



Agmatine inhibits nuclear factor- κ B nuclear translocation in acute spinal cord compression injury rat model



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Abstract Secondary damage after acute spinal cord compression injury (SCCI) exacerbates initial insult. Nuclear factor kappa-B (NF- κ B)-p65 activation is involved in SCCI deleterious effects. Agmatine (Agm) showed neuroprotection against various CNS injuries. However, Agm impact on NF- κ B signaling in acute SCCI remains to be investigated. The present study compared the effectiveness of Agm therapy and decompression laminectomy (DL) in functional recovery, oxidative stress, inflammatory and apoptotic responses, and modulation of NF- κ B activation in acute SCCI rat model. Rats were either sham-operated or subjected to SCCI at T8–9, using 2-Fr. catheter. SCCI rats were randomly treated with DL at T8–9, intraperitoneal Agm (100 mg/kg/day), combined (DL/Agm) treatment or saline ($n = 16$ /group). After 28-days of neurological follow-up, spinal cords were either subjected to biochemical measurement of oxidative stress and inflammatory markers or histopathology and immuno-histochemistry for NF- κ B-p65 and caspase-3 expression ($n = 8$ /group). Agm was comparable to DL in facilitating neurological functions recovery, reducing inflammation (TNF- α /interleukin-6), and apoptosis. Agm was distinctive in combating oxidative stress. Agm neuroprotective effects were paralleled with inhibition of NF- κ B-p65 nuclear

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translocation. Combined pharmacological and surgical interventions were proved superior in functional recovery. In conclusion, present research suggested a new mechanism for Agm neuroprotection in rats SCCL through inhibition of NF- κ B activation.

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1. Introduction

Acute spinal cord injury (SCI) frequently causes permanent motor and sensory loss below the site of injury, and disruption of the voluntary control of voiding.¹ Presently, few treatments for SCI are available; however, they do not provide satisfactory neuroprotective effect and/or significant functional improvement.²

The pathophysiology of acute SCI comprises primary immediate irreversible mechanical injury to the cord which is amplified by secondary damages induced by a variety of mechanisms including hypoxia, vascular insult, glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, inflammation, and cell death. Secondary injury takes place over days or even weeks. Indeed, most studies are aiming for modifying or preventing secondary injuries.³

Activation of nuclear factor kappa B (NF- κ B) is a hallmark of central nervous system (CNS) pathologies.⁴ NF- κ B consists of five subunits (RelA [also called p65], RelB, c-Rel, p105/50, and p100/52). In un-stimulated cells, Rel/NF κ B proteins are sequestered in the cytoplasm and bound with specific inhibitor proteins called I κ Bs that render them inactive.^{5,6} Tumor necrosis factor- α (TNF- α) and oxidative stress are activators of neuronal NF- κ B signaling.^{7,8} Once stimulated, degradation of I κ Bs allows translocation of Rel/NF- κ B to the nucleus and expression of target genes.⁹ NF- κ B may promote either protective or detrimental effects on CNS recovery, depending on which subunit is activated. Activation of the transcriptional activator subunit p65, promotes inflammatory and apoptotic genes expression. By contrast, p50 acts as a transcriptional repressor, while c-Rel is an antiapoptotic genes' activator.^{4,10} Interference with the NF- κ B pathway might hold clinical potentials to improve functional restoration following traumatic CNS injury.⁴

Agmatine (Agm), [4-(aminobutyl)-guanidine-NH₂-CH₂-C(H₂)-CH₂-NH-C(=NH)(=NH)], is an endogenous neurotransmitter-neuromodulator stored in the synaptic vesicles of the brain and spinal cord.^{11,12} Exogenous Agm administration showed neuroprotective effects in various experimental models of neurological disorders,^{13–17} with multiple molecular targets proposed including N-methyl-D-aspartate (NMDA) receptors,^{15,16} α_2 -adrenergic receptors,¹⁸ imidazoline receptors,¹⁹ inducible nitric oxide synthase (iNOS) and cAMP pathway.¹² Although Agm attenuated neuronal loss and reduced pain following SCI,²⁰ the precise cellular mechanisms by which Agm acts in SCI are not yet well established. Few studies have addressed the impact of Agm on NF- κ B signaling in the nervous system; however, controversial results were observed.^{21–23}

In the present study, we explored modulation of NF- κ B activity by Agm in an spinal cord compression injury (SCCI) rat model, and investigated whether combined

pharmacological and surgical managements would be more advantageous than providing each one individually.

2. Materials and methods

2.1. Experimental animals

Adult female Wistar rats weighing 250–300 g were used (procured from Animal Experimental Center of Alexandria University). Female animals were preferred since complete manual bladder evacuation in male rats is difficult.²⁴ Animals were maintained at room temperature (25 \pm 2 °C) under standard conditions of light–dark cycle with free access to rat chow and water. Rats were allowed to acclimatize for one week prior to experimentation. All animals received care according to national animal care guidelines which are in compliance with “*Guide for the Care and Use of Laboratory Animals* (1996, 95 National Academy Press)” and the protocol was approved by the Faculty of Medicine, Alexandria University Ethics Committee.

2.2. Experimental groups

Rats were assigned to 5 experimental groups (16 rats each) as follows: spinal cord compression injury (SCCI) group, SCCL followed by decompression laminectomy at T8–9 (SCCI + DL), SCCL treated by Agm (SCCI + Agm), SCCL followed by decompression laminectomy at T8–9 and Agm treatment (SCCI + DL + Agm), and control (sham-operated) group.

2.3. Surgical procedure: induction of spinal cord compression injury (SCCI)

As previously described,²⁵ rats were anesthetized with intraperitoneal (*i.p.*) injection of Ketamine and Xylazine (100 mg/kg and 10 mg/kg, respectively). After midline incision over L1–T10 spinous processes, a 2-French Fogarty catheter (Edwards Lifesciences) was inserted into the epidural space through a bur hole (laminotomy) drilled in the posterior arch of the T10 vertebra. The catheter was advanced cranially until the center of the balloon was resting on T8–9 spinal level and then inflated with 15 μ l water using a Hamilton syringe. The inflated balloon was left in place for 5 min. Then, the catheter was deflated and removed. Careful attention was paid not to damage the dura mater. Soft tissues and skin were sutured in anatomical layers. Animals in control (sham-operated) group underwent the same surgical procedure except for balloon insertion and inflation. For rats subjected to decompressive laminectomy (DL), laminae opposite to injury site (T8 and T9) were removed after performing SCCL. Immediately

following surgery, rats were placed on water heating pads. When recovered from anesthesia, rats were housed in pairs and given free access to food and water.

After surgery, rats were injected with ampicillin (20 mg/kg for 1 week, *i.m.*) for prophylaxis against urinary tract infections, and with meloxicam (1 mg/kg for 3 days, *s.c.*) for analgesia. Rats received 5-ml bolus of lactated ringers (*s.c.*) immediately after urine collection at 7AM, for proper hydration. After SCCI, rats were monitored daily for general health and mobility. Manual bladder compression was performed twice daily until normal autonomic bladder voiding was regained.

2.4. Drugs and reagents

For Agm and combined treated groups, Agmatine sulfate (Sigma-Aldrich, St. Louis, USA) was freshly prepared on each day and administrated *i.p.* in a dose 100 mg/kg/day in normal saline.¹³ Other groups received daily *i.p.* saline in equivalent volumes. Treatments started half an hour after SCCI and continued for 28 days. Rats died before completion of experiment was replaced by others.

2.5. Neurological assessment of motor function

Gross and skilled motor testing was performed before surgery, one day post-surgery, and weekly thereafter for 28 days.

2.5.1. Hindlimb locomotor activities (BBB open field rating scale)

The hindlimb gross locomotion was assessed using the Basso, Beattie, and Bresnahan locomotor rating scale (BBB).²⁶ Rats were placed in an open field (80 × 130 × 30 cm), monitored by 2 digital cameras, and observed individually for 4 min by 2 examiners, who were kept blind to treatment groups. Briefly, the BBB is a 21-point scale ranges from complete paralysis (score 0) to normal locomotion, balance and coordination (score 21), by assessing hindlimb joint movements, stepping, trunk position and stability, forelimb-hindlimb coordination, paw placement, toe clearance, and tail position. To control for possible asymmetric injuries, left and right hindlimbs were scored individually and the average score for each individual rat was reported. Before surgery, rats were tested to ensure that a maximum score of 21 was consistently obtained.

2.5.2. Grid walk test

This test assesses skilled walking with a focus on forelimb-hindlimb coordination. In this test, the animals had to walk on a 1.3 m-long horizontal runway of 40 metal grid rods (3 mm diameter) elevated 30 cm from the ground, for a total of three trials. To prevent habituation to a fixed rod distance, the rods were placed irregularly (1–4 cm spaced). Hindfoot placements were scored on 30 of the rods, counting the total number of errors in foot placing. Rats were trained on the grid walk prior to baseline testing one day before surgery. Normal rats place hindlimbs on alternate bars, so scoring 30 bars yields 15 steps/hindlimb for a total of 30 placements without errors. Rats that could not step with their hindlimbs received scores of 30.²⁴

2.5.3. Assessment of bladder function

Following SCCI, defective autonomic functions are reflected by an inability to completely evacuate the bladder. The amount of retained urine in the bladder is a measure of this deficit. To assess residual urine volume, bladders were emptied by manual massage on the lower abdomen twice a day at morning and evening until 14 days post-injury. The expressed urine volume was measured in ml/day.²⁴ The percentage of rats that regained bladder control by the end of 14 days was also calculated.

2.6. Spinal cord tissue sampling and assessment

Four weeks following surgery, rats were euthanized after performing the last motor testing. Then, rats were randomly subjected to either biochemical estimations ($n = 8/\text{group}$), or histological and immunohistochemical examination of the spinal cord ($n = 8/\text{group}$).

2.6.1. Biochemical assessment of spinal cord tissue inflammatory cytokines and oxidative stress

The injured segment of spinal cord was rapidly excised and homogenized in phosphate buffered saline (PBS, pH = 7.4) at 4 °C containing protease inhibitor cocktail to prevent auto-oxidation of spinal cord tissues. The homogenates were then centrifuged at 3000 rpm for 15 min, and aliquots of supernatant were stored at -80 °C for biochemical estimations. The inflammatory cytokines, IL-6 and TNF- α , tissue levels were determined using ELISA kits (WKEA MED Supplies, NY, USA), according to the manufacturers' instructions. The amount of protein in each sample was measured as described by Lowry et al.²⁷

The SC tissue total antioxidant capacity (TAC) was determined by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H₂O₂) (EGY TECH). The antioxidants in the sample eliminated a certain amount of the provided H₂O₂. The residual H₂O₂ was determined colorimetrically by an enzyme reaction which evolves the conversion of 3,5-dichloro-2-hydroxy benz-sulphonate to a colored product.²⁸ Values were expressed as mmol/l. In addition, Malondialdehyde (MDA) was measured using commercial colorimetric kits (Biodiagnostic, Egypt), according to the manufacturer's instructions, as a biochemical marker for lipid peroxidation. Values were expressed as nmol/g tissue.

2.6.2. Histopathological examination of spinal cord tissue sections

For animals undergoing histopathological processing, rats were intracardially perfused with physiological saline, followed by 4% paraformaldehyde in PBS. The vertebral column was removed and the spinal cord was left in bone overnight in 10% formalin. Three cross sections were sampled from the spinal cord, at the site of injury, rostral and caudal to it. Sections biopsied were embedded in paraffin for tissue sectioning. Alternating 3–5 μm sections were stained with hematoxylin and eosin (H&E) for histopathological analysis. The segments of each spinal cord tissue were evaluated by a histopathologist in a blinded fashion. The histopathological changes evaluated were neuronal nuclear pyknosis or loss associated with dark eosinophilic or basophilic staining of the cytoplasm,

congestion, edema, infarction as well as neutrophilic infiltration of the gray matter parenchyma. Damaged neurons were counted and scored on a 6-point scale: (0), no lesion observed; (1), gray matter contained 1 to <5 dead neurons; (2), gray matter contained 5–10 dead neurons; (3), gray matter contained more than 10 dead neurons; (4), small infarction (less than one third of the gray matter area); (5), moderate infarction (one third to one-half of the gray matter area); (6), large infarction (more than half of the gray matter area).²⁹ The scores of all the sections from each spinal cord were averaged to give a final score for each individual rat.

2.6.3. Immunohistochemical expression of NF- κ B p65 and caspase-3 proteins in the spinal cord

To investigate the expression of activated NF- κ B and Caspase-3 after SCCI, sections 3–5 μ m thick from each spinal cord were cut on coated slides. The immunostaining procedure was done following the streptavidin–biotin-immunoenzymatic antigen detection method, performed according to the manufacturer's protocol. The primary antibodies (NF- κ B-p65 subunit Rabbit Polyclonal Antibody, Cat. #RB-9034-R7 and Caspase-3 Cat. #RB-1197) as well as the detection system kit (UltraVision detection system) were purchased from Lab Vision Corporation (Neo Markers, Fremont, USA). Negative controls (where the primary antibody has been omitted) were included in all runs. Sections from normal prostate gland known to be positive for NF- κ B expression served as a positive control and were also included in each run. Similarly, sections from human tonsils known to be positive for caspase-3 expression served as a positive control and were also included in each run. Images were observed and captured on an Olympus BX53 microscope equipped with a UC-30 digital camera (Olympus, Japan). As a measure for NF- κ B activity, the numbers of cytoplasmic- and nuclear-positive neurons were separately counted and their percentages to the total number of neurons were calculated.³⁰ The percentage of caspase-3 positively stained (neuronal and non-neuronal) cells to the total number of cells was estimated in 4 non-overlapping fields in 4 sections (obtained from 1 and 2 mm length around the injury site). These regions were chosen because of the spared tissue even after SCCI.

2.7. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 20.0 for Windows (SPSS, Chicago, IL). Results were presented as means \pm S.D., and analyzed by one-way and repeated measures analysis of variance (ANOVA), as indicated. When significant, pairwise comparisons between groups were made using Post-hoc with least significant difference test. In all statistical comparisons, $P < 0.05$ was considered as significant difference.

3. Results

3.1. Neurological assessment

No significant difference was observed between-groups in the results of any test before surgery. This confirms that all rats were normal prior to experimentation.

3.1.1. Gross motor: BBB open field rating scale

On the 1st day post-injury, there was a drop of BBB score to zero, whereas sham-operated controls have mean score of 19.75 ± 0.37 . During the weekly locomotor BBB assessment to evaluate functional recovery, we observed a significant improvement of BBB scores in the medical and surgical treated groups started at the first week and progressed overtime, compared to SCCI saline-treated group. Combined therapy group showed better overall scores than mono-therapy ($P < 0.001$, versus Agm or DL groups) (Fig. 1A), and the best BBB score after 4 weeks (Table 1).

3.1.2. Fine motor: hindlimb placement in grid walk (GW) task

In the GW task, sham-operated rats crossed the grid with minimal, if any, footfalls errors. Next day following SCCI, all rats were not able to bear weight, and had scores of 30. As the rats recover, the number of footfall errors decreases in groups. However, the overall decrease in errors in the SCCI was significantly less than other treated groups ($P < 0.001$, repeated measures ANOVA). Combined Agm + DL group gained the uppermost decrease in foot miss-placement ($P < 0.001$, versus individual treatment) (Fig. 1B), and the least total number of errors four weeks following surgery (Table 1).

3.1.3. Residual urine volume

The sham-operated rats demonstrated bladder control, with no residual urine. However, in rats subjected to SCCI, the retained urine volume increased over days to peak at about 6 days post-injury, and then declined thereafter till day 14 post-injury, showing differential rates of improvement in self-voiding and bladder function. The overall residual urine volume in Agm-treated groups (alone, or combined with DL) was significantly reduced ($P < 0.001$ for both, by repeated measures ANOVA, versus SCCI group) (Fig. 1C). After 14 days post-injury, 87.5%, 68.8% and 62.5% of combined-, Agm- and DL-treated rats, respectively, established spontaneous voiding reflex, versus 37.5% of the SCCI rats.

3.2. Biochemical estimations of spinal cord tissue levels of inflammatory cytokines and oxidative stress parameters

As shown in Fig. 2, there was a significant increase in IL-6 and TNF- α levels ($P < 0.001$ for both), as well as a marked increase in free radical generation and lipid peroxidation as depicted by a significant depletion of TAC ($P < 0.001$) and rise in MDA ($P < 0.001$) levels in spinal cord tissues of the SCCI group as compared to sham group. Treatment with Agm for four weeks and/or DL resulted in a significant reduction of the inflammatory cytokines levels versus SCCI rats. Yet, no significant difference was found between pharmacological, surgical and combined treated groups. Agm supplementation alone or combined with DL caused a significant induction of TAC and inhibition of MDA levels ($P < 0.001$ for all) versus untreated SCCI. However, the effect of DL on TAC and MDA levels was insignificant ($P = 0.297$, $P = 0.163$, respectively) versus SCCI rats.

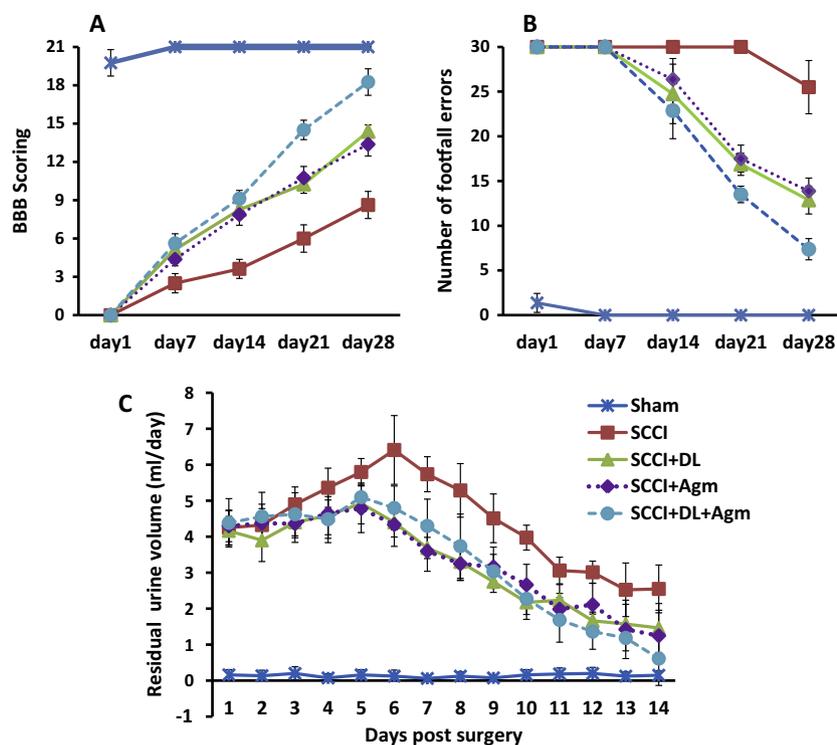


Figure 1 Effect of agmatine (Agm) and/or decompression laminectomy (DL) on neurological functions after spinal cord compression injury (SCCI); (A) the time course of hindlimb motor recovery as measured by open-field BBB score and (B) the number of foot misplacements (footfall errors) in grid walk task, where animals were evaluated weekly over 28 days after injury. (C) Assessment of daily volume of retained urine as a measure of bladder function over 14 days following SCCI. Data are expressed as means \pm SD of sixteen animals per group and assessed by repeated measures ANOVA.

Table 1 Motor performance of different groups 28 days following acute spinal cord compression injury (SCCI).

	Sham	SCCI	SCCI + DL	SCCI + Agm	SCCI + DL + Agm
Open-field BBB scoring	21 \pm 0.00	8.63 \pm 0.38*	14.38 \pm 0.18* ^{#S}	13.38 \pm 0.32* [#]	18.25 \pm 0.37* ^{#SS}
Number of Foot-fall errors	00.00	25.50 \pm 1.05*	12.88 \pm 0.55* [#]	13.88 \pm 0.52* [#]	7.38 \pm 0.42* ^{#SS}

Data are expressed as means \pm SD of sixteen animals per group and assessed by one-way ANOVA.

* $p < 0.001$ versus sham.

$p < 0.001$ versus SCCI group.

^S $p < 0.05$.

^{SS} $p < 0.001$ versus SCCI + Agm group.

3.3. Histopathological and immunohistochemical findings

3.3.1. Histopathological examination

The degree of the spinal cord injury at the perilesional segments was evaluated. Sections in the spinal cord of sham group showed normal neurons with round and pale stained nuclei. Samples of the SCCI group showed marked damage of the gray matter parenchyma in the form of variable infarction, hemorrhage, congestion and neutrophilic infiltration. After counting the dead neurons and estimating the extent of infarction, a significant damage to the spinal cord was observed in the SCCI group with a mean score 4.5 ± 1.2 , which was ameliorated after Agm or DL treatment (mean scores were 2.25 ± 1.0 and 2.5 ± 1.1 , respectively). Of note, combined Agm administration and DL resulted in the minimum histopathological damage and neuronal death with a histological score of 1.5 ± 0.5 (Fig. 3).

3.3.2. NF-κB-p65 protein immunohistochemical expression

In the sham-control group, neuronal expression of NF-κB-p65 was mainly cytoplasmic. Constitutive expression of NF-κB-p65 in nuclei of normal neurons was minimal. However, after SCCI, positive cells for nuclear staining of NF-κB-p65 were increased, compared to sham controls. This denotes translocation of from NF-κB-p65 cytoplasm to nucleus. In the DL group, NF-κB-p65 protein was expressed mainly in the nuclei of neurons. Nuclear translocation of NF-κB-p65 subunit decreased in samples of the Agm- and combined-treated groups, as the positive cells for cytoplasmic staining were increased compared to SCCI untreated rats (Fig. 4).

3.3.3. Immunohistochemical detection of caspase-3 expression

As shown in Fig. 3, immunohistochemical staining results revealed that SCCI induced caspase-3 expression in neuronal and non-neuronal cells around the injury site. However,

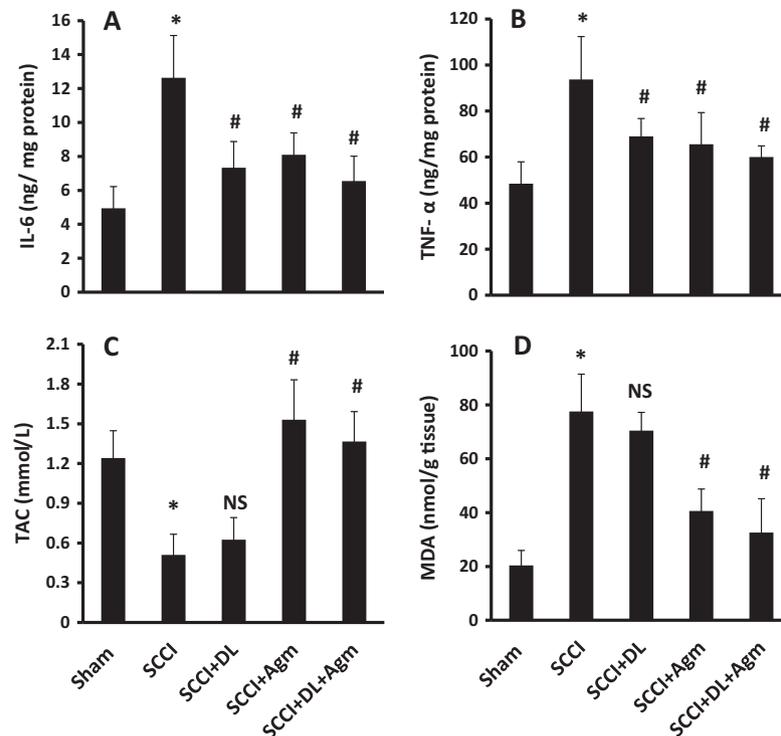


Figure 2 Effect of agmatine (Agm) and (or) decompression laminectomy (DL) on spinal cord tissue levels of inflammatory cytokines; IL-6 and TNF- α (A and B, respectively) and oxidative stress markers; TAC and MDA (C and D, respectively) in acute SCI rats. Data are presented as means \pm SD of eight animals per group. * p < 0.001 versus sham; # p < 0.001, NS, nonsignificant versus SCI group.

number of caspase-3 positive neurons was reduced in the Agm and DL treated groups compared to SCI group at 28 days post-injury. The least percentage of caspase-3 positive cells was observed in the combined-treated group.

4. Discussion

In the past, administration of high-dose steroids has been the only specific pharmacological therapy in acute SCI in human. Besides the harmful adverse effects, their therapeutic outcome was not satisfactory.² Recently, pharmacological intervention by targeting NF- κ B pathway was recommended.⁴

Several studies have focused on Agm neuroprotective potentials in CNS disorders. The present study demonstrated that Agm monotherapy or in combination with DL improved gross and fine motor activities, restored bladder function and regained micturition reflex in about two weeks following SCI in most rats. Agm functional recovery was associated with biochemical improvement in injured spinal cord tissues, reflected by reduction of inflammatory cytokines (IL-6 and TNF- α) and oxidative stress, increase in total antioxidant capacity TAC, together with decline in histopathological damage and neuronal apoptosis. Interestingly, these results were paralleled with inhibition of nuclear translocation of NF- κ B in the injured segments. Combined pharmacological and surgical treatments showed better motor and bladder recovery and less histopathological insult and neuronal apoptosis than individual treatment.

In line, previous reports showed that Agm treatment improves functional recovery and reduces tissue damage in

different models of SCI in rats.^{14,31} Several mechanisms have been proposed to stand beyond Agm beneficial effects. Agm, as an inhibitor of NMDA receptors,¹⁵ may reduce glutamate-mediated neuronal excitotoxicity involved in SCI damage. Recent studies reported an Agm enhancement of neuro-regeneration and re-myelination through modulation of expression of multifunctional growth factors in neurons, oligodendrocytes and astrocytes, such as transforming growth factor β -2 (TGF β -2) and bone morphogenetic proteins (BMPs), thereby reducing the collagen or glial scar formation at the site of lesion.^{32,33} Moreover, Agm can potentiate opioid analgesia, possibly by modulating opioid receptors, reducing subsequently pain perception and allowing better recovery of motor functions after SCI.^{20,34} All of which may help to accelerate motor recovery and restoration of bladder function that are of high priority for the patients' quality of life.

Activation of NF- κ B is thought to play a central role in SCI associated secondary effects.^{4,10} Our finding revealed that SCI led to the activation and nuclear translocation of NF- κ B-p65 subunit within the neurons of the injured segment. In line, NF κ B-p65 was increased in injured spinal cord, stimulating transcription of many genes and mediating deleterious responses after SCI,¹⁰ while inhibition of this up-regulation exerted neuronal protection.³⁵ Previous trials of treatment of SCI by inhibition of NF- κ B activity was proved effective. For example, tetramethylpyrazine improved functional recovery after contusion SCI by inhibiting the transcriptional activity of NF- κ B through increasing the expression of its cytoplasmic inhibitor I κ B α .³⁶ Similarly, DNA decoy blockade of SCI-induced p65 activity decreased SC damage, enhanced

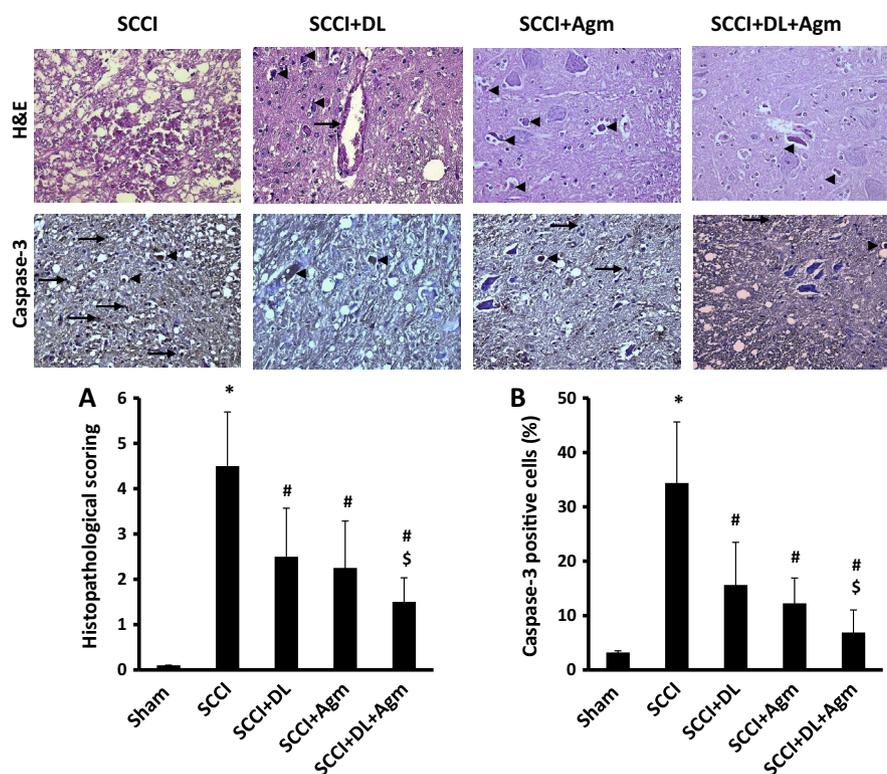


Figure 3 Effect of agmatine (Agm) and/or decompression laminectomy (DL) on histopathological findings (by H&E) and immunohistochemical expression of caspase-3 in injured spinal cord segments after 28 days of spinal cord compression injury (SCCI) (original magnification 400 \times). H&E stained spinal cord section of a rat from the SCCI saline-treated group shows marked hemorrhage, hemorrhagic infarct and vacuolation of the neuropil; the section in the spinal cord of DL treated rat shows vascular congestion (arrow) and moderate vacuolation of the neuropil. Three necrotic neurons can be discerned (arrow heads); in the section from an Agm treated rat, 5 necrotic/apoptotic neurons are seen in this field (arrow heads) with evident paraneuronal oligodendrocytes around necrotic/apoptotic neurons (satellite cells). A slight vacuolation of the neuropil is noted; in the section from the spinal cord of combined-treated rat, 2 necrotic/apoptotic neurons are evident in this field (arrow heads). Neuronal damage is scored from 0 to 6 (A) (as described in the methods). Caspase-3 immunohistochemical staining of spinal cord sections shows numerous positively stained neuronal (arrow heads) as well as neuroglial (arrows) cells of an SCCI rat. Agm and/or DL treatment significantly reduced the percentage of caspase-3 positive cells (B). A non-specific background staining is observed within the neuropil. Data are presented as means \pm SD. ($n = 8$ /group). * $P < 0.001$ versus sham group; # $P < 0.001$ versus SCCI group; NS, nonsignificant versus SCCI group.

locomotor recovery and limited inflammatory and apoptotic events.¹⁰

Although different mechanisms and multiple signaling molecules have been proposed for the neuroprotective actions of Agm, the possible contribution of NF- κ B modulation by Agm in SCI recovery was not previously investigated. Treatment with Agm alone or in combination with DL was found to inhibit neuronal activation and translocation of NF- κ B-p65 from cytoplasm to nucleus. However, DL alone did not suppress this activation. Recently, the relationship between Agm and NF- κ B was studied in different pathological animal models. Consistent with our results, Agm suppressed the phosphorylation and nuclear translocation of NF- κ B in rotenone-induced Parkinson's disease²¹ and in hypoxic-damaged retinal ganglion cells.²² In addition, Agm blocked the activation of NF- κ B and consequently, protected mice against acute lung or hepatic injury.^{37,38} However, in contrast to our findings, Agm was shown to facilitate translocation of NF- κ B-p65 from cytosol to nucleus in an in vitro model of astrocytes oxygen glucose deprivation.²³

Oxidative stress and inflammation are implicated in the pathophysiologic mechanisms underlying secondary damage in SCI.³ Consistent with previous studies,^{39,40} our findings showed a significant increase in injured tissue level of MDA, exceeding the tissue TAC, as well as, a significant increase in injured tissue level of IL-6 and TNF- α , concomitantly with obvious inflammatory histopathological insult. Lesion-induced oxidative stress and inflammatory cytokines are not only implicated as NF- κ B activators after SCI,^{7,8} but also develop as a sequel of NF- κ B activation, since it induces transcription of genes encoding pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6 and IL-12), cell adhesion molecules, iNOS, cyclo-oxygenase-2 (COX-2) and apoptotic cascades.^{4,10}

In SCI, the contribution of apoptotic cell death in tissue injury and neurological dysfunction is established through the implication of caspases as principal mediators of programmed cell death. Of concern, we reported caspase-3 expression up-regulation in neuronal and non-neuronal cells in injured spinal segments, in line with previous findings.^{41,42} Caspase-3 expression is preceded by upstream events initiated

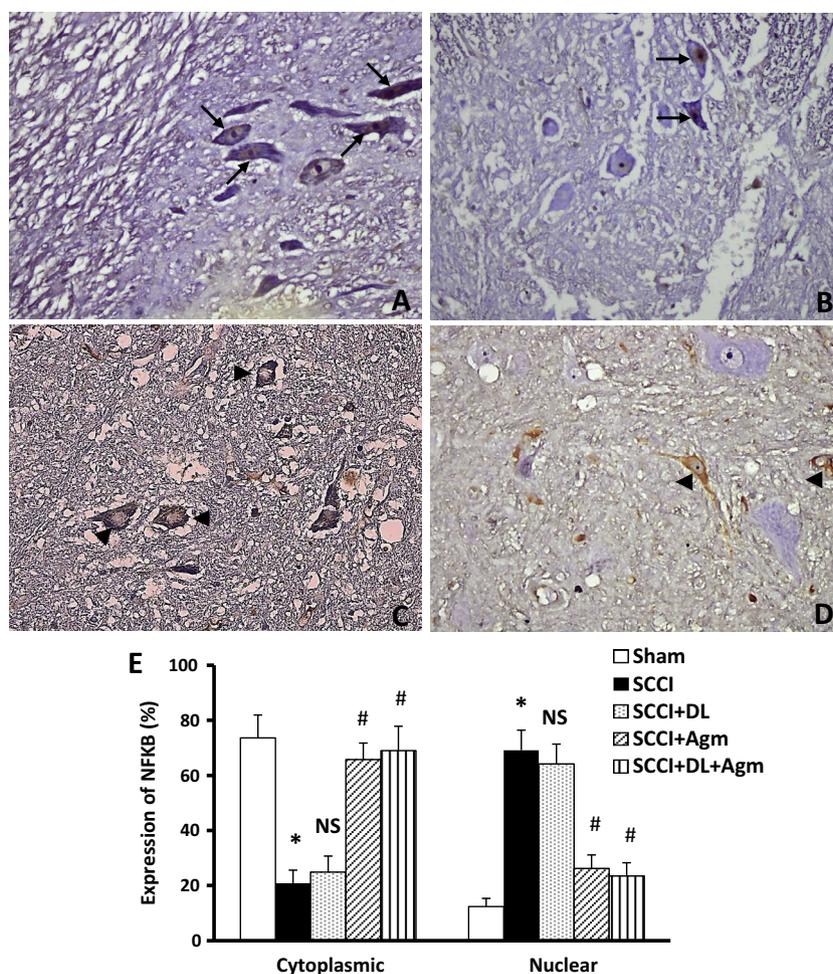


Figure 4 Effect of agmatine (Agm) and/or decompression laminectomy (DL) on NF- κ B immunohistochemical staining in injured spinal cord segments after 28 days of spinal cord compression injury (SCCI) (Original magnification 400 \times). (A) Positive nuclear staining in most of the neurons of SCCI rats; (B) Positive nuclear staining in some of the neurons of DL rats; (C) and (D) Positive cytoplasmic staining in most of the neurons of the Agm-treated and combined-treated rats, respectively; (E) Percentage of positive neurons for nuclear and cytoplasmic expression of NF κ B. Data are presented as means \pm SD. ($n = 8$ /group). * $P < 0.001$ versus sham group; # $P < 0.001$ versus SCCI group; NS, nonsignificant versus SCCI group.

by expression of TNF- α and activation of its death receptor (TNFR) mediating neuronal apoptosis.⁴³ Interestingly, the constitutive expression of the transcriptionally active member of NF- κ B family, c-Rel induces an early resistance against TNF- α -induced apoptosis; however, it has been reported that c-Rel is decreased in neurons after SCI, consistent with the observed failure of anti-apoptotic responses.^{10,44} Moreover, activation of NF- κ B is known to trigger a growth-permissive genetic program, which causes neuronal death and hampers neuronal regeneration.⁴

In the present study, Agm rather than DL significantly enhanced antioxidant defenses and combated oxidative stress. However, Agm and DL had equivalent anti-inflammatory and anti-apoptotic effects by inhibiting TNF- α and IL-6 levels in the injured segments along with decreasing caspase-3 neuronal expression. Consistent with our findings, Agm exerted in vitro and in vivo neuroprotective effects against oxidative damage, inflammation and/or apoptosis in retinal cell ganglia,²² injured peripheral nerves,⁴⁵ and even against rotenone²³ or lipopolysaccharide-induced neuronal damage.⁴⁶ On the

contrary, Feng and LeBlanc⁴⁷ demonstrated that Agm had no effect on the early release of inflammatory cytokines (IL-6 and TNF- α) in model of brain injury in rats pups after carotid artery ligation. The observed antioxidant and/or anti-inflammatory effects of Agm in some lung and liver disorders, suggested Agm may serve as a novel therapeutic strategy for diseases associated with these conditions.^{37,38,48}

Since enhancing antioxidant defenses and combating inflammation and apoptosis were reported to reduce post-traumatic lesion size and improve motor performance,^{41,46} the present observations suggest a neuroprotective role for Agm in acute SCCI in rats. The close interrelation between oxidative stress, inflammation and apoptosis with NF- κ B activation, allows us to postulate that Agm beneficial effects in SCCI were mediated, at least in part, via inhibition of NF- κ B activation. It is unlikely that Agm anti-inflammatory or antiapoptotic properties are behind its inhibition of NF- κ B activation, as the DL alone, though implementing significant anti-inflammatory, and antiapoptotic actions, did not suppress NF- κ B activation. It is possible that Agm inhibition

of oxidative stress may have a role in decreasing NF- κ B nuclear translocation. However, the exact mechanism by which Agm modulates NF- κ B activation remains to be elucidated.

5. Conclusion

The present research identified a new mechanism for the beneficial effects of Agm against SCCI in rats. Functional recovery and restoration of bladder function, observed by Agm treatment herein, were associated with reduced inflammatory response, oxidative stress and apoptosis. These neuroprotective actions of Agm in SCCI were mediated, at least in part, via inhibition of NF- κ B activation. Considering these specific functions, treatment with Agm (in combination with surgical intervention) might hold clinical potentials to improve functional recovery following traumatic SCI.

Conflict of interest

The authors declare no conflict of interest.

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