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# FINAL REPORT

## COVER PAGE

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2. Name of Organization/Institution

New Jersey Healthcare System
VA Medical Center East Orange

3. Grant Title

Effect of Erythropoietin Therapy on Acute Spinal Cord Injury and Mechanism of Action

4. Grant Number

05-3046-SCR-E-0

5. Grant Period Covered by the Report

June 15, 2005 – June 30, 2007
Note: one year budget approved with no-cost-extension in the second year

6. Date of Submission of the Report

June 15, 2008

## 1. Original aims of the project

**S. A. 1**: determine if erythropoietin therapy can favorably modify the clinical and histopathologic outcome in wild type mice after traumatic spinal cord injury. To qualify MHC II alterations and mononuclear/T cell infiltrate in treated animals versus wild type saline treated post spinal cord injured controls.

**S. A. 2**: determine if erythropoietin therapy can favorably improve the clinical and histopathologic spinal cord injury outcome in RAG1-/- immunodeficient mice versus (1) saline treated RAG1-/- cord injured mice or (2) saline treated wild type lesioned mice

**S. A. 3**: As a further test of our hypothesis, determine if erythropoietin therapy can favorably modify the clinical outcome in RAG1-/- immunodeficient mice that have received wild type mononuclear/T cells by adoptive transfer 24 hours before induction of spinal cord injury.

## 2 and 3. Project challenges and successes

Induction of mouse spinal cord injury

During the first 6-8 months of the project, we encountered technical challenges in producing a consistent contusive cord injury in mouse using the NYU Impactor; a device originally developed for rat spinal cord injury. We consulted the group at the Keck Center (Rutgers University) where attempts to establish a mouse contusive SCI model using the NYU Impactor had been in progress. In spite of our technical improvement and implementation of the BMS neurobehavioral scoring system for measuring functional recovery in SCI mice, our data still showed inconsistent injuries in groups of saline-treated control SCI mice. For this reason, we searched for a SCI impactor that worked better in mice. By reviewing the literature, we found several publications had adopted a commercially available SCI Impactor claimed to induce reproducible injuries in mice (the IH-0400 mouse/rat spinal cord impactor, Precision System and Instrumentation, Fairfax VA). We purchased this impactor and since then, we have successfully generated pretty consistent cord injuries in more than 200 wild type C57BL/6 mice.

During and after surgery, body temperature and depth of isoflurane anesthesia was closely monitored. We found it critical to use isoflurane inhalation anesthesia. This allowed us to eliminate animals with incomplete cord injury before initiating therapy, assuring that the similarly injured animals were equally divided into the different treatment arms. Following spinal cord injury, we observed the injured animals over 4-6 weeks. Locomotor recovery was assessed daily by the BMS scoring system, and we did not experience any major differences between the two independent observers in clinical scoring. In addition, we experienced little or no mortality during and post surgery (0-5% maximum).

With our improved techniques and the new SCI device described above, we pursued Specific Aim 1. A total of more than 200 mice were allocated to these experiments on EPO-treated or saline-treated control animals.

***S.A. 1:*** *determine if erythropoietin therapy can favorably modify the clinical and histopathologic outcome in wild type mice after traumatic spinal cord injury. To qualify MHC II alterations and mononuclear/T cell infiltrate in treated animals versus wild type saline treated post spinal cord injured controls.*

Effect of Epo therapy on SCI mice

We closely followed the dose levels of Epo that had been used by other investigators who reported beneficial effects of Epo in SCI. In our initial studies, Epo was administered intraperitoneally at a dose of 5,000 units/kg on Day 0, and was followed by a daily i.p. injection of 1,000 units/kg for 6 days. In other experiments, either a single dose of 5,000 units/kg i.p. or i.v. on Day 0, or daily i.p. injections of 1,000 units/kg for 7 days were employed. No excess mortality was observed from the Epo treatment, but the hematocrit was significantly elevated in Epo-treated animals within a week of therapy.

In spite of our initial impression that Epo may hasten the locomotive recovery in the early course of spinal cord injury (within a week), the repeat experiments using over a hundred of mice failed to show any significant beneficial effect of Epo when SCI mice were followed for up to 6 weeks following injury.

Immunohistochemistry and Western immunoblot assay

As reported in June 2005, we were able to demonstrate various immune and inflammatory cells in early stages of spinal cord injury using our immunohistochemical assay system (MHC class II, macrophage/microglia, GFAP+ astrocyte, CD3+ T cells, Gr-1 leukocyte antigens, etc.). We have also shown early cell demise characterized by TUNEL assay and extensive axon degeneration by SMI-32 immunoreactivity in injured cords. Blood brain barrier integrity was also assessed by the one step mouse IgG immunohistochemistry. Contrary to our expectations, none of the immune and inflammatory parameters listed above appeared to be altered by Epo therapy, with one exception that the BMS score in the early course of the disease was significantly correlated with the extent of blood brain barrier disruption in the traumatized cords whether Epo-treated or not, using mouse IgG immunohistochemistry ($p=0.012$).

In summary, despite the previous reports by other groups, we failed to demonstrate a significant beneficial effect of Epo in our mouse contusion SCI model, although there was a trend that Epo might hasten the functional recovery.

For all these reasons, we were not able to pursue the S.A 2 and S.A. 3.

**4 and 5 : Implications for future research and/or clinical treatment, and plans to continue this research, including applications submitted to other sources for ongoing support.**

We could not reproduce the early impressive results by other groups where Epo or chemically modified Epo showed dramatic effects on locomotive improvement in rodent models of SCI. In this regard, we are in accordance with a more recent publication that failed to demonstrate the neuroprotective efficacy of Epo in a rat contusion model of cord injury [1].

[1] Mann et al. Delayed treatment of spinal cord injury with erythropoietin or darbepoietin- a lack of neuroprotective efficacy in a contusion model of cord injury. Exp Neurol 2008; 211(1): 34-40.

Whereas no beneficial effect was found in a mouse spinal cord injury model, our experience in traumatic brain injury rodent models stands in marked contrast. We have found that both whole molecule Epo and low molecular Epo fragments are quite effective in reducing cell death and reducing lesional area by more than 50%. The spinal cord paradigm appears to be much more difficult to deal with than injury to the cerebral cortex.

We continue to carry out small pilot studies using the mouse spinal cord injury model and are currently testing new generation caspase inhibitors for beneficial clinical effects and reversal of spinal cord pathology.

**6. List and include a copy of all publications emerging from this research, including those in preparation.**

No publications were generated from our negative outcome data.

7. For the past two years, we have collaborated with the UMDNJ spinal cord research group headed by Dr. Heary in the Neurosurgery Department and help them set up their new laboratories. We have trained several post-docs and technicians from his group on the induction of the spinal cord injuries using our mouse contusion spinal cord injury equipment. His group has now successfully utilized this technology to lesion 150 rodent spinal cords.