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Final Report

Grant Title: Treating Spinal Cord Injury with Ginsenosides

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1. Original Aims of the Project:

Spinal cord injury (SCI) is a major cause of disability. Extensive clinical and experimental studies have shown that traumatic spinal cord injury results in neuronal loss and axonal degeneration, which may further lead to partial disability or complete paralysis. The current standard therapy for clinical acute spinal cord injury is methylprednisolone (MP). Discovered over 10 years ago to have neuroprotective effects in human spinal cord injury, this drug is a potent synthetic glucocorticoid that is also a weak free radical scavenger. MP has significantly but only modestly improved neurological recovery. We have conducted a screen for compounds that protect spinal cord neurons and identified ginsenoside Rb1 and Rg1, which are two main active components of ginseng root (*Panax ginseng* C.A. Meyer), an ancient medicinal herb used for over 2,000 years in the treatment of neurological diseases in the Orient. We hypothesized that ginsenoside Rb1 and Rg1 might be effective in treating spinal cord injuries and might be synergistic with MP. We therefore proposed to test this hypothesis with the following specific aims:

Specific Aim 1: To examine whether ginsenosides and methylprednisolone (MP) have synergistic neuroprotective effects in vitro. MP is a drug currently used in the treatment of SCI. We planned to compare the effects of ginsenosides and MP alone and in combination, to ascertain whether the drugs have synergistic effects in protecting spinal cord neurons using our established in vitro tissue culture models.

Specific Aim 2: To examine neuroprotective effects of ginsenosides and potential synergistic effects with MP in a standardized rat cord contusion model. To critically examine the effects of ginsenosides, we planned to evaluate the best doses of ginsenosides to protect spinal neurons and promote functional recovery in rats with SCI.

2. Project Successes:

(1). Isolation of active compounds for spinal cord neural protection from ginseng root.

An important strategy in the treatment of spinal cord injury is to promote neuron survival and axon outgrowth, making possible the recovery of neural connections. Using an in vitro survival assay, we have identified ginsenosides Rb1 and Rg1, extracted from ginseng root (*Panax ginseng* C. A. Meyer), as efficient neuroprotective agents for spinal cord neurons. These compounds protect spinal neurons from excitotoxicity induced by glutamate and kainic acid, as well as oxidative stress induced by H_2O_2 . The neuroprotective effects are dose-dependent. The optimal doses are 20 – 40 μ M for ginsenoside Rb1 and Rg1 respectively. The effects are specific for Rb1 and Rg1, since a third ginsenosides, Re, did not exhibit significant activity. These observations indicate that ginsenosides Rb1 and Rg1 represent potentially effective therapeutic agents for spinal cord injuries. In this grant period, we have completed the study of effects of ginsenosides on spinal cord neuron survival in vitro. We have demonstrated that several chemical components of ginseng, have protective effects on spinal cord neurons against several chemical insults similar to that in injured spinal cord. These observations suggest

that the compounds from Ginseng, ginsenosides, are potentially useful in the treatment of spinal cord injury. The data collected have now been summarized in a paper published in *Experimental Neurology* (see attached).

(2). Neural protective effects of MP and synergistic activity with ginsenosides

In addition, we have analyzed whether ginsenosides could act synergistically with MP in spinal cord neuron protection. MP is currently used in the treatment of SCI, but its ability and mechanism to protect spinal cord neurons have not been well understood. One of the aims of this study is to compare the effects of ginsenosides and MP to examine whether ginsenosides have better protective abilities than MP. Examining MP effects is the first step towards this objective. Our analysis showed only modest protection of spinal cord neurons by MP. At concentrations ranging from 40 to 160 μM , a 42% to 57% increase in surviving neurons were observed in rescuing neurons from excitotoxicity by glutamate (Fig. 1). MP appears to be less efficient than ginsenosides. However, when ginsenosides and MP were used together, they protected spinal neurons much better (Fig. 2 & 3). These studies indicate that there are synergistic effects between ginsenosides and MP, suggesting a potential combination therapy would be more effective.

(3). Efficacy of ginsenosides in promoting recovery of spinal cord injuries in rats

To test the efficacy of ginsenosides *in vivo*, we examined effects of administration of ginsenosides in rats with injured spinal cords. Two doses (5 mg/kg and 20 mg/kg) of total ginseng saponin (GTS) were administered intracally to rats with spinal cord injuries. The animals were tested for BBB scores at various times after injury to examine effects on recovery. This analysis showed that there is considerably more recovery in animals treated with GTS in the first week, suggesting that animals with ginsenosides recover faster than controls in the first week after injury (Fig. 4).

To be useful in the treatment of human patients, drugs need to be administered systemically. This requires that drugs can penetrate the blood brain barrier to enter the spinal cord. To examine whether GTS can pass through the blood brain barrier, we developed HPLC methods to measure the concentration of GTS in spinal cord samples after *i.p.* administration. By comparing 5 different methods of extraction, it was determined that the MeOH deprotein-Solid phase combination method was the best for spinal cord samples (Figure 5). The procedure is as follows: The whole spinal cord was dissected out and homogenized in 0.8 ml of distilled water with a glass homogenizer. After defatting of the homogenate with 3.0 ml of benzene, 3.5 ml of methanol was added to aqueous phase. The precipitate was removed by centrifugation at 14,000 rpm for 5 min and the supernatant was dehydrated in a centrifuge heater in vacuum. The resulting residue was subsequently suspended in water and applied to a Sep-Pak C₁₈ cartridge pre-treated by washing with MeOH and H₂O. The cartridge was washed with water and subsequently eluted with MeOH. The MeOH eluate was dried and reconstituted with 80 μl of MeOH. A 40 μl aliquot was injected into HPLC system. In this method the recoveries of -Rg1 and -Rb1 is more than 70% and 60% respectively.

The rats was administered at dose of 450 mg/kg (*i.p.*) and sacrificed at 30 min, 1h, 4h, 8h and 12h. The spinal cord was immediately taken out from the rats within 10 min

and frozen in -80°C . According to the aforementioned method the contents of ginsenoside-Rg1 and Rb1 were analyzed by HPLC. The result showed that these two compounds were not detected in the spinal cords within 12 h. after *i.p* administration. This indicated that the amount of Rg1 and Rb1 in rat spinal cord was less than $1\ \mu\text{g/g}$ tissue weight which was the lower limit of detection in our method. So it is difficult for ginsenosides to diffuse the blood-brain barrier.

3. Project challenges. The main challenge now we are facing is to identify ginsenoside derivatives that are effective in neural protection and can pass efficiently through the blood brain barrier. We are teaming up with Longqin Hu, an organic chemist, to explore the potential of designing and synthesizing novel ginsenoside derivatives with improved blood-brain barrier penetration.

4. Implications for future research and/or clinical treatment. Our analyses suggest strongly that a combination of ginsenosides and MP could improve recovery of patients with SC injuries. The challenge now is to identify related compounds with better blood-brain barrier penetration.

5. Plans to continue this research. We are actively exploring the possibility of synthesizing ginsenoside derivatives, and we plan to submit proposals to the NIH and other funding agencies once we have more preliminary results on these experiments.

6. Publications from this research.

Liao B, Newmark H, Zhou R. Neuroprotective effects of ginseng total saponin and ginsenosides Rb1 and Rg1 on spinal cord neurons in vitro. *Exp Neurol.* 2002 Feb;173(2):224-34.

Figure 1. MP has mild neuroprotective activity *in vitro*. Glutamate toxicity of cultured spinal cord neurons was induced in the presence of various concentrations of MP. 3 days after glutamate treatment, the cultures were stained with anti-NSE antibody and quantified for surviving neurons. * indicates $p < 0.05$ compared to controls.

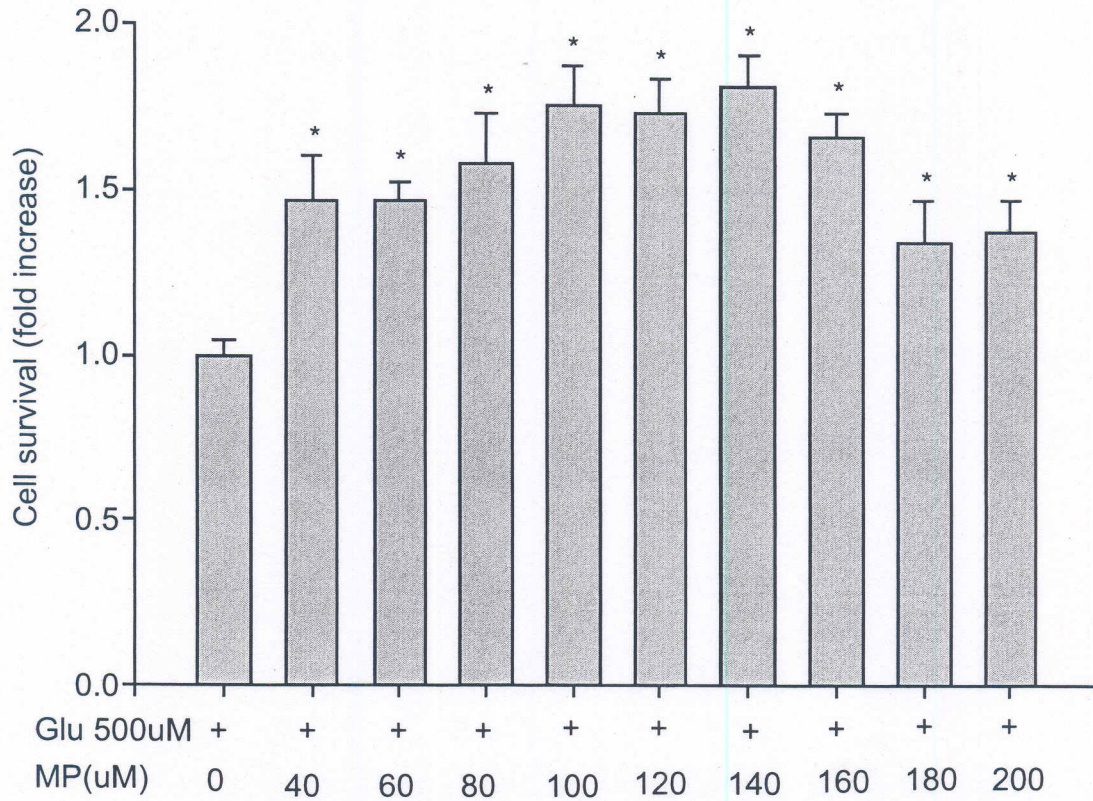


Figure 2. Synergistic effects of MP and ginseng total saponin. Cultured spinal cord neurons were treated with glutamate (500 mM) for 1 hour in the presence of 50 $\mu\text{g/ml}$ of GTS and various concentrations of MP. Neuron survival was dramatically improved in the presence of both GTS and MP.

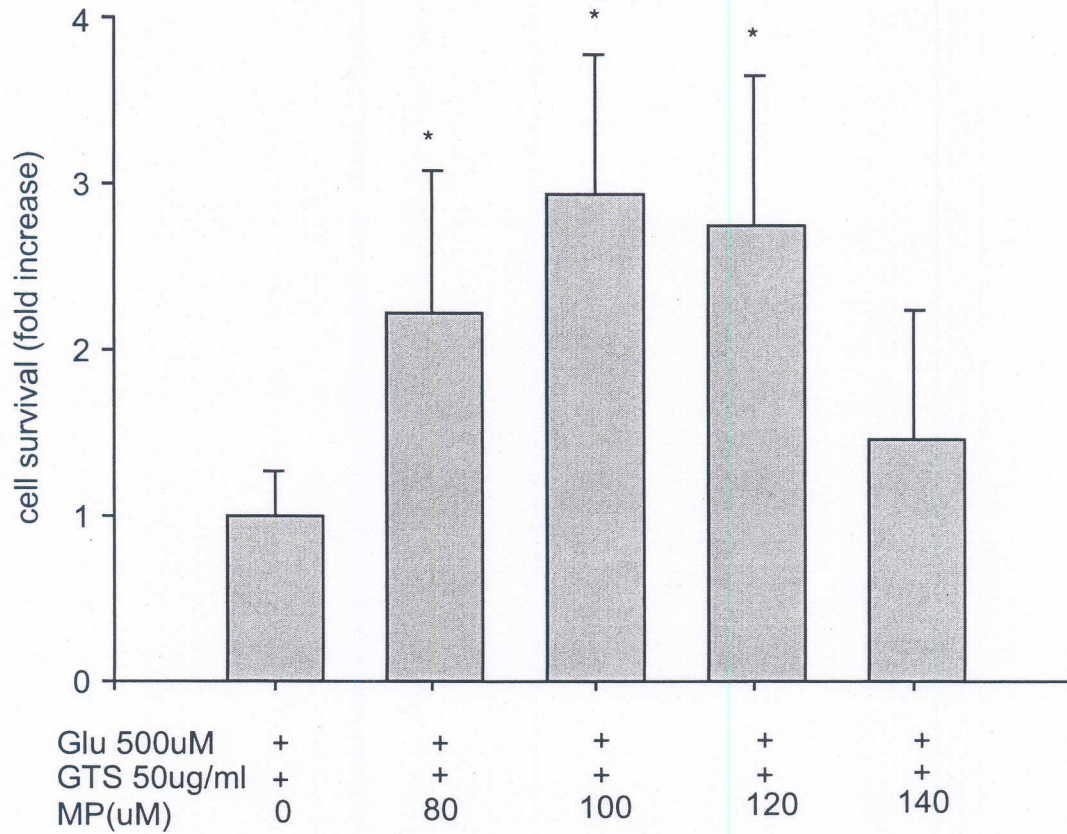


Figure 3. Synergistic effects of ginseng total saponin and MP in preventing spinal neuron death. Neurons were treated similarly as in Figure 2, except with a fixed concentration of MP (150 μ M) and various concentrations of GTS.

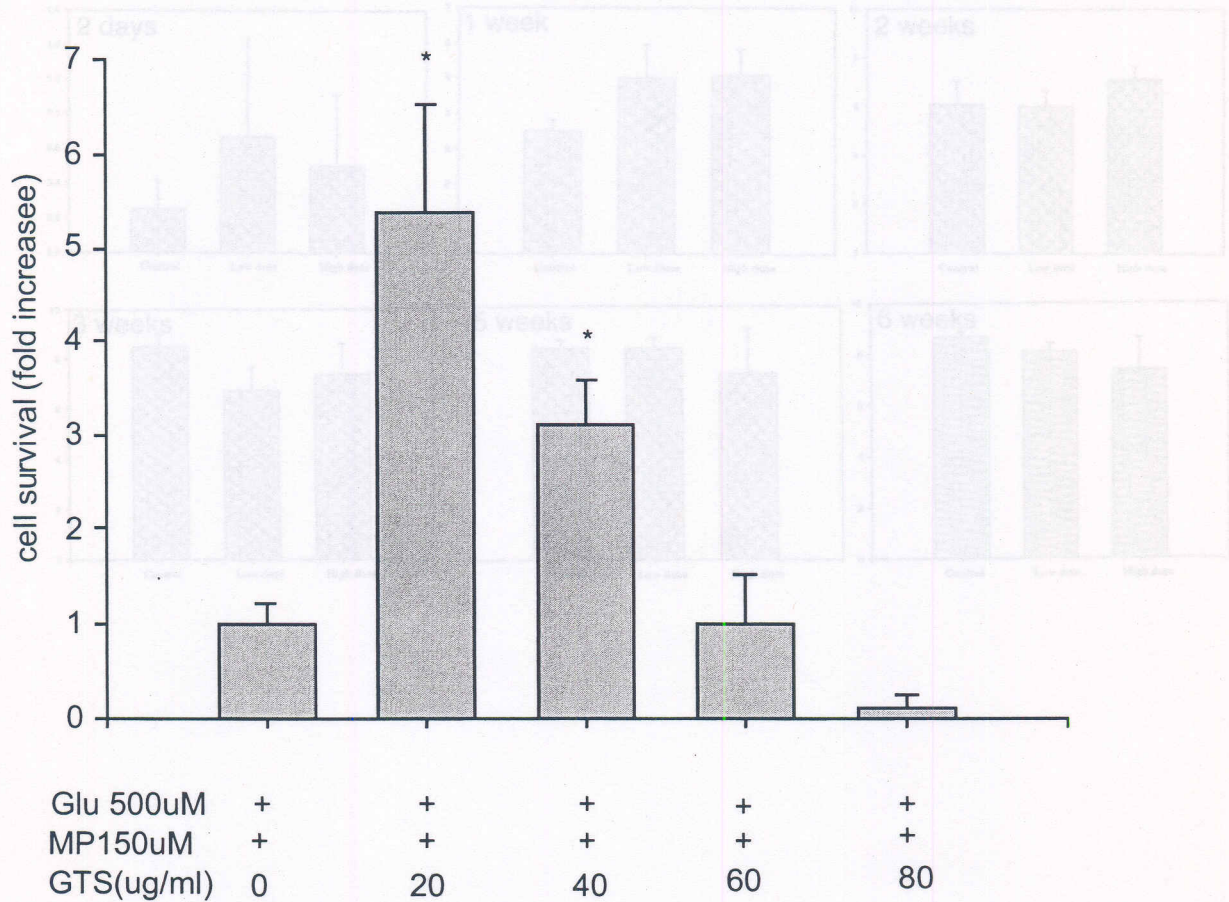


Figure 4. Effects of GTS on Recovery of rats with injured spinal cord. Rats with injured spinal cord were administered two different doses intracereally (low dose: 5 mg/kg body weight; high dose: 20 mg/kg body weight), and the BBB scores were measured at various times post-treatment.

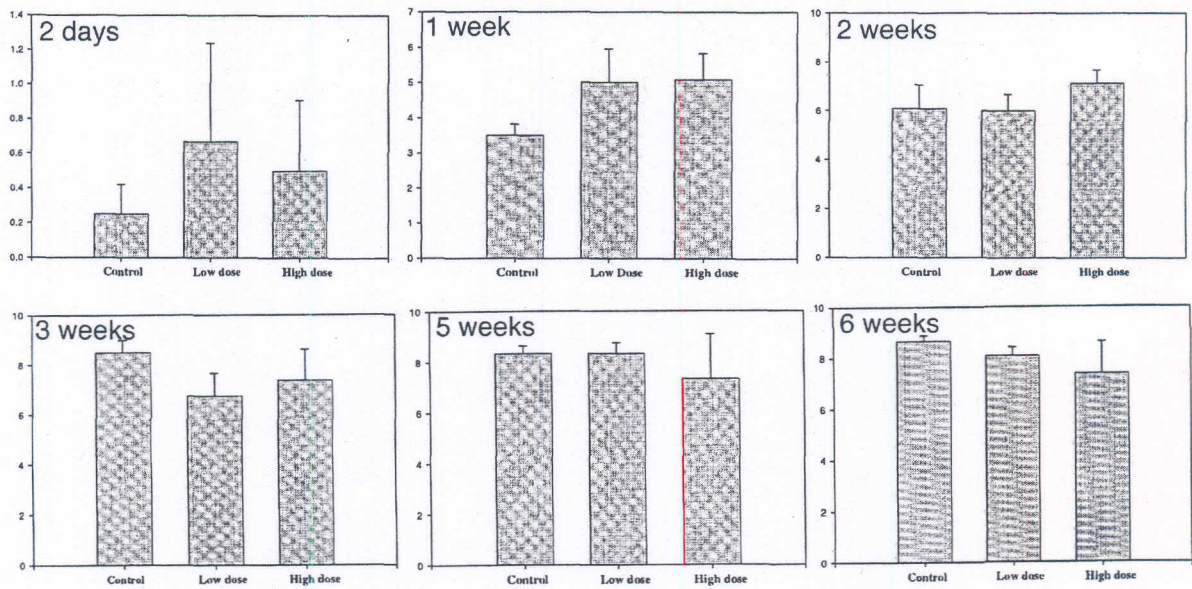


Figure 5. HPLC Conditions for detecting Rg1 and Rb1 in animal tissues. The HPLC separation were made with a RP-18 column and a linear gradient elution system using eluents A and B [A=CH₃CN-0.02% H₃PO₄ solution (15:85); B=CH₃CN-H₂O (85:15)] according to the following profile: 0-15 min, 98-96% A, 2-4% B; 15-25 min, 96-85% A, 4-15% B; 25-55 min, 85-65% A, 15-35% B; 55-65 min, 98% A, 2% B. The flow rate was kept constant at 1.0 ml/min and the peaks were monitored at 203 nm. The following is the HPLC chromatogram under this gradient system.

