Effect of atosiban on rabbit embryo development and human sperm motility

Piotr Pierzynski, M.D., Ph.D.,[a] *Barbara Gajda, M.Sc., Ph.D.,*[b] *Zdzislaw Smorag, M.Sc., Ph.D.,*[b] *Allan Dahl Rasmussen, Ph.D.,*[c] *and Waldemar Kuczynski, M.D., Ph.D.*[a]

[a] Center for Reproductive Medicine KRIOBANK, Bialystok, Poland; [b] National Research Institute of Animal Production, Department of Animal Reproduction Biotechnology, Balice/Krakow, Poland; and [c] Ferring Pharmaceuticals A/S, International PharmaScience Center, Department of Non-Clinical Development, Copenhagen, Denmark

Objective: To investigate embryotoxic potential and effects on human sperm motility of the mixed vasopressin V_{1a}/oxytocin receptor antagonist atosiban considered for novel indication of improvement of uterine receptivity in embryo-transfer recipients.
Design: One-cell rabbit embryo bioassay and human sperm motility bioassay were performed in control media or in media containing atosiban.
Setting: Private center of reproductive medicine and academic research institute of reproduction biotechnology.
Animal(s): Rabbit females (New Zealand and California, N = 15) aged 4.5–6.5 months.
Intervention(s): In vitro exposure of one-cell rabbit embryos and human sperm to atosiban in the range of therapeutic concentrations clinically occurring in human beings.
Main Outcome Measure(s): Embryo development and sperm motility.
Result(s): Preimplantation development of one-cell rabbit embryos was not affected by atosiban in the concentrations ≤15,000 nM, which was 50-fold higher than the mean plasma concentration reached during regular therapy (300 nM). Atosiban did not affect human sperm motility in concentrations of ≤3,000 nM, in other words, 10 times the human mean plasma concentration.
Conclusion(s): Clinical application of atosiban in the proposed indication may be safe for embryos because it is compatible with preimplantation rabbit embryo development and human sperm motility. (Fertil Steril® 2007;87: 1147–52. ©2007 by American Society for Reproductive Medicine.)

Key Words: Embryotoxicity, rabbit embryo bioassay, human sperm motility, atosiban, oxytocin antagonists, embryo transfer, IVF

Atosiban (TRACTOCILE; Ferring Pharmaceuticals A/S, Copenhagen, Denmark) is a uterine-specific, mixed vasopressin V_{1a} and oxytocin-receptor antagonist that is registered for tocolysis in imminent premature birth (1). It also has been demonstrated to inhibit uterine contractility in nonpregnant women (2). For the novel clinical application of embryo transfer (ET), atosiban is suggested to decrease uterine contractions and promote uterine receptivity in patients undergoing ET (3).

Pregnancy rates after IVF-ET decrease in a stepwise fashion with increasing frequency of uterine contractions (4). In ET recipients, in addition to spontaneous contractions, the uterus is also exposed to strong contractile stimuli arising from transcervical passage of the transfer catheter and, in some cases, from using a tenaculum (5, 6). Exaggerated uterine contractile activity may last as long as 1 hour after ET and cause expulsion of embryos from the uterus (6). It has been shown that up to 50% of embryos could be found in the uterine cavity after ET (7).

Application of oxytocin antagonists like atosiban or barusiban (8) during ET may decrease uterine contractions and prevent embryo expulsion. Prolonged application may also facilitate embryo implantation that takes place 48–72 hours after the ET (9, 10).

A good safety profile of atosiban already has been demonstrated in human studies preceding its registration (11, 12), although these were conducted after the 24th gestational week (the time of completion of fetal organogenesis). Atosiban showed no postimplantation fetal toxicity in studies performed in rats and rabbits (13, 14). Also, maternal treatment with atosiban caused no fetotoxicity in a rat cross-fostering study (15).

However, these studies did not cover the preimplantation phase of the embryonic development. Because the possibility of contact between early embryos and maternal blood cannot

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Reprint requests: Piotr Pierzynski, M.D., Ph.D., Center for Reproductive Medicine KRIOBANK, Stoleczna 11, 15-889 Bialystok, Poland (FAX: 48857441378; E-mail: piotr.pierzynski@wp.pl).

be excluded during ET (16), clinical application of atosiban before and during ET should be preceded with evaluation of potential risks for patients and transferred embryos. To acquire embryotoxic data that could support such a novel clinical application, an embryotoxicity study was performed. Embryotoxic potential of atosiban was assessed in rabbit one-cell embryos (see Rabbit Embryo Bioassay) and human sperm (see Human Sperm Motility Bioassay). This is the first study on the early embryotoxicity of atosiban.

MATERIALS AND METHODS

Rabbit Embryo Bioassay

The experiments were carried out after receiving consent from the local ethics committee in 262 one-cell rabbit embryos. Rabbit females (New Zealand and California, N = 15) 4.5–6.5 months of age and weighing 3–4 kg were used as donors. Females were superovulated by IM injection of 100 IU of postmenopausal gonadotropin (Serogonadotropin; Biowet, Poland), followed by 100 IU of hCG (Biogonadyl, Biomed, Poland), injected IV 72 hours later. Immediately after hCG injection, donors were inseminated. The embryos at the one-cell stage were collected at 18 to 20 hours after insemination. The embryos were recovered at room temperature by flushing the Fallopian tubes with 10 mL of Dulbecco's solution (PBS, Sigma) supplemented with 20% fetal calf serum (Sigma). The recovered embryos were examined morphologically under a stereomicroscope.

Morphologically normal embryos were divided into six groups: one control and five experimental groups. Experimental embryos were cultured in vitro in B2 medium (INRA, Ménézo, bioMerieux, France) (17) with addition of atosiban (Ferring Pharmaceuticals A/S, Denmark) at concentrations of 300 nM, 1,000 nM, 3,000 nM, 6,000 nM, and 15,000 nM (groups I–V, respectively). Control embryos (group VI) were cultured in B2 medium. The embryos were washed in a B2 medium and cultured in 1.0 mL of the medium in four-well multidishes (Nunc, Denmark) in a CO_2 incubator (5% CO_2 in air at 38°C) for 3 or 4 days. The cultured embryos were evaluated at 24-hour intervals (18).

The viability of embryos was determined by estimating the percentage of expanded or hatched blastocysts. Assessments were carried out under a stereomicroscope (Nikon). Moreover, a sample of the embryos that developed to the blastocyst stage were assessed for cell number. They were stained with 2 μg/mL of bisbenzimide (Hoechst 33342) in PBS for 15 minutes and put on a glass slide with a drop of the dye solution (19, 20). Counts were taken with a fluorescence microscope (Nikon).

Analysis of repeated marginal probabilities was used for the assessment of influence of atosiban concentration and exposure time on the development of embryos. A variance analysis test was used for comparison of numbers of cells in blastocysts. Test assumptions were verified by using the Kolmogorov-Smirnov and Bartlett tests. All statistical procedures were undertaken with the SAS STAT 8 package.

Human Sperm Motility Bioassay

Assessments of human sperm motility were performed on fresh samples taken from 15 healthy donors with perfect seminal parameters. Initial evaluation of parameters of sperm motility and velocity showed no significant difference between consecutive donors. Swim-up ascending migration on human sperm preparation medium (21) was applied to select alive and motile spermatozoa. After such a selection, each semen sample was divided into eight equal aliquots (about 200 μL each, volume adjusted to maintain the spermatozoa concentration at 10 million/μL) that were transferred to sterile Eppendorf tubes. In addition to two control tubes, six tubes had atosiban added at concentrations of 300 nM, 1,000 nM, and 3,000 nM (two aliquots per concentration).

Throughout the time of the experiment (24 hours), tubes were incubated in a 5% CO_2 environment, with constant temperature and humidity conditions. Sperm motility was assessed in a computer-assisted sperm analyzer (Hobson Sperm Tracker, Model 7V1B; Hobson Tracking Systems Ltd., Sheffield, United Kingdom) connected to a contrast phase microscope (Olympus BX-40; Olympus Corp, Japan) that was equipped with a heating table. Computer-aided sperm assessments were performed at a $\times$400 magnification after 1, 8, and 24 hours of exposure to atosiban. For each assessment, 4 μL of each aliquot was added to a warmed Cell Vue Glass Semen Analysis Chamber (Fertility Technologies Inc). The sperm remained in the calibrated frame for $\geq$3 seconds (minimum track time) before their tracks were accepted in the system.

Microscopy was performed at $\times$400 magnification in a search radius of 12.50 μm, at a framing rate of 25 Hz (PAL video). Computer-aided sperm assessment was performed over a fixed time interval of 120 seconds. All the assessments were performed by the same technician. The kappa value of intraobserver variability was equal to 0.8; accuracy and reproducibility of the Hobson Sperm Tracker System was 97%.

In total, 120 samples were assessed by 375 measurements (3 measurements at 1, 8, and 24 h per sample + 15 control measurements at zero timepoint). In the analysis, parameters of sperm motility, that is, total percentages of motile, actively motile, and hyperactivated spermatozoa (Motile%, Active% and Hyper%, respectively) as well as parameters of sperm velocity, that is, curvilinear (total) and straight-line velocity (VCL and VSL, respectively), were included. Statistical analysis involved two-factor repeated measures analysis of variance. When data did not satisfy the assumption of Type H covariance (test for sphericity) (22), the P value was calculated for the adjusted test (23). P values of $>.05$ were accepted as statistically significant.