Lack of effect of a single i.v. dose of oxytocin on sperm output in severely oligozoospermic men

Maria M. Byrne, Claus Rolf, Marion Depenbusch, Trevor G. Cooper and Eberhard Nieschlag

Institute of Reproductive Medicine, University of Münster, Domagkstrasse 11, D-48129 Münster, Germany

BACKGROUND: ICSI into the oocyte is the only treatment currently available for most male patients with severe oligozoospermia who wish to father children. In order to perform ICSI, motile sperm need to be recovered from the ejaculate and, if no sperm or not enough motile sperm are recovered on the day of ICSI, testicular sperm extraction (TESE) must be performed. Oxytocin stimulates epididymal contractility and may be important for the release of stored sperm. The aim of this randomized single-blind cross-over study was to establish the effects of oxytocin on sperm output in severely oligozoospermic men. METHODS: Forty-nine infertile men with sperm concentrations <0.2 × 10⁶/ml were studied on two occasions after 3–4 days of sexual abstinence. They received an i.v. injection of saline or oxytocin 0.75 IU in random order, and commenced masturbation within 5 min. Ejaculate analysis was performed according to the WHO 1999 guidelines. RESULTS: A single i.v. dose of oxytocin resulted in no change in ejaculate volume (P = 0.4), total sperm count (P = 0.14) or sperm motility (P = 0.9). There was no significant correlation between the change in total sperm count and FSH levels (r = −0.32, P = 0.2), or the change in total sperm count and estradiol levels (r = −0.02, P = 0.9). Similar results were found in a subgroup of men with total sperm counts of <100. CONCLUSIONS: Our data indicate that a single-dose of i.v. oxytocin has no detectable effect on seminal parameters in men with severe oligozoospermia.

Key words: ejaculation/ICSI/male infertility/oxytocin/sperm count

Introduction

ICSI into the oocyte is the only treatment currently available for most male patients with severe oligozoospermia who wish to father children (Kamischke and Nieschlag, 1999). In order to perform ICSI, motile sperm need to be recovered from the ejaculate. In azoospermic patients, testicular sperm extraction (TESE) must be performed prior to ICSI. As it has been demonstrated that the fertilization rate after ICSI is higher with fresh sperm from the ejaculate when compared with ICSI–TESE (Ubaldi et al., 1995; Tournaye, 1999), there is considerable interest in the therapeutic possibilities of increasing the number of motile sperm in the ejaculate of severely oligozoospermic men prior to ICSI.

Several substances are known to stimulate epididymal contractility and may therefore be important factors in determining sperm transport through or expulsion from the epididymis. These include prostaglandins, transforming growth factor-β (TGF-β), nitric oxide (NO)/cGMP and oxytocin (Maekawa et al., 1996). In humans, oxytocin receptors are located in the testes, smooth muscle cells and the epithelium of the epididymal tubules and the prostate (Frayne and Nicholson, 1998). The action of oxytocin is mediated via activation of the oxytocin receptor (a typical class I G protein-coupled receptor) present in the peritubular smooth muscle cells of the epididymis stimulating contractility in vivo (Melin, 1970; Hib, 1977) and in vitro (Hib, 1974; Filippi et al., 2002b). Oxytocin acts directly on smooth muscle cells throughout the entire epididymis and indirectly by stimulating the release of endothelin-1 from epithelial cells of the caput epididymidis (Peri et al., 1997; Filippi et al., 2002a). Endothelin-1 is a potent stimulator of epididymal contraction.

Epididymal contractility stimulated by oxytocin may be important for the release of sperm stored in the cauda epididymidis (Studdard et al., 2002). In several species including humans, a pulse of oxytocin, presumably of hypothalamic origin, appears to be associated with ejaculation (Sharma et al., 1972; Ogawa et al., 1980; Peelers et al., 1983; Stoneham et al., 1985; Murphy et al., 1987). Results from animal studies with single-dose i.v. administration of oxytocin resulted in increased sperm output in Holstein bulls (Berndtson and Igboeli, 1988) and sheep (Nicholson et al., 1999). In a preliminary study with a small number of azoospermic or severely oligozoospermic patients (n = 14), i.v. oxytocin 0.5 IU given before a second ejaculation improved emptying of spermatozoal stores and reduced the necessity for TESE (Rolf et al., 2000). In a recent study of five severely oligozoospermic men (mean total sperm count 19.5), i.v. oxytocin (2.5 IU) resulted in a non-significant tendency to increased sperm...
output (Filippi et al., 2002a). The effect of a single i.v. dose of oxytocin on sperm output in a large cohort of severely oligozoospermic men has not been studied. Therefore, this study was undertaken to establish the effects of oxytocin on sperm output in 49 severely oligozoospermic men.

Materials and methods

Subjects

Studies were performed on 49 infertile men who were recruited from the infertility clinic of the Institute of Reproductive Medicine. Male infertility was defined as the inability to induce a pregnancy after 1 year of unprotected intercourse. Men were included with sperm concentrations >0 and <0.2 $10^6$ ml, scrotal testes and no infection of the accessory glands. The primary diagnosis included severe idiopathic oligoasthenoteratozoospermia (OAT) ($n = 29$), a past history of undescended testes treated with HCG or orchidopexy in childhood ($n = 16$), microdeletions of the Y chromosome in the AZFc region ($n = 2$), and a history of mumps orchitis ($n = 2$).

The subjects underwent a complete history and physical examination prior to entering the study, as previously described (Behre et al., 2000). The subjects were examined at least twice prior to recruitment into the study. Baseline characteristics of the 49 subjects included a mean age of 34 ± 1 years, mean FSH of 15.0 ± 1.6 U/l, mean LH of 5.9 ± 0.5 U/l, mean total testosterone of 15.9 ± 0.9 nmol/l, mean sex hormone-binding globulin (SHBG) of 35.3 ± 2.0 nmol/l, mean free testosterone of 316.3 ± 15.8 pmol/l, mean estradiol (E2) of 65.9 ± 2.7 pmol/l, mean prolactin of 196.3 ± 11.3 mU/l, mean bilateral testicular volume of 28.3 ± 1.9 ml, mean glucosidase of 38.3 ± 4.1 mU/ejaculate, mean fructose of 71.5 ± 7.9 mmol/ejaculate and mean zinc of 5.2 ± 0.5 mmol/ejaculate. All studies were performed in the Institute of Reproductive Medicine of the University of Münster. The protocol was approved by the Ethics Committee of the University of Münster and the State Medical Board, and all subjects gave written informed consent.

Experimental protocol

The subjects were studied on two occasions after 3–4 days of sexual abstinence. The single-blind studies were performed with each patient receiving an i.v. injection of 2 ml of saline or oxytocin 0.75 IU (Syntocinon 3 IU, Novartis GmbH, Nürnberg, Germany) in random order with at least 1 week between the two studies. The subjects commenced masturbation within <5 min post-i.v. injection. Blood pressure and pulse were recorded pre- and post-i.v. injection.

Semen analysis

Ejaculate analysis was performed according to the WHO guidelines (World Health Organization, 1999). The sample was allowed to liquefy in an incubator at 37°C for 30 min and was then analysed by light microscopy. Seminal volume, sperm concentration, sperm count, sperm motility, sperm morphology, zinc, fructose, α-glucosidase and sperm vitality were measured when the sperm count was >0.1 $10^6$/ml. When the sperm count was <0.1 $10^6$/ml, the ejaculate was centrifuged for 10 min at 390 g and the pellet was resuspended in 50–200 µl according to pellet size, and total sperm were counted. Internal quality control of semen parameters was carried out according to Cooper et al. (1992). The laboratory is also enrolled in an external quality assurance scheme (Cooper et al., 1999, 2002). The laboratory technicians were unaware of the source of the specimen.

<table>
<thead>
<tr>
<th>Table I. Ejaculate parameters in response to placebo or oxytocin (0.75 IU) administration in severely oligozoospermic men</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
</tr>
<tr>
<td>($n = 49$)</td>
</tr>
<tr>
<td>Ejaculate volume (ml)</td>
</tr>
<tr>
<td>Total sperm count/ejaculate</td>
</tr>
<tr>
<td>Progressive motile 'a' sperm (%)</td>
</tr>
<tr>
<td>Progressive motile 'b' sperm (%)</td>
</tr>
</tbody>
</table>

* Motility classified according to World Health Organization (1999).
Statistical analysis

All results are expressed as mean ± SEM. Data analysis was performed using the Statistical software package SPSS for Windows version 6.1 (SPSS, Chicago, IL). The significance of differences within individuals was determined using paired \( t \)-tests. Differences were considered to be significant if \( P < 0.05 \). When data were not normally distributed, the Mann–Whitney rank sum and Wilcoxon tests were performed.

Results

No major side effects occurred. Minor side effects included feeling flushed (\( n = 14 \)) and a slight transient headache (\( n = 1 \)), with symptoms disappearing in all subjects within 5 min after injection of oxytocin. Pre- and post-oxytocin, mean systolic and diastolic blood pressure (135 ± 2 versus 134 ± 2 mmHg \( P = 0.5 \); 82 ± 1 versus 84 ± 2, \( P = 0.3 \)), respectively, did not differ, and mean pulse was significantly increased 73 ± 2 versus 79 ± 2 beats/min, \( P < 0.001 \). Pre- and post-saline, mean systolic and diastolic blood pressure (138 ± 3 versus 134 ± 2 mmHg, \( P < 0.3 \); 83 ± 1 versus 84 ± 1 beats/min, \( P = 0.7 \)), respectively, did not differ, and mean pulse rate increased significantly 73 ± 2 versus 77 ± 2 beats/min, \( P < 0.02 \).

Mean ejaculate parameters for all subjects after placebo or oxytocin administration are shown in Table I and Figure 1. I.v. oxytocin resulted in no change in ejaculate volume (\( P = 0.4 \)), total sperm count (\( P = 0.14 \)) or World Health Organization (1999) grades ‘a’ or ‘b’ sperm motility (\( P = 0.9 \)). There was no significant correlation between the change in total sperm count and FSH levels (\( r = -0.32, P = 0.2 \)), and the change in total sperm count and E2 levels (\( r = -0.02, P = 0.9 \)).

In a subset of subjects with total sperm counts of <100, the mean ejaculate parameters are shown in Table II and Figure 2. There was no significant change in total sperm count (\( P = 1.0 \)) or sperm motility (\( P = 0.7 \)). One subject was not included in the analysis as he had a dramatic response to oxytocin, with the total sperm count increasing from 18 to 180 000. This subject had idiopathic OAT, a low ejaculate volume of 0.7 ml and normal FSH of 1.9 U/l. There was no correlation between change in total sperm count in response to oxytocin and FSH levels (\( r = -0.32, P = 0.2 \)), and between change in total sperm count in response to oxytocin and E2 levels (\( r = -0.02, P = 0.9 \)).

When ejaculate parameters were analysed according to diagnosis, in the subgroup of 29 men with OAT, i.v. oxytocin did not increase sperm output; similar results were found in the subgroup of 16 men with undescended testes. No significant response in sperm output was seen in the 14 men who experienced flushing (\( P = 0.13 \)). A subanalysis of 17 men with FSH levels <8 U/l resulted in no increase in total sperm count after i.v. oxytocin (\( P = 0.21 \)).

Discussion

A pulse of oxytocin presumably of hypothalamic origin is associated with ejaculation. Oxytocin is also produced locally in the testes and possibly also the epididymis and prostate (Ivell et al., 1997). Local oxytocin is important for regulating basal contractility of the cauda epididymis, and also increases the conversion of testosterone to dihydrotestosterone (Nicholson et al., 1991). In this study, we attempt to mimic the pulse of oxytocin associated with ejaculation in order to increase epididymal contractility and hence the release of stored sperm in men scheduled to undergo ICSI.

Our results in this study demonstrate that after a single i.v. dose of oxytocin, there is no significant increase in total sperm count.

Table II. Ejaculate parameters in response to placebo or oxytocin (750 mU) administration in men with a total sperm count <100 (\( n = 16 \))

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Oxytocin</th>
<th>( P \text{-value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejaculate volume (ml)</td>
<td>4.2 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Total sperm count/ejaculate</td>
<td>55 ± 24</td>
<td>54 ± 49</td>
<td>1.0</td>
</tr>
<tr>
<td>Progressive a motile sperm (%)</td>
<td>7.9 ± 3.0</td>
<td>9.3 ± 2.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Progressive b motile sperm (%)</td>
<td>13.4 ± 3.5</td>
<td>11.5 ± 3.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

One subject not included in the analysis had an increase in total sperm count from 18 after placebo to 180 000 after oxytocin.
count or motility in severely oligozoospermic men. Our findings are therefore consistent with previous studies in humans which failed to reveal any correlation between plasma oxytocin levels and sperm parameters (van Rijen et al., 1996). In a more recent study (Goverde et al., 1998), serum oxytocin levels and oxytocin seminal plasma levels were found to be similar in normozoospermic versus astheno/oligo/teratozoospermic versus azoospermic patients.

Previous studies in humans have shown no effect of i.v. oxytocin (10 IU) on sperm output in five men with a mean sperm count of 8.9 × 10⁶/ml (Powers et al., 1982). A single intranasal dose of oxytocin (16 IU) in healthy men resulted in no effect on seminal parameters or time to ejaculation (Walch et al., 2001). In a recent study of five severely oligozoospermic men (mean total sperm count 19.5), i.v. oxytocin (2.5 IU) resulted in a non-significant tendency to increased sperm output (Filippi et al., 2002a). Results from animal studies are contradictory, with increased sperm output in Holstein bulls (Berndtson and Igboeli, 1988) and sheep (Nicholson et al., 1999) in response to i.v. oxytocin, but no effect on sperm output in rabbits (Agmo, 1975).

Prior to commencing this study, we examined the effects of a single i.v. dose of 50 or 500 mIU of oxytocin on ejaculate parameters in 13 men with oligozoospermia (sperm counts between 1.5 and 10 × 10⁶/ml). After 3 days of sexual abstinence, patients delivered a semen sample within 5 min of the i.v. injection. There was no increase in mean sperm count after oxytocin injection compared with placebo. However, in four of the 13 patients, there was an increase in sperm counts after 50 mIU of oxytocin and also an increase after 500 mIU of oxytocin. Only the four subjects who responded to 50 mIU also responded to 500 mIU, suggesting that only those responding to lower doses also respond to higher doses. Based on these preliminary results, we chose a slightly higher dose of 750 mIU, to stimulate epididymal emptying and to minimize side effects such as feeling flushed, headache and a peculiar taste which have been described in male volunteers after i.v. injection of 1 or 10 IU (Powers et al., 1982; De Groot et al., 1995). The pharmacokinetics of oxytocin after an i.v. dose of 1 IU raise plasma oxytocin levels markedly above the baseline level (De Groot et al., 1995). This increase is greater than the 5-fold rise in plasma oxytocin described at ejaculation (Murphy et al., 1987). After i.v. administration of 1 IU to men, the distribution half-life was 0.049 ± 0.106 h and the elimination half-life was 0.33 ± 0.23 h (De Groot et al., 1995). This corresponds to a plasma half-life of 8–10 min. However, it remains possible that higher doses may be more effective, and this should be explored in the future.

During sexual arousal in men, there is a selective release of oxytocin at the time of ejaculation. Plasma oxytocin levels rise ~5-fold at the time of ejaculation (Ogawa et al., 1980; Murphy et al., 1987) and then fall to basal in 30 min. However, no differences have been found in the oxytocin levels at ejaculation in fertile compared with infertile men (Ogawa et al., 1980). With regard to seminal parameters, Zavos et al. (1985) reported that the quality of an ejaculate produced during sexual intercourse is superior to that obtained by masturbation with respect to semen volume, sperm concentration, progressive motility and morphology. Improved sexual stimulation may result in improved (post-testicular) emptying of spermatozoal stores in men.

Male mice deficient in oxytocin generated using embryonic stem cell technology are fertile and do not have any reproductive behavioural or functional defects in the absence of oxytocin (Nishimori et al., 1996). In order to establish the role of oxytocin in the process of spermiation and sperm transfer, oxytocin knockout (OTKO) mice deficient in testicular oxytocin and mice containing an oxytocin transgene (bOT4.2) that overexpress testicular oxytocin were compared with wild-type mice (Assinder et al., 2002). No difference was found in epididymal weight or diameter and area of seminiferous tubules. Germ cell development was similar in all genotypes. Oxytocin was important to promote sperm release into the epididymis as a result of early spermiation and transport of immotile sperm from the testes into the epididymis.

In conclusion, this study demonstrates that i.v. oxytocin did not improve the release of sperm in this group of severely oligozoospermic men. Perhaps this subgroup studied have intact ejaculatory function and the defect does not lie in the epididymis. It is also possible that endogenous oxytocin is functioning at maximal capacity and contractility is not enhanced by increasing plasma levels further. However, it also remains possible that higher doses of oxytocin may be more effective, and this should be explored in the future.

Acknowledgements
The authors thank Raphaele Kürtén, Daniela Schmidt and Sabine Rehr for their excellent technical support.

References

Oxytocin treatment for male infertility


Submitted on March 28, 2003; resubmitted on May 19, 2003; accepted on June 30, 2003