

Brief Report

Oral probenecid improves sperm motility in men with spinal cord injury

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Study Design: Prospective cohort study (twenty men with spinal cord injury [SCI]).

Objective: Determine if administration of oral probenecid results in improved sperm motility in men with SCI.

Setting: Major university medical center.

Methods: Twenty men with SCI were administered probenecid for 4 weeks (250 mg twice a day for 1 week, followed by 500 mg twice a day for 3 weeks). Semen quality was assessed at three time points: pre-treatment, post-treatment (immediately after the 4-week treatment), and follow-up (4 weeks after the last pill was ingested).

Result(s): Probenecid was well-tolerated by all subjects. Sperm motility improved in each subject after 4 weeks of oral probenecid. The mean percent of sperm with progressive motility increased from 19% to 26% ($P < 0.05$). A more striking increase was seen in the mean percent of sperm with rapid linear motility, from 5% to 17%, ($P < 0.001$). This improvement continued into the four week follow up period. Similar improvements were seen in the total motile sperm count (15 million, 28 million, and 27 million at pre-treatment, post-treatment, and follow-up, respectively). Sperm concentration was not significantly different at pre-treatment, post-treatment, and follow-up, (52 million, 53 million and 53 million, respectively).

Conclusion: This study showed that administration of an oral agent (probenecid) known to interfere with the pannexin-1 cellular membrane channel, can improve sperm motility in men with spinal cord injury. It is the first study to report improved sperm motility after oral medication in men with SCI.

Keywords: Probenecid, Inflammasome, Spermatozoa, Spinal cord injuries, Infertility

Introduction

Most men with spinal cord injury (SCI) are infertile due to a combination of erectile dysfunction, ejaculatory dysfunction, and abnormal semen quality. Therapies are available for erectile dysfunction and ejaculatory dysfunction in this patient population.^{1,2} To date, however, no therapy is available for their abnormal semen quality, which is characterized by normal sperm count, but abnormally low sperm motility and viability.^{3,4} Evidence suggests that inflammatory factors in the semen contribute to the problem. For example, inflammatory cytokines are elevated in semen of men with SCI.^{5,6} Neutralization of these cytokines improves sperm motility.^{7,8} Furthermore, proteins contributing to the release of these cytokines (i.e. constituents of the

inflammasome signaling mechanism) are more prevalent in semen of men with SCI than controls.⁹ Blocking these proteins *in vitro* improves sperm motility.¹⁰

An important pathway leading to activation of the inflammasome is the pannexin-1 channel.¹¹ Activation of the pannexin-1 channel allows intracellular entry of various molecules or proteins which activates the inflammasome, leading to a cascade of events, ultimately resulting in the release of inflammatory cytokines.¹² We hypothesized that blocking the pannexin-1 channel would lead to improved sperm motility in men with SCI. We confirmed this hypothesis using probenecid *in vitro* (unpublished data). Probenecid is an established medication that inhibits the pannexin-1 channel. This drug has been safely used for decades in the treatment of gout.¹³ The goal of the present study was to determine if probenecid, administered orally to men with SCI, would lead to improved sperm motility.

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Materials and methods

Patients/semen collection

Twenty male subjects with SCI were enrolled in this study. All subjects had been injured for more than one year. Their mean age \pm standard error of the mean (SEM) was 38.0 ± 1.8 years (range 25–55 years). Their level of injury ranged from C2 to T12. None of the subjects had taken any medication known to interfere with fertility within the 6 months prior to their participation.

All patients were anejaculatory. Semen was obtained by the standard methods of penile vibratory stimulation (PVS, $n = 14$ subjects) or electroejaculation (EEJ, $n = 6$ subjects).¹⁴ Antegrade fractions only (no retrograde fractions) were used in the study. The same method of semen collection (either PVS or EEJ) was used for all semen collections of an individual patient.

Study design

All subjects were participants in the Male Fertility Research Program of the Miami Project to Cure Paralysis at the University of Miami School of Medicine in Miami, Florida. Eligibility criteria for the present study were stable semen parameters on 3 assisted ejaculation procedures performed prior to participation in the study.

Oral probenecid

Subjects were administered oral probenecid in a regimen typical for a patient with gout.¹⁵ Probenecid tablets (Watson Pharma Inc., Corona, CA, USA) were given by mouth, 250 mg twice daily for 1 week. If no adverse effects were observed and the drug was well-tolerated, subjects were then directed to take 500 mg of probenecid twice daily for the next three weeks. The subjects were contacted weekly for these ensuing 3 weeks and queried about any side effects. Semen was collected and analyzed at three time points:

Pre-treatment: Two to three hours before the first dose of probenecid.

Post-treatment: At the conclusion of the 4-week trial of probenecid.

Follow-up: One month after the last dose of probenecid was ingested.

Semen analysis

Sperm motility was assessed based on WHO 1999 criteria in which four grades of motility were assessed: (a) rapid linear motility; (b) sluggish motility; (c) non-progressive motility; and (d) immotility.¹⁶ Categories (a) and (b) were summed to calculate progressive

motility. Additionally, semen volume (cc) and sperm concentration ($10^6/\text{cc}$) were measured. Total motile sperm count was calculated (semen volume \times sperm concentration \times percent of sperm with progressive motility).

Statistical analysis

GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Statistical significance was considered at $P \leq 0.05$. Each subject served as his own control. Repeated measures *t*-tests were used to compare sperm motility at pre-treatment, post-treatment and follow-up.

Results

Probenecid was well tolerated by all 20 subjects. No changes were reported in their general health while taking the medication or during the follow up period. All subjects completed the study.

Sperm motility improved in each subject after treatment with probenecid. The mean percent of sperm with progressive motility improved significantly after four weeks on probenecid and declined slightly from that point to the follow-up period. A more striking increase was seen in the percent of sperm with rapid linear motility, from 5.7% to 17.0%. This improvement continued into the follow-up period (Table 1).

Sperm concentration remained unchanged during the study. The total motile sperm count improved significantly after treatment with probenecid, and remained improved into the follow up period, but did not reach statistical significance (Table 2).

Table 1 Sperm motility after oral administration of probenecid.

Grade of sperm motility:	Pre-Treatment (Mean \pm SEM)	Post-Treatment (Mean \pm SEM)	Follow-Up (Mean \pm SEM)
Progressive motility (%)	19.0 \pm 2.8	26.0 \pm 3.7	23.0 \pm 4.1
Compared to pre-treatment:		$P < 0.05$	NS
Rapid linear motility (%)	5.7 \pm 1.5	17.0 \pm 3.3	17.0 \pm 3.7
Compared to pre-treatment:		$P < 0.001$	$P < 0.001$

Notes: Oral probenecid was administered to 20 men with spinal cord injury. Sperm motility was assessed prior to the first dose (Pre-Treatment), after a four-week course of probenecid (Post-Treatment), and four weeks after the last pill was ingested (Follow-Up). The percent of sperm showing progressive motility (rapid linear motility + sluggish motility) is presented as mean \pm standard error of the mean (SEM). The sub-fraction of sperm showing the grade of rapid linear motility is also presented as mean \pm SEM. NS, not significant.

Table 2 Sperm concentration and total motile sperm count after oral administration of probenecid.

Sperm parameter:	Pre-Treatment (Mean ± SEM)	Post-Treatment (Mean ± SEM)	Follow-Up (Mean ± SEM)
Sperm concentration (10 ⁶ /cc)	52.0 ± 8.5	53.0 ± 9.3	53.0 ± 10.0
Compared to Pre-Treatment:		NS	NS
Total motile sperm count (10 ⁶ /ejaculate)	15.0 ± 4.4	28.0 ± 6.5	27.0 ± 10.0
Compared to pre-treatment:		P < 0.05	NS

Notes: Oral probenecid was administered to 20 men with spinal cord injury. Sperm concentration and total motile sperm count were determined prior to the first dose (Pre-Treatment), after a four-week course of probenecid (Post-Treatment), and four weeks after the last pill was ingested (Follow-Up). Sperm count and total motile sperm count are presented as the mean ± standard error of the mean (SEM). NS, not significant.

Discussion

This study showed a statistically and clinically significant improvement in sperm motility of men with SCI after treatment with oral probenecid. *This study represents the first report of improved sperm motility from an oral medication in men with SCI.*

These findings follow previous studies investigating causes of low sperm motility in men with SCI. This condition does not seem to be due to factors such as scrotal hyperthermia, endocrinopathy, infrequency of ejaculation, methods of bladder management, methods of semen retrieval, or aging post-injury.^{2,3} Instead, studies indicate that an abnormal seminal plasma environment contributes to the problem. For example, seminal plasma from men with SCI inhibited motility of sperm from non-injured men.¹⁷ Further, sperm aspirated from the vas deferens of men with SCI had significantly higher motility than sperm from those same men's ejaculates.¹⁸ Seminal plasma from most men with SCI had elevated concentrations of activated T-lymphocytes,^{6,19,20} which are known to secrete cytokines.²¹ Elevated concentrations of pro-inflammatory cytokines were found in semen of men with SCI.⁵ When these cytokines were neutralized *in vitro* using monoclonal antibodies or receptor interference agents, sperm motility improved,^{7,8} indicating a role of the semen cytokines in lowering sperm motility in men with SCI. The inflammasome signaling mechanism can trigger release of cytokines.²² Components of the inflammasome signaling mechanism were found to be elevated in semen of men with SCI.⁹ When these components were blocked *in vitro*, sperm motility improved.¹⁰

This study sought to interfere with a mechanism that participates in triggering activation of the inflammasome, namely, the pannexin-1 channel. Oral administration of probenecid, a pannexin-1 channel blocker, led to improved sperm motility in men with SCI. Our interpretation of this finding is that the process of inflammasome activation is ongoing in the seminal plasma, and that interfering with the pannexin-1 channel by administration of probenecid creates an improved seminal plasma environment that is protective, to a degree, to the fresh sperm being added to the ejaculate.

The significance of this finding has ramifications for the clinical management of infertility in men with SCI. Reproductive function is a high priority for persons with SCI.²³ The ability to reliably and easily increase the number of motile sperm in the ejaculate of men with SCI will increase the options for the couple trying to achieve pregnancy. For example, intrauterine insemination or intravaginal insemination become more viable options when higher numbers of motile sperm are available.²⁴ Since the subjects had stable semen parameters from prior ejaculations at our center, the improvements seen in our study cannot be attributed to an increase in the frequency of ejaculations.

Further research will establish the optimal dosage and duration of probenecid administration in men with SCI. Early studies of the proteomics of the seminal plasma in men with SCI versus controls, as well as specific changes in the proteomic profile of semen from men with SCI who took probenecid, have been informative regarding mechanisms of infertility in these men.²⁵

With the demonstration of a simple intervention that improves sperm motility in men with SCI, this treatment holds promise for improving fertility options in this severely affected patient population.

Conclusion

This study has shown that administration of an oral agent (probenecid) known to interfere with the pannexin-1 cellular membrane channel, can improve sperm motility in men with spinal cord injury. It suggests that at least one cellular pathway contributing to impairment of sperm motility can be easily altered clinically with a positive result. This is the first study to report improved sperm motility after oral medication in men with spinal cord injury.

Disclaimer Statements

Contributors EI, CML and NLB participated in study design, execution, analysis, manuscript drafting and critical discussion. TCA participated in study design, execution and analysis.

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Conflicts of interest The authors report no conflict of interest.

Ethics approval This study was approved by the University of Miami Institutional Review Board.

Consent Informed consent was obtained from all subjects.

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