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Sperm Motility in Men With Spinal Cord Injuries Is Enhanced by Inactivating Cytokines in the Seminal Plasma

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ABSTRACT: The goal of this study was to determine whether inactivating specific cytokines in seminal plasma improves sperm motility in men affected by spinal cord injury (SCI). For this purpose, we used monoclonal antibodies to interleukin 6 (IL6), interleukin 1 beta (IL1-β), and tumor necrosis factor alpha (TNF-α), all 3 cytokines having been previously detected at high concentrations in the seminal plasma of patients with SCI. In a group of 17 SCI men with low sperm motility (mean ± SE, 20.1% ± 3.1%), treatment with the 3 monoclonal antibodies at the median neutralization dose concentrations for 1.0 to 1.5 hours improved sperm motility in all cases. Effectiveness was higher in those specimens with a pretreatment sperm motility between 11% and 30% (from 19.3% ± 1.4% to 41.9% ± 4.9%, $P < .0002$), suggesting that pretreatment sperm motility might represent an indicator of cell damage and, therefore, a factor that influences monoclonal antibody effectiveness. To the best of our knowledge, these results represent the first rational treatment for improving low sperm motility in these severely affected patients.

Key words: Infertility, ejaculation, semen, IL1-β, IL6, TNF-α.

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Patients with spinal cord injury (SCI) have impaired sexual function and a unique sperm profile characterized by normal to high sperm concentrations and abnormally low sperm motility (Brackett et al, 1996b, 1997). The cause of this condition is unknown but might be related to abnormalities in the seminal plasma. Activated T-cell subpopulations were identified in the semen of these patients (Basu et al, 2002), and abnormal concentrations of cytotoxic cytokines were found in seminal plasma of SCI men with low sperm motility (Basu et al, 2004). To determine whether elevated cytokines contribute to low sperm motility in men with SCI, this study evaluated the usefulness of specific monoclonal antibodies to improve sperm motility by blocking cytokine activity in fresh semen samples.

Materials and Methods

Subjects

All subjects (n = 17) were men with SCI who were participants in the Male Fertility Research Program of the Miami Project to Cure Paralysis at the University of Miami (School of Medicine, Miami, Fla). The mean (±SEM) age of subjects was 35.2 ± 2.2 years (range 21 to 43 years). All subjects were past the acute phase of injury, and their mean time postinjury was 13.3 ± 3.8 years (range 3 to 32 years). Levels of injury were C5 to C6 in 5 subjects, T1 to T7 in 6 subjects, and T8 to T12 in 6 subjects. Each subject had undergone at least 4 ejaculations spaced 4 to 8 weeks apart prior to semen collection for this study. All subjects were in good health and did not have any condition, other than SCI, known to interfere with fertility.

Semen Collection and Analysis

Only antegrade semen (ie, no retrograde semen) was collected from subjects by the standard method of penile vibratory stimulation (Brackett, 1999) because semen quality can be altered in retrograde ejaculates or if semen is collected by electroejaculation (Brackett and Lynne, 2000). Semen analysis was performed according to World Health Organization criteria (1999). Each semen specimen was first allowed to liquefy at room temperature. Sperm parameters were assessed by placing 6 μL of the semen specimen on a disposable semen analysis chamber (Cell-Vu; Fertility Technologies, Natick, Mass). Sperm motility was evaluated in semen specimens before exposure to monoclonal antibodies. The study was evaluator blind, in which the operator did not know the treatment conditions of the specimen being evaluated. Sperm motility was calculated by adding the percentage of rapid and sluggish sperm with forward movement. The same operator evaluated all specimens.

Monoclonal Antibodies

Specific monoclonal antibodies to human cytokine interleukin 1 beta (IL1-β), interleukin 6 (IL6), and tumor necrosis factor alpha (TNF-α) were used to neutralize cytokine activity in the seminal

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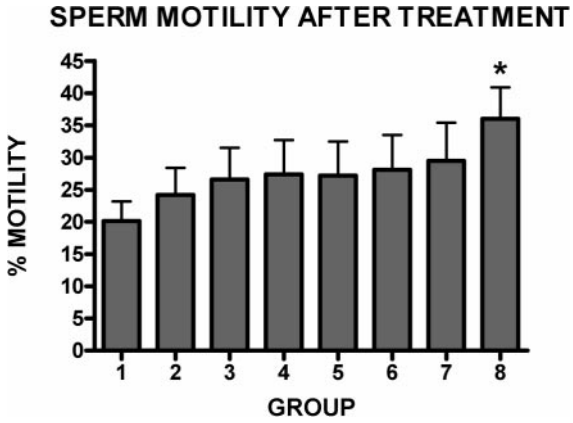

Group 1 indicates untreated (control group); group 2, mAb to TNF-α; group 3, mAb to IL1-β; group 4, mAb to IL6; group 5, mAb to TNF-α + IL1-β; group 6, mAb to TNF-α + IL6; group 7, mAb to IL1-β + IL6; group 8, mAb to all 3 cytokines. * $P < .01$. Bars show mean ± SEM. Numeric data for the Figure is shown as ''All Groups'' in the Table.

plasma. These agents were selected according to our previous finding that concentrations of these specific cytokines are elevated in the seminal plasma of patients affected by SCI (Basu et al, 2004).

Monoclonal antibodies to human IL1-β, IL6, and TNF-α (catalog numbers MAB 601, MAB 206, and MAB 610, respectively; R&D Systems, Minneapolis, Minn) were reconstituted in sterile phosphate-buffered saline (PBS, pH 7.2), and a stock solution of 500 μg/mL of each cytokine was prepared. Aliquots (200 μL) of the stock solution were transferred to sterile microfuge tubes and frozen at −20°C. The stock solution was further diluted to 10 μL/mL and 1 μL/mL with PBS and frozen at −20°C until used.

Experimental Design

Each semen specimen was separated into eight 50-μL aliquots, with each aliquot placed in a 1.5-mL microfuge tube. Specific monoclonal antibodies to IL1-β, IL6, and TNF-α were added directly to the semen aliquots in the tubes. Monoclonal antibodies were added singly and in all possible combinations as described below. Doses were adjusted according to the median neutralization dose information provided by the manufacturers. There were 8 different treatment groups for each specimen: 1) 50 μL semen + 10 μL buffer (untreated control), 2) 50 μL semen + 10 μL monoclonal antibody (mAb) to TNF-α (1 μg/mL), 3) 50 μL semen + 10 μL mAb to IL1-β (1 μg/mL), 4) 50 μL semen + 10 μL mAb to IL6 (1 μg/mL), 5) 50 μL semen + 10 μL mAb to TNF-α (1 μg/mL) + 10 μL mAb to IL1-β (1 μg/mL), 6) 50 μL semen + 10 μL mAb to TNF-α (1 μg/mL) + 10 μL mAb to IL6 (1 μg/mL), 7) 50 μL semen + 10 μL mAb to IL1-β (1 μg/mL) + 10 μL mAb to IL6 (1 μg/mL), 8) 50 μL semen + 10 μL mAb to TNF-α (1 μg/mL) + 10 μL mAb to IL1-β (1 μg/mL) + 10 μL mAb to IL6 (1 μg/mL). Concentrations of all cytokines were 1 μg/mL.

After 1.0 to 1.5 hours incubation at room temperature, sperm motility was analyzed in each preparation. Mean sperm motility in untreated vs treated preparations was compared by analysis of variance.

Results

Similar to previously published reports of semen quality obtained by penile vibratory stimulation (Brackett et al, 1997), the mean antegrade sperm concentration of SCI subjects was 77.6 ± 11.5 × 10^6 sperm/mL (millions of sperm per milliliter of ejaculate), and the mean sperm motility was 20.1% ± 3.1% (percentage of sperm with forward progression). The Figure shows the mean sperm motility in the 8 treatment groups. Sperm motility increased in all groups treated with monoclonal antibodies; however, statistical significance was achieved only in the group receiving monoclonal antibodies against all 3 cytokines (group 8).

Further analysis revealed that treatment success was related to pretreatment sperm motility (PSM). The Table shows the ranges of PSM and the resulting sperm motility following treatment with monoclonal antibodies. The groups with a PSM between 11% and 30% showed the greatest improvement after treatment with monoclonal antibodies, whereas the groups with a PSM below 10% or above 31% showed smaller improvements.

Results grouped by pretreatment sperm motility (PSM)

		Group*							
PSM, %	n	1	2	3	4	5	6	7	8
≤10	3	1.6 ± 1.6	3.0 ± 2.1	3.0 ± 2.1	3.0 ± 2.1	3.0 ± 2.1	2.3 ± 0.3	3.0 ± 1.5	4.0 ± 2.6
11–19	6	17.3 ± 3.5	23.3 ± 4.0	27.0 ± 7.6	27.0 ± 7.7	25.8 ± 5.8	30.3 ± 8.3	29.0 ± 8.7	37.2 ± 5.3†
20–30	5	21.4 ± 4.7	26.8 ± 7.3	26.8 ± 6.4	29.2 ± 9.7	27.6 ± 8.1	27.4 ± 9.0	30.2 ± 8.5	47.6 ± 8.8‡
≥31	3	41.6 ± 2.9	45.6 ± 8.4	49.3 ± 12.1	49.6 ± 12.5	54.0 ± 15.8	51.0 ± 11.3	56.3 ± 16.6	46.3 ± 5.7
All groups	(17)	21.2 ± 4.0	24.2 ± 4.2	26.6 ± 5.0	27.7 ± 5.4	27.3 ± 5.3	28.1 ± 5.5	29.6 ± 6.0	36.0 ± 4.0†

* Group 1 indicates untreated (control group); group 2, mAb to TNF-α; group 3, mAb to IL1-β; group 4, mAb to IL6; group 5, mAb to TNF-α + IL1-β; group 6, mAb to TNF-α + IL6; group 7, mAB to IL1-β + IL6; and group 8, mAb to all three cytokines. Data are expressed as mean ± SEM for each treatment group. Data for ''All groups'' is depicted graphically in the Figure.

† $P < .01$.

‡ $P < .03$.

Discussion

The cause of male infertility after SCI is an area of active research. The majority of men with SCI have impaired semen quality characterized by low sperm motility and leukocytospermia. It is possible that these characteristics are associated because most of the white blood cells present in semen specimens from men with SCI are activated T cells with a capacity to synthesize cytokines (Basu et al, 2002). The relationship between cytokines and human reproduction represents a growing area of investigation because of its involvement in several aspects of human infertility. Cytokines affect human sperm motility, increase the production of reactive oxygen species, and reduce ability to penetrate ova (de Lamirande et al, 1995). Our group has determined that men with SCI have elevated concentrations of inflammatory cytokines in the seminal plasma, specifically TNF-α, IL1-β, and IL6 (Basu et al, 2004). The actual source of these cytokines in the semen is unknown, but apparently the cytokines are restricted to the urogenital system because they are not detected in patient blood samples.

Additional studies suggest that seminal plasma contributes to low sperm motility in men with SCI. For example, seminal plasma from men with SCI rapidly reduces sperm motility of normal men (Brackett et al, 1996a). Another study showed that sperm collected from the vas deferens of men with SCI (ie, sperm that had not been in contact with the seminal plasma) had higher motility than sperm from their ejaculates, whereas control subjects had similar sperm motility in their vas deferens vs their ejaculates (Brackett et al, 2000).

We investigated whether low sperm motility in men with SCI could be improved by inactivating cytokines in seminal plasma with the use of specific monoclonal antibodies to TNF-α, IL1-β, and IL6. In vitro treatments were based on the 3 monoclonal antibodies mentioned above, and 8 different treatment groups were designed to compare all possible treatment combinations. The results showed that the low sperm motility of the untreated control group could be improved by treatment with monoclonal antibodies, singly and in all possible combinations; however, statistical significance was reached only in the group treated with all 3 monoclonal antibodies (group 8).

Although the number of subjects studied is small, treatment success appears to be related to PSM (Table). The groups at either extreme (ie, the group with PSM $\leq$ 10% or the group with PSM $\geq$ 31%) showed small but nonsignificant improvement in sperm motility in all treatment combinations. The groups with PSMs of 11% to 19% and 20% to 30% showed significant improvement when treated with the combination of all 3 monoclonal antibodies (ie, from 17.3% to 37.2% and from 21.4% to 47.6%, respectively).

The reason for these differences is currently not known. Possible causes for these observations are topics for further investigation. For example, in the group with the highest mean PSM (41.6% $\pm$ 2.9%), with motility close to normal, other mechanisms at play might be higher concentrations of stimulatory cytokines, lower concentrations of reactive oxygen species, or different cytokine receptor expression (Fierro et al, 2002). For the group with the lowest mean PSM (1.6% $\pm$ 1.6%), these low sperm motilities might represent overwhelming irreparable damage related to any of the above-mentioned factors or simply to higher concentrations of toxic cytokines in the seminal plasma.

Cytokines rarely act alone and generally are expressed in groups or patterns in response to a stimulus. Their effects on other cells and cytokine pathways could be stimulatory or inhibitory (Tanaka, 2000; Petty, 2003). How cytokines negatively affect sperm motility is not fully understood. Cytokines are thought to modulate pro-oxidant and antioxidant activities in the seminal plasma, with resultant negative effects on sperm motility and fertilization (Sanocka et al, 2003). Sperm membrane phospholipid composition might be affected by oxidative damage with a resultant negative effect on fertilization potential (Zalata et al, 1998). There have been strong associations in other systems between elevations of certain seminal plasma cytokines and poor sperm motility (Sikka et al, 2001).

A relationship between reactive oxygen species (ROS) and sperm parameters has been well reported. ROS might act as intracellular signals to mediate the effects of cytokines (Sanocka et al, 2003). Our study looked only at a group of cytokines noted to be greatly elevated in the seminal plasma of SCI men. It did not account for other cytokine populations less profoundly elevated or even those found markedly lower in this group of subjects. Finally, the testing method used did not allow for analysis of each subject's seminal plasma cytokine concentrations. This information would have allowed the individualization of the amount of monoclonal antibodies for specific cytokines in each subject. Given these constraints, it is remarkable that simple addition of a combination of monoclonal antibodies resulted in significant improvement in sperm motility.

Conclusion

Neutralization of specific cytokines via monoclonal antibodies enhances sperm motility in men with SCI, and although the exact mechanism of the cytokine-sperm relationship remains unknown, to the best of our knowl-

edge, this is the first intervention that significantly improves sperm motility in these severely impaired patients.

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