The Combination of Selective Inhibition of the Cannabinoid CB_1 Receptor and Activation of the Cannabinoid CB_2 Receptor Yields Improved Attenuation of Motor and Autonomic Deficits in a Mouse Model of Spinal Cord Injury

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Spinal cord injury (SCI) is a tremendous public health problem in the United States and worldwide. The Centers for Disease Control and Prevention and the University of Alabama National Spinal Cord Injury Statistical Center estimate the annual incidence of SCI in the United States is between 11,000 and 12,000 injured per year.[14,31] There are approximately 250,000 people in the United States living with disability related to an SCI.[14,31] The cost of SCI to society is considerable, with a monetary estimate of more than $9,700,000,000 per year.[14] Considering that more than half of individuals affected by SCI are young males between the ages of 15 and 29 years, the cost to society from loss of productivity may even be greater. Internationally, the incidence of SCI is increasing at an alarming rate, as motorization and in many regions violence increases.

Although there have been advances in the care and rehabilitation of patients with SCI, currently there are unfortunately very few, if any, medical treatments for acute SCI that effect functional outcome.[3,17,22] The current mainstay in medical therapy for acute injury is high-dose methylprednisolone.[3,10–12,22,25] Many experts, however, believe that the risk of adverse events associated with high-dose steroids may outweigh the potential benefits gained through its use.[22,25] According to Hurlbert, the continued use of steroids in acute SCI is "primarily out of peer pressure and fear of litigation."[22]

Just as in traumatic brain injury, a complex array of secondary insults is responsible for ongoing neuronal damage after SCI.[3,28] Neuroprotection is defined by Anderberg et al.[3] as measures to "counteract secondary injury mechanisms and/or limit the extent of damage caused by self-destructive cellular and tissue processes." Neuroprotective medications may be able to interrupt this destructive progression and theoretically have the potential to yield improved functional recovery.[3] The search for neuroprotective agents that demonstrate efficacy in SCI is of paramount importance given the increasing incidence and devastating nature of the disease.

Recently there has been an explosion of interest in the use of cannabinoids in treatment of central nervous system (CNS) diseases.[2,4,8,13,15,18,20,32,34,36–38] Croxford[15] identified multiple sclerosis, Parkinson's disease, neuroprotection, analgesia, emesis, and anorexia and obesity all as areas with potential for the clinical application of cannabinoids. Our group has been exploring the role of cannabinoid receptor modulation in murine models of several CNS disorders such as stroke, multiple sclerosis, traumatic brain injury, and SCI.[30,37,38] The term *cannabinoid* refers to any natural or synthetic compounds that resemble in structure and/or function those found naturally in the plant *Cannabis sativa*. Two types of cannabinoid receptors in the mammalian endocannabinoid system have been identified to date. The CB_1 and CB_2 receptors both work through G_i protein–coupled mechanisms on adenylyl cyclase function, as well as through other mechanisms.[1,13,15,32]

The CB_1 receptor is found throughout the CNS and peripheral nervous system where it is localized to axon terminals.[1,2] Activation leads to inhibition of neurotransmitter release and therefore works via presynaptic inhibition of neurotransmission. The CB_1 receptor is constitutively active and subject to endogenous tone by circulating endocannabinoids. The receptor has been shown to participate in control

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of behavior, cognition, cardiovascular responses, feeding, and pain.[1] It has also been shown that CB_1 can tonically regulate *N*-methyl-D-aspartate glutamate receptors and thus *N*-methyl-D-aspartate glutamate–induced excitotoxicity.[2] Because the CB_1 receptor has the potential to modulate excitotoxic injury, it has been investigated as a target for intervention in animal models of neuronal injury.[15,32]

In contrast, the CB_2 receptor has been shown to be expressed primarily by immune cells such as lymphocytes and neutrophils.[13,15] Recent evidence showed that the CB_2 receptor is expressed by resident inflammatory modulating cells of the CNS, including microglia.[13] Activation of CB_2 results in attenuation of the inflammatory response.[18] Unlike the CB_1 receptor, activation of CB_2 lacks any psychotropic effects.[13,20,27,33]

Hama and Sagen[21] in 2007 demonstrated that the nonselective cannabinoid agonist WIN 55,212-2 has antinociceptive properties in rats with SCI pain.[21] In addition, they showed that this effect was localized to the CB_1 receptor. To our knowledge, we are the first to explore the use of selective CB_1 and CB_2 receptor drugs as neuroprotective agents in SCI. Our previous work in SCI demonstrated a significant improvement in both motor and bladder function recovery in mice treated with a selective CB_2 agonist (O-1966; 1 mg/kg) one hour before injury.[8] When we explored CB_1 inhibition with a selective CB_1 antagonist (SR141716; 20 mg/kg), we again found significant improvement over control. In this study, we explore the combined effects of selective CB_1 inhibition and CB_2 activation in a murine model of SCI to determine whether there is an additive effect.

MATERIALS AND METHODS

Animals

A murine SCI contusion model was performed on 7- to 12-week-old female C57BL/6 mice weighing approximately 16 to 21 g (Taconic, Hudson, NY). All procedures, interventions, and animal care were done in accordance with protocol approved by the Temple University Institutional Animal Care and Use Committee following the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed for 1 week before surgical intervention for acclimation and observation. A 12-hour light/dark cycle was maintained, and mice were allowed free access to food and water including hydrogel at all times. Mice were trained on the Rota Rod (Ugo Basile Biologic Research Apparatus, Comerio, Italy) at a constant speed of 10 rpm before intervention.

Group Design and Drug Preparation

Mice were randomized into four groups: two experimental and two control. Both the principal investigator who preformed all procedures and evaluations and the laboratory assistant who participated in animal care and motor function evaluation were kept blinded to treatment throughout the experiment. Drug was prepared and injected by a laboratory assistant who randomized the animals and did not participate in mice evaluation. The CB_1 antagonist (SR141716) was dissolved in a dimethyl sulfoxide:Cremophor:saline mixed solution at 1:1:18. The CB_2 agonist (O-1966) was dissolved in a pure ethanol:Emulphor:saline solution at 1:1:18.

Mice in the preinjury experimental group (n = 18) received a combined intraperitoneal (IP) injection of CB1 antagonist (SR141716; 10 mg/kg) and CB_2 agonist (O-1966; 1 mg/kg) at one hour before injury and 24 hours post-injury. Mice in the postinjury experimental group (n = 14) received combined IP injection of CB_1 antagonist (SR141716; 10 mg/kg) and CB_2 agonist (O-1966; 1 mg/kg) at one hour and 24 hours post-injury. The preinjury (n = 43) and postinjury (n = 31) control groups received equal volume of vehicle (0.9% saline; 0.2 mL) by IP injection at the same time points.

Surgical Procedure

Mice were anesthetized using an IP injection of a 1:1 combination of ketamine (100 mg/mL) and xylazine (20 mg/mL) at a dose of 1 mL/kg. Once under anesthesia, back hair was shaved, ears and tails were definitively marked, and protective eye gel applied. Body temperature was maintained at 37 ± 5°C during the procedure and the recovery period with a heating pad and lamp. The surgical site was prepped with povidone-iodine solution. Appropriate depth of anesthesia was confirmed before surgery by the lack of a withdrawal response to toe pressure. A midline dorsal thoracic incision was made using a number 15 blade scalpel in the mid portion of the back. The incision was completed from approximately the upper scapula to just beyond the dorsal hump using sharp dissecting scissors. Skin was undermined using a cotton swab to allow for easier retraction. The dorsal fat pad was dissected from its caudal attachment using scissors and then reflected cephalad. Care was taken not to injure or avulse the large dorsal vein found in the cephalad portion of the fat pad located at approximately T5. Ribs were used to localize the T8 and T9 lamina. Using a combination of sharp and blunt dissection, the paraspinal musculature was dissected free from the lamina from T7 to T10. Mice were then carefully held by the lateral aspects of the T7 vertebra using Adson forceps. Using the operative microscope for better visualization, laminectomies were performed at the T8 and T9 levels using fine microscissors and laminectomy forceps. The ligamentum flavum was gently dissected free using a cotton swab. Care was taken not to injure the spinal cord and to ensure adequate width of laminectomy.

Mice were then transferred to the Infinite Horizons (IH) impactor device (PSI Inc., Lexington, KY) where they were suspended via modified Adson forceps clamped to the lateral aspect of the vertebra above and below the level of the

laminectomies. The impactor tip was positioned directly above exposed dura and then raised to a height of 5 mm. The IH impactor is a computer-driven contusion device that delivers a set force. Sensors on the impactor tip return nearly instantaneous information regarding actual force delivered, displacement, and velocity. Information is returned both numerically and graphically as a function of time. This information allows exclusion of mice who received either too minor or too severe of an impact or for which impact was not delivered cleanly (i.e., the tip hit the bone or soft tissue) as was evident by an irregular curve on the graph. The device was set to deliver a 60 kdyne force to the spinal cord. The actual force, displacement, velocity, and injury time were recorded as well as injury characteristics such as presence and absence of tail flip at the time of injury and severity of the bruise seen. The wound was irrigated with 0.9% normal saline solution (NSS). Spinal musculature was reapproximated with 4-0 silk suture and the dorsal fat pad placed back in its normal position. The skin was closed with clips.

Postoperative Care

At the conclusion of surgery, the mice were given subcutaneous injections of fluid (0.9% NSS; 1 mL), antibiotic (Baytril [enrofloxacin]; 2.5 mg/kg), and analgesic (buprenorphine; 0.03 mg/kg). The mice were placed in a recovery cage under a heating lamp until they were well recovered from anesthesia. All mice cages were kept on a heating pad on the first postoperative night. Mice were housed in cages of five or fewer and at all times allowed free access to food and water. They were also given subcutaneous injections of fluid (0.9% NSS; 1 mL) and analgesic (buprenorphine; 0.03 mg/kg) twice daily and antibiotic (Baytril; 2.5 mg/kg) once daily for the first three postoperative days. The mice had their bladders emptied twice daily via the Credé maneuver until recovery of autonomic function (discussed below).

Motor Function Evaluation

The mice were evaluated for motor function recovery using two widely accepted scales for open-field assessment of locomotion, the 9-point Basso Mouse Scale (BMS) and the 17-point Basso, Beattie, and Bresnahan locomotor scale (BBB) modified for mice by Dergham.[5–7,16] Scoring in each of these scales relies on lower limb movement, the ability to plantar place the hindpaw, stepping with weight support, coordination in ambulation, and trunk stability. Each mouse was evaluated on postoperative days one, three, seven, and 14. Scoring was done by the principal investigator and a laboratory assistant who agreed on the final score to be given.

On postoperative day 14, the mice were tested using the Rota Rod (Ugo Basile Biologic Research Apparatus), which we have introduced as an objective measure of mouse motor function recovery after SCI (J.E.H., unpublished data, 2009). The Rota Rod is a continuously spinning rod on which mice need to walk. To stay on the rod for an extended period of time, mice need to have recovered plantar stepping with coordination and have only mild trunk instability. When mice are no longer able to walk on the rod, they fall off, tripping a sensor and thus recording the total time. In our efforts to validate the Rota Rod as an objective measure of mouse motor function recovery, 195 mice were subjected to a thoracic contusion SCI using the same technique described above. Of these, 157 were able to be evaluated on postinjury day 14 with open-field assessment (BMS and modified BBB) and Rota Rod testing. We found through Spearman correlation coefficient analysis that the ability to perform on the Rota Rod correlated very well with higher BMS and BBB scores.

For this experiment, the Rota Rod was set to spin at a constant speed of 10 rpm. A mouse was deemed to have passed the Rota Rod test if it could stay on the rod for 500 seconds or longer.

Autonomic Function Evaluation

All mice had autonomic impairment with urine retention after SCI. To relieve their bladders and to assess for autonomic function recovery, urine was expressed twice daily via suprapubic pressure (Credé maneuver) and urine mass determined. Mice were considered to have recovered autonomic function once the total urine mass expressed was less than 500 mg/d for three consecutive days. The first day of less than 500 mg was considered the day of passing.

Exclusion Criteria

As mentioned earlier, the IH impactor device provides information regarding actual force delivered, displacement, and velocity. We have found that displacement correlates most with severity of injury and ultimately the ability to recover greater than other parameters. To produce as standard of an injury as possible, we set numerical exclusion criteria based on force delivered ($\leq$70 kdyne) and displacement (400–550 μm). In addition, mice that scored more than one on the BMS on postoperative day one (too minor an injury) and mice that lost more than 5% of their body weight (too severe of an injury) were excluded from evaluation. Mice that had an adverse event during the procedure, such as a forceps injury, were also excluded (n = 2).

Of the 43 animals randomized to the preinjury control group, seven died either during surgery or soon thereafter (16.3% mortality). Thirteen (30.2%) were excluded based on information provided by the IH impactor device (displacement: high, six [14.0%]; low, seven [16.3%]). An additional seven animals (16.3%) were excluded based on excessive weight loss of more than 15% of the initial body weight, and two animals (4.7%) were excluded because they scored more than 1 on the BMS 1 on postinjury day 1 open-field assessment. The total number of animals in this group included for evaluation was 14.

Thirty-one animals were randomized to the postinjury control group. Of these, eight died (25.8% mortality), 12 (38.7%) were excluded based on IH compactor device information (displacement: high, six [19.4%]; low, six [19.4%]). One (3.2%) was excluded based on weight loss, and one (3.2%) was excluded for scoring more than 1 on the BMS on postinjury day one. Nine mice from this group were included in the final assessment.

The preinjury experimental group consisted of 18 animals. None died (0% mortality). Five animals (27.8%) were excluded based on IH impactor device data (displacement: high, three [16.7%]); low, two [11.1%]). Three mice (16.7%) were excluded based on excessive weight loss. One mouse (5.6%) was excluded because of an intraoperative event requiring resuscitation. Nine mice were therefore included in the final evaluation.

Fourteen animals were randomized to the postinjury experimental group. One animal died (7.1% mortality). Two mice (14.3%) were excluded based on IH impactor device information (displacement: high, two [14.3%]). Two animals (14.3%) were excluded based on excessive weight loss. One mouse (7.1%) was excluded because of an obvious intraoperative forceps injury. Eight animals were included in the final evaluation.

Statistical Analysis

Analysis of variance was used to compare the difference in motor function scores between experimental and control groups. Data are presented as the mean ± the standard error of the mean. Fisher's exact test was used to determine significance of Rota Rod function and bladder function recovery. The log-rank test was also used to evaluate Kaplan-Meier curves of bladder recovery among groups. A difference was considered statistically significant if $P < 0.05$. For the purpose of analysis, the preinjury and postinjury control groups were combined after it was determined that there was no significant difference between them and hereafter together are referred to simply as the control group.

RESULTS

Motor Function: Open-Field Assessment

Results for open field assessment of motor function on the BMS and BBB modified for mice are displayed in *Table 16.1* and represented graphically as a function of time in *Figures 16.1* (BMS) and *16.2* (BBB modified for mice).

Mice in the preinjury experimental group demonstrated statistically significant improvement over the control group in both BMS and modified BBB scores for open-field assessment of locomotion at three, seven, and 14 days post-injury. The difference in average BMS and modified BBB score between experimental and control groups at the conclusion of the study (postoperative day 14) was 7.67 ± 0.24 versus 6.30 ± 0.18 ($P < 0.0001$) and 14.67 ± 0.37 versus 12.35 ± 0.36 ($P < 0.0001$), respectively.

Mice in the postinjury experimental group demonstrated improvement over the control group with statistically significant differences on postinjury day 7 (BMS, 5.67 ± 0.67 versus 3.74 ± 0.24 [$P = 0.04$]; BBB, 10.13 ± 1.13 versus 7.61 ± 0.31 [$P = 0.01$]). A statistically significant difference was also evident at the conclusion of the study in modified BBB testing, but not in BMS testing. The difference in postoperative day 14 BMS and modified BBB scores was 6.75 ± 0.41 versus 6.30 ±

TABLE 16.1. Open-Field Assessment of Motor Function[a,b]

	POD 1		POD 3		POD 7		POD 14	
	BMS	**BBB**	**BMS**	**BBB**	**BMS**	**BBB**	**BMS**	**BBB**
Control group								
Preinjury (n = 14)	0.07 ± 0.07	0.07 ± 0.07	0.93 ± 0.20	2.29 ± 0.68	3.43 ± 0.29	7.07 ± 0.30	6.43 ± 0.23	12.29 ± 0.47
Postinjury (n = 9)	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.33	3.11 ± 0.95	4.22 ± 0.36	8.44 ± 0.53	6.11 ± 0.31	12.50 ± 0.52
ANOVA (P value)	0.74	0.76	0.6	0.88	0.31	0.25	0.46	0.94
Combined	0.04 ± 0.04	0.04 ± 0.04	1.09 ± 0.18	2.61 ± 0.55	3.74 ± 0.24	7.61 ± 0.31	6.30 ± 0.18	12.35 ± 0.36
Experimental groups								
Preinjury (n = 14)	0.44 ± 0.18	0.44 ± 0.18	2.22 ± 0.52	4.89 ± 0.95	5.67 ± 0.67	11.22 ± 1.08	7.67 ± 0.24	14.67 ± 0.37
ANOVA (P value)	**0.046**	0.064	**0.02**	**0.023**	**0.0003**	**0.0003**	**<0.0001**	**<0.0001**
Postinjury (n = 10)	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.46	3.50 ± 1.15	5.00 ± 0.60	10.13 ± 1.13	6.75 ± 0.41	13.50 ± 0.68
ANOVA (P value)	0.83	0.85	0.43	0.43	**0.041**	**0.0097**	0.098	**0.03**

[a]POD, postoperative day; BMS, Basso Mouse Scale; BBB, Basso, Beattie, and Bresnahan locomotor scale; ANOVA, analysis of variance.

[b]BMS and BBB modified for mice scores are displayed for each group at every time point ± standard error of the mean. Statistical significance is considered for ANOVA; $P < 0.05$ shown in bold. There was no significant difference between the preinjury and postinjury control groups (as shown in the upper portion of the table), so they were combined for analysis purpose. The preinjury experimental group demonstrated statistically significant improvement in both BMS and modified BBB motor function scores on PODs 3, 7, and 14. The postinjury experimental group had higher motor function scores than the control group, and this difference was statistically significant in the modified BBB testing on PODs 7 and 14.

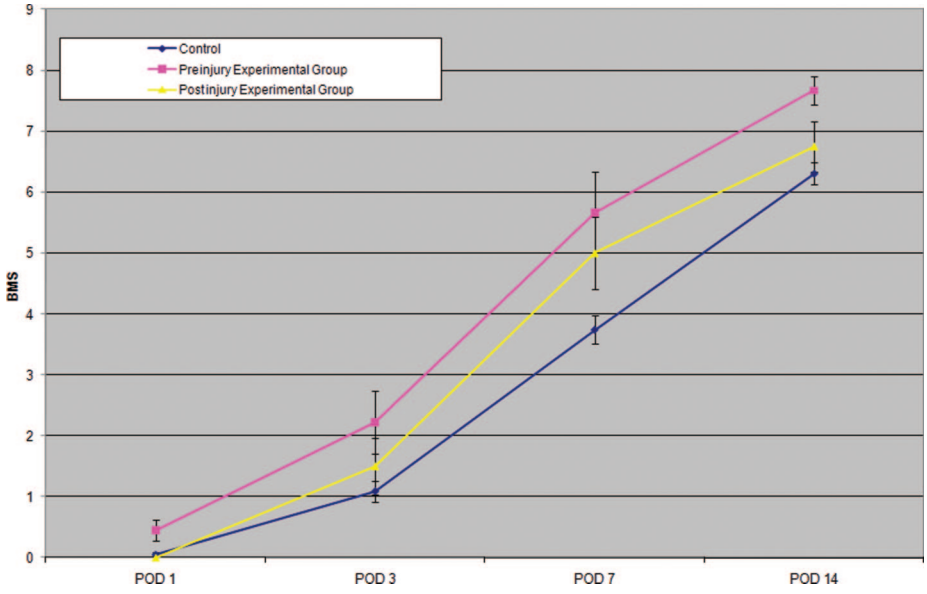

FIGURE 16.1. The Basso Mouse Scale (BMS) as a function of time. BMS results for the preinjury experimental group reveal statistically significant improvement over the control group at every time point. BMS results for the postinjury experimental group reveals a trend toward improvement over the control group, which was statistically significant on postoperative (POD) 7 but not POD 14.

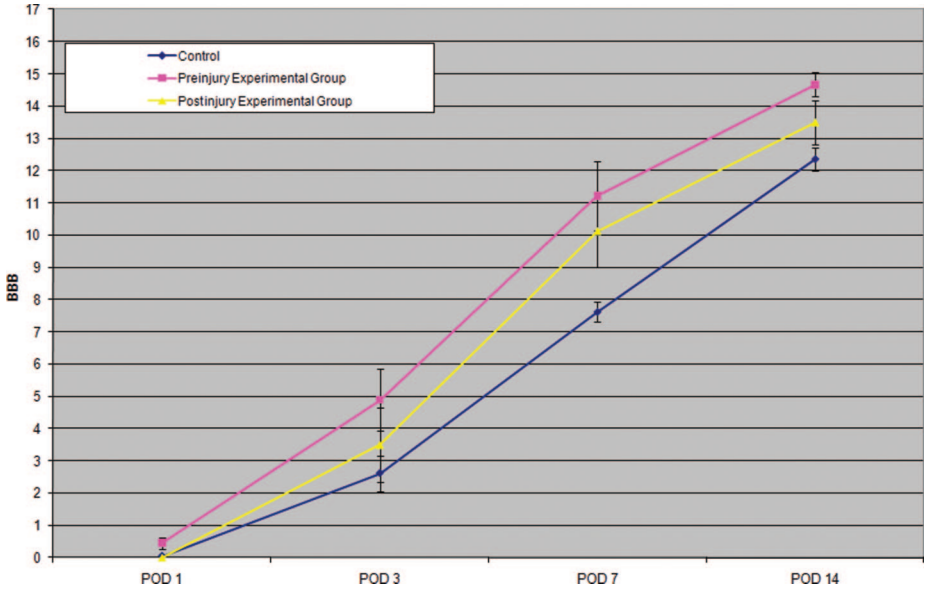

FIGURE 16.2. Basso, Beattie, and Bresnahan (BBB) (modified for mice) as a function of time. Results for the preinjury experimental group reveal statistically significant improvement over the control group at every time point. Results for the postinjury experimental group reveals statistically significant differences on postoperative day (POD) 7 and POD 14.

0.18 ($P = 0.098$) and 13.50 ± 0.68 versus 12.35 ± 0.36 ($P = 0.03$), respectively.

Motor Function: Rota Rod Assessment

The percentage of animals in each group that were able to perform on the Rota Rod is displayed graphically in *Figure 16.3*.

The percentage of mice in the preinjury experimental group able to walk on the Rota Rod for more than 500 seconds was different from the percentage of animals in the control group (77.8% versus 39.1%). This difference approached but did not reach significance when using Fisher's exact test ($P = 0.057$).

The percentage of mice in the postinjury experimental group able to walk on the Rota Rod for more than 500 seconds was not different from the percentage of animals in the control group (37.5% versus 39.1%; $P = 0.69$).

Autonomic Function Assessment

The percentage of animals in each group that recovered bladder function is displayed graphically as a function of time in *Figure 16.4*.

The percentage of mice in the preinjury experimental group that recovered bladder function as determined by less than 500 mg of urine expressed daily over three consecutive days was significantly different from the percentage of the control group animals that recovered (66.7% versus 26.1%) (Fisher's exact test; $P = 0.04$). When the difference in

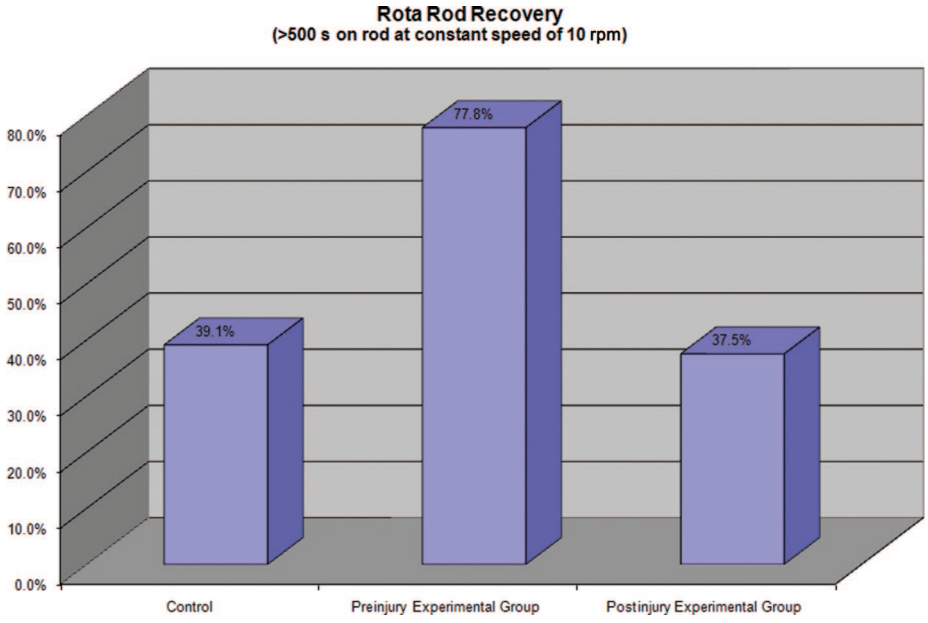

FIGURE 16.3. Rota Rod recovery: The percentage of mice in the preinjury experimental group able to walk on the Rota Rod for more than 500 seconds was different from the percentage of animals in the control group (77.8% versus 37.5%). There was no difference in Rota Rod performance between the postinjury experimental group and the control group.

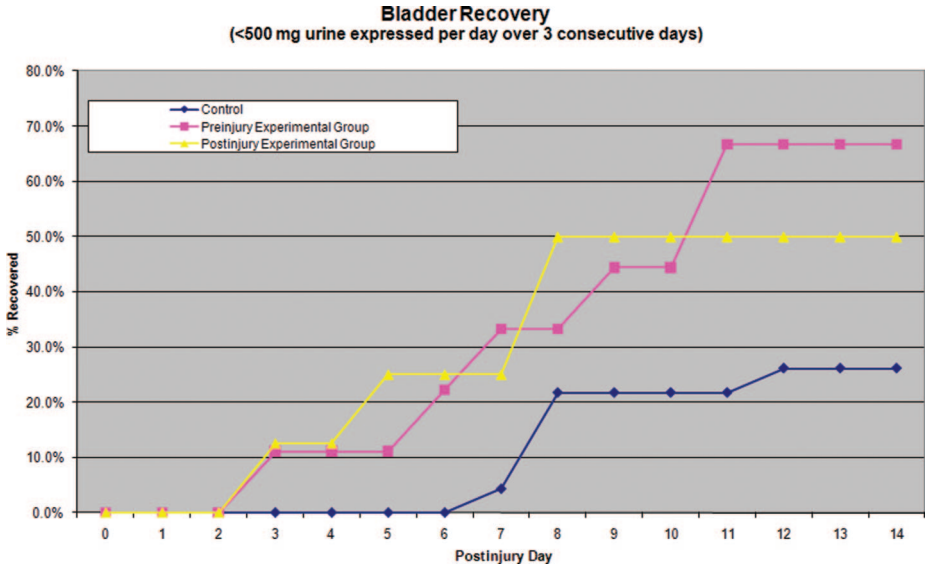

FIGURE 16.4. Autonomic function recovery. The percentage of mice in the preinjury experimental group that recovered bladder function as determined by less than 500 mg of urine expressed daily over 3 consecutive days was significantly different from the percentage of control group animals that recovered (66.7% versus 25.0%; Fisher's exact test, $P = 0.04$). The percentage of mice in the postinjury experimental group that recovered bladder function was increased compared with the control group; however, this difference did not reach statistical significant (50.0% versus 25.0%; $P = 0.2$).

bladder recovery was expressed as a Kaplan-Meier curve and evaluated using the log-rank test, significance was approached but not reached (*Fig. 16.5*).

The percentage of mice in the postinjury experimental group that recovered bladder function was increased compared with the control group; however, this difference did not reach statistical significance (50.0% versus 26.1%; Fisher's exact test, $P = 0.2$).

DISCUSSION

The search for neuroprotective agents to be used as first-line therapies in acute SCI is critical given the devastating nature of the disease and current lack of effective treatment strategies. William Donovan[19] postulated in his Donald Munro Lecture given at the 52nd Annual Meeting of the American Paraplegia Society that in the future, SCI will be regarded as an "ailment to be cured." He identified reduction of the effects of the damage through maintenance of circulation and oxygenation and reduction of neurotoxins, free radicals, inflammation, and ultimately apoptosis as the first steps toward achieving this goal. Mitchell and Lee,[28] in a recent report on the pathology of secondary injury after SCI equated the dynamic concept of interaction of insults as the rate-limited "fire," which is rapidly followed by a "flood."

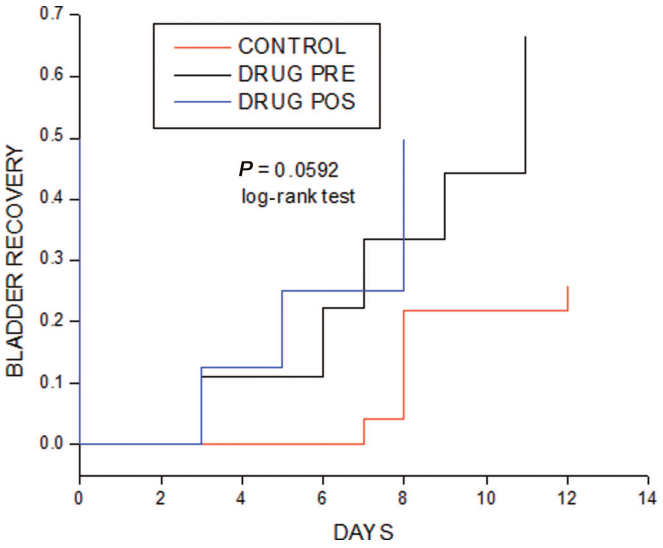

FIGURE 16.5. Autonomic function recovery. Kaplan-Meier curves for bladder recovery. Log-rank test; $P = 0.59$. PRE, pre-injury; POS, post-injury.

The "flood" describes "the accumulation dynamics in which the accumulation of independent factors drives the propagation of the secondary insult process." Their map of the summary of secondary injury pathology dynamics lends added justification for the rationale of using a combination of multiple neuroprotective agents aimed at targeting the different simultaneous processes ongoing in the spectrum of insults.[28]

In this study, we attempted to demonstrate the additive neuroprotective effects afforded through the use of a combination of selective cannabinoid receptor–modulating agents in a mouse model of SCI. To date, two receptors have been identified in the endocannabinoid system. The CB_1 receptor is widely dispersed throughout the CNS and peripheral nervous system where it is localized to axon terminal where it functions in the presynaptic control of neurotransmission. The CB_1 receptor and circulating endocannibinoids have been shown to effect neuron function and thus effect excitotoxicity. The CB_2 receptor is expressed primarily by inflammatory cells including CNS microglia. CB_2 stimulation has been shown to have immunomodulatory properties, such as decreasing the activity of antigen-presenting cells and downregulating cytokine (interferon-γ and tumor necrosis factor α) production during inflammatory processes.[9,23,26,35] The hypothesis that modification of the endocannabinoid system can influence outcome after neuronal injury is supported by several previous reports that cannabinoids have direct effects on neuronal function and inflammatory responses.[2,4,13,29,32,34]

Our laboratory has devoted most of its efforts over the past five years to test this hypothesis through several animal models of CNS disease including multiple sclerosis, cerebral ischemia, and SCI.[29,37,38] Our previous work in SCI demonstrated a significant improvement in both motor and bladder function recovery in mice treated with a selective CB_2 agonist (O-1966; one mg/kg) one hour before injury.[8] When we explored CB_1 inhibition with a selective CB_1 antagonist (SR141716; 20 mg/kg), we again found significant improvement over control.

For this study, we hypothesized that the combined effects of CB_1 inhibition and CB_2 activation would yield improved recovery of motor and autonomic function. To test this theory, we randomized mice to receive a combination of drugs, the CB_1 antagonist (SR141716; 10 mg/kg) and the CB_2 agonist (O-1966; 1 mg/kg) both in the preinjury and postinjury setting in a mouse model of SCI. The preinjury experimental group received an IP injection of drugs at one hour before and 24 hours after injury. The postinjury experimental group received an IP injection of drugs one and 24 hours post-injury. The postinjury group was included in this study so as to better model actual clinical conditions. We used strict exclusion criteria based on information provided by the IH impactor device plus clinical information such as the percentage of body weight lost and the BMS score on postinjury day one to better standardize our injury.

Mice in the preinjury experimental group had statistically significant recovery of both motor and autonomic function. In open-field testing of locomotive function and both BMS and modified BBB, mice in this group had statistically significantly higher function on postinjury days three, seven, and 14. Using an objective measure of murine motor function recovery, the Rota Rod, the percentage of mice in the preinjury experimental group able to walk on the Rota Rod for more than 500 seconds was greater than the percentage of animals in the control group who recovered this ability. We have introduced the Rota Rod as an objective means with which to test mice motor recovery because of the inherent difficulty in assessing the highest grade of recovery via open-field testing techniques and to remove any suspicion of bias.[24] Mice in this group also demonstrated superior recovery of bladder function compared with the control group.

Some have criticized the use of preinjury experimental groups in animal studies of SCI because such cohorts do not adequately represent real-world situations. This argument stems from the fact that it is highly improbable for a patient to have a neuroprotective agent in their circulation, at the appropriate dose, before an unforeseen SCI, such as the result of trauma. The benefit of preinjury neuroprotection should not be discounted. Spine surgeons face situations in which iatrogenic SCI is a possible complication of their intervention, such as in severe stenotic cervical myelopathy, scoliosis, and intramedullary spinal cord tumors. If an agent is shown to be neuroprotective and is safe to use, then it could be given preoperatively or nearly immediately after an injury has occurred.

Mice in the postinjury experimental group demonstrated a trend toward improvement in motor and autonomic

function recovery. In open-field assessment of motor function, the difference remained statistically significant in modified BBB testing on postinjury day 14. BMS and Rota Rod testing demonstrated no significant difference between the postinjury experimental group and the control group. It is possible that the greater degree of neuroprotection afforded to mice in the preinjury experimental group is related to the neuromodulator effects of the CB_1 receptor at the time of injury. The trend in the postinjury experimental group is promising, and we suspect that significance would be achieved if the number of mice in this group were increased.

In our assessment of autonomic function recover, it is interesting to note that not only did more animals in the treatment groups recovery, but they did so earlier.

CONCLUSION

Our study demonstrates that the additive effect of CB_1 inhibition and CB_2 activation with selective cannabinoid receptor modulators yields significant improvement in motor and autonomic function recovery after SCI when given in the preinjury setting. We also observed a trend toward significant improvement in motor and autonomic function when a combination of these drugs was given in the postinjury setting. In addition, when we compare these data with data from our previous work, the combined effect of CB_1 inhibition and CB_2 activation seems to be greater than the results obtained using either agent alone.[8] Further research is needed to delineate the true nature of these effects as well as to determine the appropriate dosing and timing of intervention.

Acknowledgments

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Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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