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Mechanisms for poor sperm motility after spinal cord injury

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Background: Spinal cord injury-related male infertility is associated with abnormal semen qualities including poor sperm motility.

Objective: The current experiment examined the relationship between the extents of cord injury and motility-related sperm parameters in the rat at different phases of cord injury.

Methods: Spinal cord injury was induced in adult Sprague-Dawley rats by surgical transection of the cord (SCI), or contusion by weight-drop from different heights (SCC), at the level of the 9^{th} thoracic vertebra. At various times after the injury, epididymal sperm were recovered and vedio-taped for analysis of sperm motility, stained with fluorescent dyes for viability and mitochondrial potential assays, or stored frozen for measurement of cAMP and protein tyrosine phosphorylation.

Results: There were significant decreases in sperm motility in SCI rats 4-8 weeks post injury; significant recovery of sperm motility was only observed in <20% of the animals after > 12 weeks of recovery period. Sperm motility was also reduced in SCC rats during the first 4 weeks post injury. Thereafter, sperm motility recovered in those rats received weight-drop from 12.5 or 25 mm heights, but not in those received weight-drop from 50 or 75 mm heights. These effects were associated with parallel changes in sperm viability and mitochondrial potential. These results indicated that deterioration of sperm motility after cord injury is related to the extent of cord injury and time post-injury, and suggested that both cell death and abnormal energy metabolism were contributing factors for such effects. Western blotting revealed lower protein tyrosine phosphorylation in sperm of SCI and SCC rats. On the other hand, sperm cAMP contents were elevated in SCI and SCC rats. These results were consistent with altered cAMP signaling in the sperm after spinal cord injury.

Conclusion: Multiple factors are involved in deterioration of sperm motility after spinal cord injury.

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ABNORMAL SPERM NUCLEAR CONDENSATION IN SPINAL CORD INJURED RATS

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Abnormal sperm morphology has been implicated as one of the causes for spinal cord injury (SCI)-related male infertility. It is well documented that condensation of spermatid nuclei dictates normalcy of sperm head morphology, and this process is accomplished by formation of disulfite bonds between spermatid-specific nuclear proteins [transition protein (TP)-1 and 2, protamine (Pm)] that are expressed during specific steps of spermiogenesis. In this study we examined de-condensation of sperm heads of SCI rats after they were exposed to SDS and a reducing agent, dithiothreitol (DTT). In addition, we compared the expression of spermatid nuclear proteins in the testes of sham control and SCI rats using immunohistochemistry. Incubation of sperm of SCI rats with SDS/DTT for 5-15 minutes resulted in more extensive sperm head de-condensation compared to that of sham control rats. These results were consistent with abnormal spermatid nuclear condensation after SCI. Feeding of SCI rats with vitamin E (10 mg/kg) for 8 weeks attenuated the DTT-induced de-condensation of sperm heads, suggesting that oxidation-related mechanisms might be involved in impairment of sperm head maturation after SCI. Immuno-histochemistry revealed decreases in the expression of TP-1 and Pm in elongated spermatids of SCI rats, consistent with reduced mRNA transcripts for these proteins. In conclusion, SCI resulted in reduced expression of spermatid nuclear proteins. Such effects might impair the condensation of spermatid nuclei essential for morphogenesis of the sperm head, and could contribute to increases in sperm with abnormal morphology after SCI. Attenuation of such effects by vitamin E feeding offers a simple and effective approach to improve sperm quality of SCI men. This study was supported by VARR&D Service and New Jersey Commission for Spinal Cord Research.

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Impaired sperm function after spinal cord injury in the rat is associated with altered cAMP signaling

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Spinal cord injury is known to result in male infertility due to impairment of spermatogenesis and/or abnormal sperm function. Our previous experiments using cord-transected (SCI) rat have demonstrated changes in the characters of the seminiferous epithelium during the regression and restoration of spermatogenesis at various times after cord injury. In contrast, we found a persistence of spermatogenesis in spinal cord-contused (SCC) rats regardless of the extent of cord injury. Significant changes in the expression and cellular distribution of cAMP responsive element modulator (CREM) in spermatogenic cells in SCI and SCC rats suggest that altered cAMP signaling may underlie some effects of spinal cord injury on spermatogenesis. To further understand the impact of altered cAMP signaling in spermatogenic cells upon sperm functions, we further examined the normalcy of cAMP-related sperm functions in SCC and SCI rats. In addition, we also examined whether these effects could be prevented by exogenous testosterone or vitamin E. The results of multiple experiments revealed persistently lowered sperm motility in SCC and SCI rats which was associated with elevated sperm cAMP contents but overall reduced sperm protein phosphorylation. These results were consistent with altered sperm cAMP signaling after cord injury. Flow cytometric analyses revealed decreases in the uptake of fluorescent dyes specific for viability and mitochondrial function in the sperm of SCC and SCI rats, suggesting that decreases in viable cells and impaired sperm metabolism were the contributing factors for poor sperm motility after cord injury. Administration of exogenous testosterone lowered sperm motility in sham control rats in a dose-dependent manner; these effects were associated with a slight but significant increase in sperm cAMP level. Identical testosterone regimens also lowered sperm motility in SCI rats in which sperm cAMP levels were further increased by low doses of testosterone, but lowered by high doses of testosterone. Steady state sperm protein phosphorylation in SCI rats was stimulated slightly by exogenous testosterone to the extent comparable to that achieved in sham control rats. Vitamin E supplementation resulted in significant improvement of sperm functions in SCI rats during the chronic phase of the injury, suggesting that oxidative damage may contribute to abnormal sperm function. Failure to improve sperm function by vitamin E feeding during the acute phase of the injury, however, suggests that non-oxidative mechanisms were involved in abnormal sperm function during the early phase of cord injury. **Conclusion:** Abnormal sperm functions after spinal cord injury were associated with altered cAMP signaling that could be attributed to multiple mechanisms. Attenuation of these changes by exogenous testosterone and vitamin E suggests that these agents might have therapeutic roles in the preservation of sperm function after SCI.

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Cause for Abnormal Sperm Function After Spinal Cord Injury and Its Prevention Shulun Wang, Guichun Wang, Beverly E. Barton, Hosea F. S. Huang*, Department of Surgery, New Jersey Medical School, Newark and DVA New Jersey Health Care System, East Orange, NJ

Introduction: Previously we postulated that cAMP responsive element modulator (CREM)/cAMP signaling events might contribute to the effect of SCI on spermatogenesis and perhaps sperm functions. Clinical studies also suggested a role of reactive oxygen species (ROS) in abnormal sperm function after SCI. Current study examined the effects of vitamin E and testosterone (T) and on sperm functions in cord injured rats.
Methods: The spinal cord of adult male rats was injured by cord-transection (SCI) or cord-contusion (SCC) at the level T9. These rats were given subcutaneous implants of T capsules (TC, 1-10 cm), or daily dose of vitamin E (2 or 10 mg/kg), during the acute phase or chronic phase of the injury for 8-10 weeks.
Results: Sperm motility was reduced in SCC and SCI rats, and was associated with elevated sperm cAMP contents but reduced sperm protein phosphorylation, consistent with altered cAMP-related signaling events in abnormal sperm functions. These changes were associated with lower uptake of fluorescent dyes SYBR-14 and JC-1, indicating that reduced sperm viability and mitochondrial function were the contributing factors for poor sperm motility after cord injury. Exogenous T improved sperm viability and mitochondrial potential but further reduced sperm motility in SCI rats. A dose-dependent change in sperm cAMP levels in these rats implied a role of cAMP-related events in these effects. Vitamin E feeding improved sperm viability and mitochondrial potential but failed to maintain sperm motility in SCI and SCC rats during the acute phase of the injury; such effects were observed only in SCI rats during the chronic phase of the injury. These effects were not associated with changes in sperm cAMP levels.
Conclusion: Both cAMP and ROS related mechanisms are involved in the effects of spinal cord injury on sperm function. While these effects were attenuated by exogenous T and vitamin E, they are likely to benefit sperm functions though different mechanisms.

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