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In vitro contractile effects of agents used in the clinical management of postpartum haemorrhage

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Syntometrine (PubChem CID: 11979741)
Misoprostol (PubChem CID: 5282381)

ABSTRACT

Uterine atony is a major cause of postpartum haemorrhage and maternal mortality. However, the comparative pharmacology of agents used to treat this condition is poorly understood. This study evaluates, using human pregnant myometrium *in vitro*, a range of contractile parameters for agents used in the clinical treatment of atonic postpartum haemorrhage. The effects of oxytocin, carbetocin, ergometrine, carboprost, syntometrine and misoprostol were investigated in 146 myometrial strips from 19 donors. The potency and maximal response values were obtained, and compared, using both maximal amplitude and mean contractile force as indices of contraction. Single, EC_{50} concentrations of the agents were administered and both force and contraction peak parameters were compared during a 15-min exposure. Differences were considered significant when $P < 0.05$. There were no significant differences in the peak amplitude of response between agents, except for misoprostol, which was inactive. There was a wide difference in potencies using both measures of contractility, with oxytocin and carbetocin being the most potent. The most important difference between the agents was in their ability to increase the mean contractile force, with oxytocin superior to all agents except syntometrine. In single dose experiments, mean contractile force was the parameter that separated the agents. In this respect, oxytocin was not statistically different from carboprost or syntometrine, but was superior to all other agents. These findings support a clear role for oxytocin as the first line agent for treatment of postpartum haemorrhage and raise doubts about the potential clinical usefulness of misoprostol.

1. Introduction

Obstetric haemorrhage is a leading cause of maternal mortality in the developed and under-developed world. Excessive bleeding within 24 h of delivery of an infant, *i.e.* primary postpartum haemorrhage (PPH), is the most common form of major obstetric haemorrhage (Royal College of Obstetricians & Gynaecologists, 2009). Approximately one quarter of all maternal deaths worldwide are due to PPH (Carroli et al., 2008), and it is a major cause of maternal morbidity (Shields et al., 2015).

Uterine atony, the inability of the uterus to contract effectively after delivery, is the main cause of PPH, and accounts for approximately 70–80% of all cases (Gizzo et al., 2013; Bateman et al., 2010). The first line treatment approach involves the use of uterotonic agents in a preventative or therapeutic way (Royal College of Obstetricians & Gynaecologists, 2009; World Health Organization, 2012; Bohlmann and Rath, 2014). For prevention of PPH oxytocin is generally regarded as the first-line agent, but ergometrine, syntometrine (a combination of methylergometrine and oxytocin) and misoprostol are also used. (Royal College of Obstetricians & Gynaecologists, 2009; World Health Organization, 2012; Bohlmann and Rath, 2014) For the treatment of atonic PPH intravenous oxytocin is widely recommended as the initial agent, but if the bleeding does not stop the use of other uterotonic agents such as ergometrine, syntometrine, the injectable prostanoid analogues carboprost, and misoprostol, or the long acting oxytocin receptor agonist carbetocin, should be employed (Royal College of Obstetricians & Gynaecologists, 2009; World Health Organization, 2012; Gizzo et al., 2013; Bohlmann and Rath, 2014). There are, however, inconsistencies between different guidelines internationally, with no clear consensus in relation to the appropriate order of clinical efficacy of these second line compounds. Clinical trials that compare the different uterotonic agents available for treatment of PPH are not adequately powered to assess their impact on outcomes (Mousa et al., 2014), and hence clinical guidelines are based on a low level of evidence (Royal College of Obstetricians & Gynaecologists, 2009; World Health Organization,

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2012). It is for all of these reasons that there is great variation in clinical practice worldwide (Bohlmann and Rath, 2014).

Efforts to understand the effectiveness of uterotonic compounds in human myometrium should begin with *in vitro* studies (Crankshaw and Morrison, 2011). Unfortunately, there are no comparative pharmacological data for all of the various uterotonic compounds used clinically. Therefore, the aim of this study was to determine a complete pharmacodynamic profile, in human pregnant myometrium, for the uterotonic agents used clinically in the treatment of atonic PPH by evaluating a range of contractile parameters.

2. Materials and methods

2.1. Tissue collection and mounting in vitro

The study was performed at Galway University Hospital, Ireland. Myometrial biopsies were obtained after having obtained informed written consent and with hospital ethical committee approval. Biopsies were excised from nineteen women undergoing elective caesarean delivery at term. The biopsies were excised after delivery of the infant and prior to administration of the routine bolus dose of oxytocin that is given peri-opertaively. The biopsies were dissected and 140 strips were prepared for recording contractility by being mounted in 20 ml tissue baths exactly as previously described (Crankshaw and Morrison, 2011; Crankshaw et al., 2015; Crankshaw et al., 2014). Strips (usually eight per biopsy) were stretched to a resting tension of 20 mN. After 30 min, fresh physiological salt solution (PSS) was introduced to the baths and tension was readjusted to 20 mN followed by a further 15 min equilibration period. Thereafter, activity was recorded isometrically for 130 min to allow for the development of spontaneous activity. Strips were then challenged with potassium chloride (KCl, 60 mmol/l). After a 10-min exposure period baths were washed with fresh PSS and strips were allowed 15 min for recovery.

2.2. Concentration-effect experiments

For concentration-effect studies the subsequent period of the experiment was divided into seven intervals of 15 min duration, the first of which served as a control. Uterotonics were then added in concentrations that increased cumulatively by approximately one log unit every 15 min until six doses had been applied. The starting concentrations (determined from dose-finding experiments) were as follows: oxytocin (Sigma-Aldrich, Dublin, Ireland), 10 pmol/l; carbetocin (Tocris Bioscience, Bristol, UK), 10 pmol/l; ergometrine (Hameln, Gloucester, UK), 1.1 nmol/l; carboprost tromethamine (Pfizer, Sandwich, Kent UK), 0.5 nmol/l; syntometrine (Alliance Pharmaceutical, Chippenham, Wiltshire UK) 126 pmol/l expressed here and throughout in terms of the oxytocin content; and misoprostol (Cayman Chemical, Ann Arbor, MI, USA), 2.6 nmol/l. Each agent was tested on two strips from the same donor and agents were rotated through experiments in a Latin Square design until the appropriate number of observations was accumulated.

2.3. Single dose experiments

For single dose experiments agents were added at bath concentrations equivalent to their EC_{50} values for increasing mean contractile force (MCF) as determined from pooling all the data obtained from the concentration-effect experiments just described. These were as follows: oxytocin, 2.4 nmol/l; carboprost, 0.17 nmol/l; ergometrine, 13 nmol/l; carboprost, 1.2 μmol/l; syntometrine, 33 nmol/l. In this case, activity was recorded for 15 min. Each agent was tested on two strips from the same donor and agents were rotated through experiments in a Latin Square design until eight observations were made.

2.4. Contractile activity measurements and data analysis

Contractile activity was recorded as we have reported previously (Crankshaw and Morrison, 2011; Crankshaw et al., 2015; Crankshaw et al., 2014), using a PowerLab/8SP recording unit and Chart v4.0 software (AD Instruments, Oxford, UK). The raw digital files were analysed using LabChart v7.3.7 (AD Instruments, Oxford, UK). The Peak Analysis Module (AD Instruments, Oxford, UK) with the threshold set at 2 mN was used to identify contraction peaks for single-dose experiments. Custom macros controlled data extraction and results were exported to Microsoft Excel for final analysis. The following parameters were determined for each 15 min block of concentration-effect experiments: the minimum amplitude of contractions, the maximum amplitude (MAMP) and the mean contractile force above baseline (MCF). For single-dose experiments the following parameters were also determined: the time to MAMP, the maximum rate of rise of contractions, the minimum rate of relaxation of contractions, the time to the first contraction peak, the number of peaks during the 15 min post-treatment period and the total peak area.

All forces were normalised to the response to the KCl challenge. Concentration-effect curves were constructed by fitting the data to Eq. (1):

$$E = Emax/\left(1 + \left(10\text{^}\left(-pEC_{50} - logC\right)\right)\right) \quad (1)$$

where E is the effect of the uterotonic, Emax is the maximum response, C is the molar concentration of the uterotonic, and pEC_{50} is the negative log of the molar concentration of the uterotonic that produces a half-maximal response. This was done in two ways. In the first case, and to obtain an overall picture of the relative effectiveness of the various agents, all data from a given agent were pooled into a single concentration-effect curve. In the second case, when it became clear that significant differences between the agents were likely, each individual strip was analysed separately so that robust statistical comparison of parameters could be made. In this latter case, data from some individual strips were quite noisy resulting in meaningless values when fitted to Eq. (1) and were disregarded. All values are expressed as the arithmetic mean ± the standard error of the mean. Results with different uterotonics were compared by a single-factor analysis of variance (ANOVA) using $P < 0.05$ as the cut-off for significance. If the ANOVA indicated a significant difference between groups it was followed by *post-hoc* analysis using Tukey's HSD test at $P > 0.05$ to determine which treatments were different from each other.

3. Results

There were n=19 women recruited to donate myometrial tissue at caesarean delivery. The mean maternal age was 34.7 ± 1 years (range 23–41). The median parity was 1 (range 0–7) and the median gestation at delivery was 39+1 weeks (range 36+0 to 40+3). The primary reasons for caesarean delivery were as follows: previous caesarean delivery n=12, breech presentation/unstable lie of the fetus n=4, complications of pregnancy and/or maternal request n=3.

Representative recordings showing the response of strips of human pregnant myometrium to the KCl challenge, followed by cumulative addition of the uterotonics (one agent per strip), are

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