−0.24 (−0.36 to −0.13)	<0.001	−0.60 (−0.81 to −0.38)	<0.001
Mg PT/Mg + Oxy DR	Mg + Oxy PT/Oxy DR	0.11 (−0.48 to 0.69)	1.00	0.97 (−2.15 to 4.09)	0.99	0.04 (−0.09 to 0.17)	0.99	0.13 (−0.18 to 0.43)	0.97
Mg PT/Mg + Oxy DR	Mg + Oxy PT/Mg + Oxy DR	−0.61 (−1.02 to −0.20)	0.04	−0.93 (−3.50 to 1.65)	0.98	−0.04 (−0.14 to 0.07)	0.98	−0.32 (−0.50 to −0.14)	0.007
Mg + Oxy PT/Oxy DR	Mg + Oxy PT/Mg + Oxy DR	−0.71 (−1.22 to −0.20)	0.07	−1.90 (−4.54 to 0.74)	0.72	−0.08 (−0.19 to 0.03)	0.73	−0.45 (−0.73 to −0.16)	0.03

Values are expressed as estimated difference (95% confidence interval). These are presented as the summation of the measurements captured over the dose-response period. *P values using Tukey-Kramer method for multiple comparisons adjustment. $P < 0.05$ was considered statistically significant. Amp = amplitude ($\sqrt{g}$); AUC = area under the curve ($\sqrt{g \cdot s}$); DR = dose-response; EST = estimated difference; Freq = frequency ($\sqrt{\text{contractions/10 min}}$); Mg = $MgSO_4$; MI = motility index ($\sqrt{g \cdot \text{contractions/10 min}}$); Oxy = oxytocin; PT = pretreatment

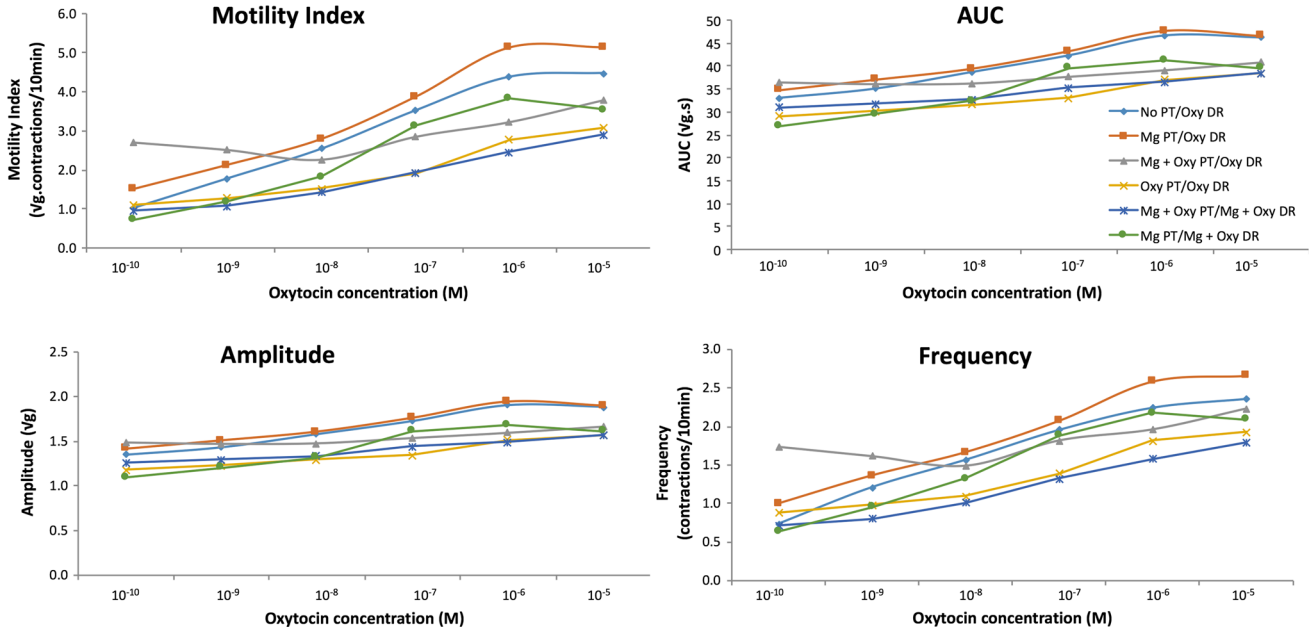

Fig. 3 Dose-response curves for the myometrial contractile parameters in various groups. AUC = area under the curve ($\sqrt{}$g·s); DR = dose-response; PT = pretreatment

Effect of $MgSO_4$ on contractile parameters in oxytocin-naïve myometrium

In oxytocin-naïve myometrium, the mean (SE) MI was not affected by $MgSO_4$ pretreatment (Mg PT/Oxy DR) compared with control [3.31 (0.20) *vs* 3.01 (0.19) $\sqrt{}$g·c/10 min, respectively; estimated difference, 0.29 $\sqrt{}$g·c/10 min; 95% CI, −0.23 to 0.81; $P = 0.88$). This MI effect was also the case when $MgSO_4$ was continued during the dose-response period (Mg PT/Mg + Oxy DR) compared with control [2.50 (0.19) *vs* 3.01 (0.19) $\sqrt{}$g·c/10 min, respectively; estimated difference, −0.52 $\sqrt{}$g·c/10 min; 95% CI, −1.06 to 0.02; $P = 0.41$]. Similarly, there was no difference when these two groups were compared with each other (estimated difference, −0.81 $\sqrt{}$g·c/10 min; 95% CI, −1.39 to −0.24; $P = 0.06$). Nevertheless, the mean (SE) AUC was reduced as compared with control [35.55 (1.14) *vs* 40.15 (0.77) $\sqrt{}$g·s, respectively; estimated difference, −4.61 $\sqrt{}$g·s; 95% CI, −7.48 to −1.73; $P = 0.02$] when $MgSO_4$ was continued during the dose-response period, but not with $MgSO_4$ pretreatment only (Tables 1 and 2).

Effect of $MgSO_4$ on contractile parameters in oxytocin-desensitized myometrium

When $MgSO_4$ was administered along with oxytocin for pretreatment, the mean (SE) MI was found to be reduced only when $MgSO_4$ was continued during the dose-response period, i.e., in Mg + Oxy OT/Mg + Oxy DR group, as compared with control [1.89 (0.13) *vs* 3.01 (0.19) $\sqrt{}$g·c/10 min, respectively; estimated difference, −1.13 $\sqrt{}$g·c/10 min; 95% CI, −1.58 to −0.67; $P < 0.001$] and not when it was discontinued in the Mg + Oxy PT/Oxy DR group, as compared with control [2.60 (0.21) *vs* 3.01 (0.19) $\sqrt{}$g·c/10 min, respectively; estimated difference, −0.42 $\sqrt{}$g·c/10 min; 95% CI, −0.97 to 0.14; $P = 0.68$]. Nevertheless, AUC was reduced in both of these groups when compared with the control group (Table 2).

Discussion

Our study tested the *in vitro* effects of $MgSO_4$ on the contractility of oxytocin-naïve and desensitized myometrium. The reduced myometrial contractility (i.e., MI) observed with oxytocin pretreatment may be explained by the phenomenon of OTR desensitization, which we have previously characterized.[14,18] Pretreatment with $MgSO_4$ *per se* did not reduce subsequent oxytocin-induced contractility; however, continuing $MgSO_4$ during oxytocin dose-response testing significantly reduced contractility in both oxytocin-naïve and desensitized myometrium. This suggests that the tocolytic effects of $MgSO_4$ are likely dependent on an extracellular mechanism.

The interpretation of our results was based on both MI and AUC findings. Although the primary outcome of this

study was the MI, the AUC offers similarly important information. The MI is derived from the amplitude and frequency of contractions and reflects uterine activity,[21] while the AUC reflects the overall strength of contractions over time.[23] When $MgSO_4$ was continued during oxytocin dose-response testing, the MI was reduced in desensitized myometrium but not in oxytocin-naïve myometrium; however, the AUC was reduced in both oxytocin-naïve and desensitized myometrium. These results are complementary, and the difference may be explained by a more appreciable effect of $MgSO_4$ on the amplitude rather than on the frequency. Our findings suggest that $MgSO_4$ may have a more important influence on the amplitude of oxytocin-induced contractions than on the frequency of the contractions. We have reported similar differences in these parameters in previous studies.[14,22]

Our results showed reduced oxytocin-induced myometrial contractility when $MgSO_4$ was present during dose-response testing, but not when present only in the pretreatment period. This implies an extracellular mechanism of action, or at least that any intracellular component has minimal tocolytic impact. If the tocolytic effect of $MgSO_4$ were dependent on an intracellular mechanism, the effects on the myometrium would have persisted after washing the pretreated strips prior to dose-response testing. Hence, a reduction in contractility would have been observed even in groups with only $MgSO_4$ pretreatment. Magnesium sulfate is thought to have both intracellular and extracellular effects on the myometrium. Studies have shown that $MgSO_4$ plays a role in reducing acetylcholine release at the neuromuscular junction,[24] as well as antagonizing the effects of calcium (Ca^{2+}), reducing entry of Ca^{2+} into the myometrial cell and possibly preventing the release of intracellular Ca^{2+} from the sarcoplasmic reticulum.[14] The resultant decreased intracellular availability of calcium may, in turn, decrease the force of spontaneous and oxytocin-induced contractions.[24] An opposing proposed mechanism is that $MgSO_4$ interacts with cholesterol as an essential allosteric modulator of the OTR, increasing the response of the myometrium to oxytocin.[25] This pathway would predict increased oxytocin-induced myometrial contractions in the presence of $MgSO_4$. We suggest from our data that this pathway may not be active in human myometrium.

The changes in contractility that we have shown, if consistent *in vivo*, would have important clinical implications for the postpartum management of women on $MgSO_4$ who have delivered, either vaginally or by CD, following oxytocin induction or augmentation of labour. This is particularly important in preeclampsia where $MgSO_4$ therapy is usually continued for up to 24 hr after delivery. Even in scenarios where $MgSO_4$ is discontinued at delivery, such as in threatened preterm labour or fetal neuroprotection, there is a possible risk of PPH since its effects last up to an hour.[26,27]

The interaction of $MgSO_4$ and oxytocin has not been extensively studied in humans. Bloss *et al.*[28] looked at the effect of oxytocin on the action of intramuscular $MgSO_4$ and showed that its pharmacokinetics were not affected by oxytocin. One study found that a higher dose of oxytocin was required in patients with mild preeclampsia receiving $MgSO_4$, suggesting that the tocolytic effect of $MgSO_4$ may be overcome by increased doses of oxytocin.[17] Nevertheless, the study did not state whether or not this specific finding was in women requiring oxytocin for augmentation, and hence, information regarding the effect of $MgSO_4$ on oxytocin-desensitized myometrium cannot be inferred from this study.

Yildirim *et al*. have studied the *in vitro* effect of $MgSO_4$ on the myometrium in a rat model with induced preeclampsia.[29] The study found that oral $MgSO_4$ 600 $mg{\cdot}kg^{-1}$ had no significant effect on the amplitude of spontaneous or oxytocin-induced contractions using increasing oxytocin doses (0, 0.1, 0.2, 0.4, 0.8, and 2.5 $mIU{\cdot}mL^{-1}$). Magnesium sulfate did however reduce the frequency of spontaneous contractions. Fomin *et al.*[30] observed that $MgSO_4$ 5 and 10 mM reduced both spontaneous and oxytocin-induced contractions (oxytocin at 0.1 $\mu mol{\cdot}L^{-1}$) *in vitro* in human myometrial strips in a time and concentration-dependent manner. A study by Tang *et al.*[31] indicated that $MgSO_4$ at concentrations of 4.06×10^{-2} mM, 4.06×10^{-1} mM and 4.06 mM prolonged the myometrial contractile interval, while at a concentration of 4.06 mM, inhibited the myometrial contractile force. They further showed that oxytocin at a dose of 0.01 $U{\cdot}mL^{-1}$ could reverse this $MgSO_4$ effect *in vitro*, as has been similarly shown *in vivo*.[17] Although these studies show the opposing actions of oxytocin and $MgSO_4$, they do not investigate the interaction between the two in myometrium in the desensitized state.

One limitation of our study is the use of $MgSO_4$ 3.5 mM in the *in vitro* model. Although a previous study[31] showed a significant reduction in myometrial contractility with $MgSO_4$ at 4 mM, the upper limit of the therapeutic plasma level for seizure management in preeclampsia is 3 mM, with toxicity occurring at > 3.5 mM. There is a loss of the patellar reflex at 3.5-5 $mMol{\cdot}L^{-1}$, respiratory paralysis at 5-6 $mMol{\cdot}L^{-1}$, cardiac conduction defects at > 7.5 $mMol{\cdot}L^{-1}$, and cardiac arrest at > 12.5 $mMol{\cdot}L^{-1}$.[27,31] Hence, our rationale for using $MgSO_4$ 3.5 mM was to provoke a response that could potentially occur at the upper end of its use. In normal clinical practice, where infusion rates are generally set at 1 $g{\cdot}hr^{-1}$ after a bolus of 2-4 g, it is rare to observe levels > 2 mM,[27] unless in the context of renal impairment. Nevertheless, with the organ bath method, higher concentrations are needed to produce

similar effects to what is seen *in vivo*. Conversely, it cannot be assumed that the contractile changes shown *in vitro* would also be seen *in vivo*. It is important to point out that, in this study design, the samples were washed prior to dose-response testing, which is unrepresentative of a true clinical situation. For example, in fetal neuroprotection, oxytocin may be administered for augmentation while the effects of $MgSO_4$ persist for up to an hour after the infusion has ended.[26,27]

In conclusion, our study suggests that $MgSO_4$ pretreatment *per se* does not impair oxytocin-induced myometrial contractility; however, the presence of $MgSO_4$ during dose-response testing significantly impairs contractility in both oxytocin-naïve and desensitized myometrium. Furthermore, our study suggests that the tocolytic action of $MgSO_4$ may be extracellular. Our *in vitro* observations support previous studies in the literature that highlight the association between $MgSO_4$ and PPH. Clinical protocols should be developed to optimize the interaction between $MgSO_4$ and oxytocin to reduce the risk of PPH.

Conflicts of interest None declared.

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