Research article

Effects of progesterone and vitamin D on outcome of patients with acute traumatic spinal cord injury; a randomized, double-blind, placebo controlled study

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Background: Steroid hormones offer promising therapeutic perspectives during the acute phase of spinal cord injury (SCI) while the role of progesterone and vitamin D remain controversial. The aim of the current study was to investigate the effects of progesterone and vitamin D on functional outcome of patients with acute traumatic SCI.
Methods: This was a randomized clinical trial including 64 adult patients with acute traumatic SCI admitted within 8 hours of injury. All the patients received methylprednisolone on admission according to standard protocol (30 mg/kg as bolus dose and 15 mg/kg each 3 hours up to 24 hours). Patients were randomly assigned to receive intramuscular injection of 0.5 mg/kg progesterone twice daily and 5μg/kg oral vitamin D3 twice daily up to 5 days (n = 32) or placebo (n = 32). Patients were visited 6 days, 3 and 6 months after injury and motor and sensory function was assessed according to American Spinal Injury Association (ASIA) score.
Results: There was no significant difference between two study groups regarding age (P = 0.341), sex (P = 0.802) and therapy lag (P = 0.609). The motor powers and sensory function increased significantly after 6 months in both study groups. Those who received progesterone and vitamin D had significantly higher motor powers and sensory function after 6 months of therapy. Those who received the therapy within 4 hours of injury, had significantly higher motor powers and sensory function 6 months after treatment in progesterone and vitamin D group. Therapy lag was negatively associated with 6-month motor powers and sensory function in progesterone and vitamin D group.
Conclusions: Administration of progesterone and vitamin D in acute phase of traumatic SCI is associated with better functional recovery and outcome.

Keywords: Spinal cord injury, Progesterone, Vitamin D, Functional recovery, Outcomes

Introduction

Traumatic spinal cord injury (SCI) is among the devastating outcomes of trauma leading to severe motor and sensory deficit along with high socioeconomic burden.[1] Following traumatic SCI, early degeneration and necrosis or apoptosis of anterior spinal horn cells is observed leading to partial or complete dysfunction of motor neurons.[2] To date, several strategies have been introduced and applied for minimizing the extent of damage and apoptosis of motor neurons following traumatic injury including transplant of peripheral nerves, olfactory ensheating cells, stem cells or schwann cells and enhancement of axonal growth using fibronectin conduits.[3] Delivery of neurotrophic factors, antioxidant compounds, antiglutamatergic drugs and steroids have also been applied as pharmacological approaches for treatment of spinal cord injury.[4–6]

Several lines of evidence have showed that steroid hormones encompass protective and therapeutic effects for traumatic SCI especially during the acute phase of the

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injury.[5,7] It has been well demonstrated that glucocorticoids are highly effective in preserving the neurological function after SCI in human subjects and in cord transection and contusion in animal models of rat and rabbit.[8,9] In the same way, it has been postulated and proved that gonadal steroids including 17β-estradiol and progesterone have neuroprotection effects in brain cortex lesions, traumatic brain injury and stem motor nuclei injuries as well as spinal injuries.[6,10] The mechanism of action for progesterone has been shown to be prevention of neuronal loss following contusion, ischemia and edema of the brain, and preservation of neurons after section of the hypoglossal and facial motor nuclei.[9,11] Experimental studies have demonstrated that administration of progesterone increase the survival of motoneuron after injury, protect against glutamate toxicity and oxidative stress and repair the defects of the nerve conduction.[6]

Vitamin D is also considered a neurosteroid with neuroprotective effects. Vitamin D protects the neurons from the early oxidative stress and prevents the apoptosis and neuroinflammation.[12] Vitamin D and progesterone might encompass synergic effects in neuroprotection. Recently it has been showed that at 24 hours after traumatic brain injury (TBI), administration of progesterone and Vitamin D demonstrates greater efficacy in reducing neuroinflammation compared to progesterone and Vitamin D given separately.[13] We have previously shown that simultaneous administration of Vitamin D and progesterone is associated with synergic effects on outcome of patients with TBI.[14] However, data regarding the synergic effects of progesterone and vitamin D on outcome of spinal cord injury is scarce. As clinical study on this issue is scarce, we performed this human study in order to determine the effects of progesterone and vitamin D on outcome of patients with acute traumatic spinal cord injury.

Materials and methods

Study population

This was prospective randomized double-blind placebo controlled trial being performed in Al-Zahra and Kashani hospitals, both tertiary healthcare centers affiliated with Isfahan University of Medical Sciences during a 1-year period from September 2012 to September 2013. We included patients with acute traumatic spinal cord injury between 18 and 65 years of age who are referred within 8 hours after injury. The study protocol was approved by both institutional review board (IRB) and ethics committee of Isfahan University of Medical Sciences and all the participants gave their informed written consents. The trial was registered with the Iranian Clinical Trials Registry (IRCT201310082445N2; www.irct.ir).

We included 64 adult patients (>18 years) suffering from acute traumatic spinal cord injury being randomized within 8 hours of the injury. Those less than 18 or more than 65 years of age, involvement of the nerve roots, cauda equina only, gunshot wounds, life-threatening morbidity and those who were pregnant were excluded from the study. We also excluded the patients who were addicted to narcotics, receiving maintenance steroids for other reasons, those who had received more than 100 mg of methylprednisolone or its equivalent, or 1 mg of naloxone before admission to the center. Those in whom follow-up was difficult, pure sensory loss, complete motor deficit and those who were randomized more than 8 hours after injury were further excluded from the study.

Randomization and intervention

Those who entered the study (n = 64) were randomly assigned to two study groups based on their registration numbers using a computer-based random digit generator. The progesterone, methylprednisolone and vitamin D were administered after inclusion in the study as soon as the patients entered the emergency room and the diagnosis was confirmed. The window period between receiving the intervention and the injury was less than 8 hours in all the patients. All the patients received standard treatment with methylprednisolone (30 mg/kg intravenously as bolus dose and 15 mg/kg each 3 hours till 24 hours). Those assigned to treatment group received intramuscular injection of progesterone (Fertigest®, Aburaihan Co., Tehran, Iran) 0.5 mg/kg twice a day for 5 days in addition to oral vitamin D3 (D-VIGEL 50000IU®; 40IU equal to 1μg; Dana Co, Tehran, Iran) 5 μg/kg twice a day for 5 days on admission (n = 32) while those in placebo group receive placebo injections and tablets in the same manner (n = 32). Progesterone, Vitamin D tablets and placebo were all prepared by the pharmaceutical school of Isfahan University of Medical Sciences. The progesterone and placebo injections were provided in 50-ml bottles with identical appearance, containing a lipid emulsion consisting of 6% soybean oil and 1.2% egg lecithin phospholipids, with the addition of 25 mg of progesterone per milliliter for the active treatment. The vitamin D and placebo tablets were also prepared in enteric coated hard gelatin capsules with identical appearance. The methylprednisolone was administered using a precise programmable syringe pump (New Era Pump Systems Inc., New York, USA). Patients were not aware of the medication they receive as treatment.

The nurse prescribing the drug was also be blinded toward the type of medication.

Study protocol and measurements

All the included patients underwent a complete history and physical examination after enrolment and the demographic information (age, sex) as well as clinical characteristics (neurological deficit, sensory level, muscle powers) were recorded. We also withdrew 5mL venous blood to measure the blood indices including complete blood count (CBC) and biochemistry information. All the patients received methylprednisolone during the first 4 hours of admission which means within 8 hours from injury. The rehabilitation started after 24 hours of admission. Patients were visited 6 days, 3 and 6 months after the injury and were assessed regarding neurologic function. Neurologic function was assessed using the American Spinal Injury Association (ASIA) score rating for sensory and motor function.[15] The percentage of neurologic recovery was defined as the "actual neural recovery" (final follow-up score minus preoperative score) divided by the "potential neural recovery" (maximal score minus preoperative score). According to ASIA score, the motor score is based on the examination of 10 key-muscles on each side. For each movement, force is measured and assigned a coefficient from 0 (absence of muscle contraction) to 5 when contraction creates a movement in all the joint amplitude against a complete resistance. The maximal total score is so 100 (50 on the Right and 50 on the Left). The sensory score is established after studying tact and prick sensitivity on a key point in each of 28 dermatomes on each side. Absence of sensitivity is quoted: 0, the hypo or the hyperesthesia: 1 and normal sensitivity: 2. The sensory evaluation was started by testing the light touch and the lower part of the body. The physician recording the outcome were blinded toward the study groups. All the patients and those visiting the patients postoperatively were blind to the study groups. Only the statisticians were aware of the study groups.

Statistical analysis

In order to have 80% power to detect significant differences between mean functional outcome with effect size of 3.5 ± 0.4 with α equal to 0.05, 30 patients were required in each study group ($P < 0.05$, two-sided). To compensate for possible nonevaluable patients and those who would possibly exit the study, we enrolled 64 participants. The Statistical Package for Social Science, SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. For comparing the ASIA scores within the groups we used Wilcoxon signed rank sum while Mann-Whitney *U* test was used for comparing the results between groups. To compare proportions between groups χ^2 test was used. Repeated measure test was used to compare the trends in changes of ASIA scores between two study groups. We also compared the results according to the time of receving the treatment. For this purpose, independent *t*-test was used to compare the results between those receiving treatment ≤ 4 hours and those getting the therapy after that. We also performed bivarient correlation analysis in oder to determine the linear correlation between therapy lag and functional recovery. Data are reported as means $\pm$ SD and proportions as appropriate. A two-sided P-value less than 0.05 was considered statistically significant.

Results

A total number of 79 patients were found eligible for the study out of whom, 11 had the exclusion criteria and 4 did not accepted to participate in the study. Thus 64 patients were randomized into 2 study groups to receive progesterone and vitamin D or placebo. As none of the patients were lost to follow-up, the final number of patients finishing the study and being included in the final analysis was 32 in each study group (Fig. 1).

The baseline characteristics of the patients in two study groups are summarized in Table 1. There was no significant difference between two study groups regarding the baseline characteristics including age ($P = 0.341$), sex ($P = 0.0802$), site of vertebral fracture ($P = 0.163$), therapeutic approach ($P = 0.793$) and time to between injury and intervention ($P = 0.835$). T12 was the most involved segment being recorded in 11 (17.2%) patients followed by L1 in 10 (15.6%) patients. Most of the patients (65.6%) were managed without surgical intervention. Most of the patients (59.3%) suffered from thoracic spinal cord injury. The surgical intervention consisted of canal decompression and spinal fixation (anterior or posterior) for restoring the alignment of the column.

Table 1 also compares the baseline ASIA scores of patients receiving progesterone and vitamin D with those receving placebo. As demonstrated, there was no significant difference between two study groups regarding the baseline ASIA scores. The ASIA motor and sensory score of the right upper and lower as well as left upper and lower extremities increased significantly 6 months after the injury in both study groups (Table 2). As demonstrated in Table 2, the ASIA scores are comparable between two study groups at 6

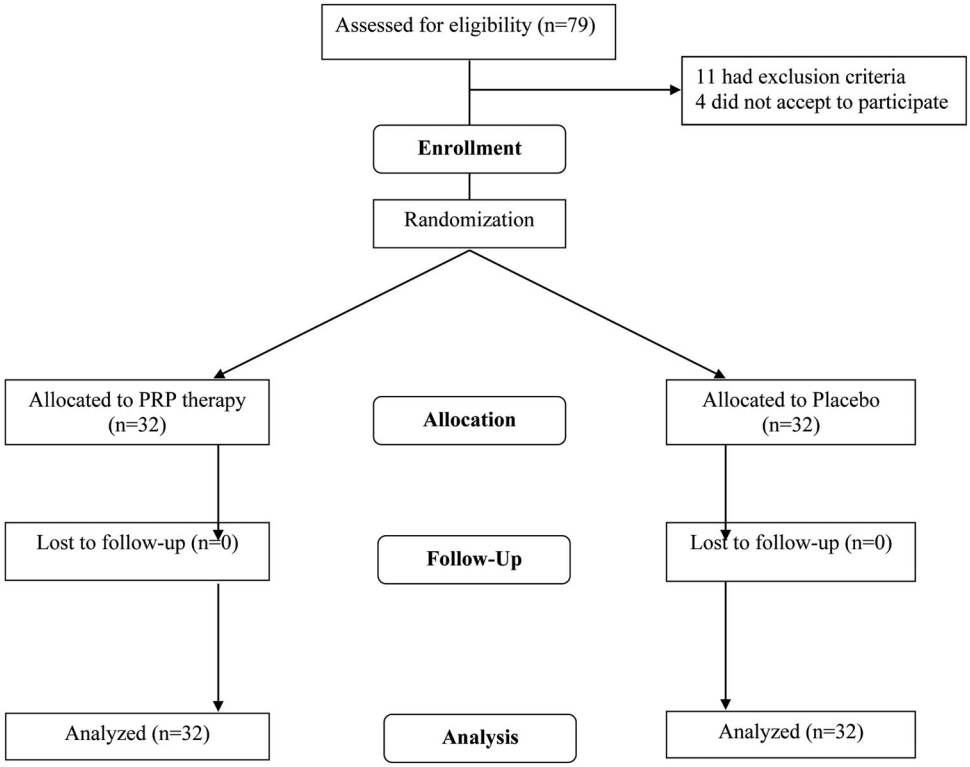

Figure 1 CONSORT flow diagram of the study.

Table 1 Baseline characteristics of 64 patients with acute traumatic spinal cord injury who received progesterone and vitamin D or placebo

	Progesterone + Vitamin D (n = 32)	Placebo (n = 32)	P-value
Age (years)	41.88 ± 13.6	45.2 ± 13.7	0.341
Sex			
Male (%)	19 (56.2%)	16 (50.0%)	0.802
Female (%)	14 (43.8%)	16 (50.0%)	
Injury Severity Score	28.9 ± 8.9	30.6 ± 9.4	0.224
Time between injury and intervention (hr)	3.62 ± 1.75	3.53 ± 1.83	0.835
≤4 hours	23 (71.9%)	22 (68.7%)	0.609
>4 hours	9 (28.1%)	10 (31.3%)	
Fracture site			
Cervical	7 (21.9%)	6 (18.8%)	0.652
C4 (%)	3 (9.4%)	0 (0.0%)	
C5 (%)	0 (0.0%)	3 (9.4%)	
C6 (%)	4 (12.5%)	0 (0.0%)	
C7 (%)	0 (0.0%)	3 (9.4%)	
Thoracic	19 (59.5%)	19 (59.5%)	0.998
T1 (%)	0 (0.0%)	3 (9.4%)	
T2 (%)	3 (9.4%)	0 (0.0%)	
T4 (%)	2 (6.2%)	3 (9.4%)	
T5 (%)	0 (0.0%)	4 (12.5%)	
T6 (%)	3 (9.4%)	0 (0.0%)	
T11 (%)	3 (9.4%)	6 (18.8%)	
T12 (%)	8 (25.0%)	3 (9.4%)	
Lumbar	6 (18.8%)	7 (21.9%)	0.652
L1 (%)	6 (18.8%)	4 (12.5%)	
L2 (%)	0 (0.0%)	3 (9.4%)	
Treatment			
Surgery (%)	12 (37.5%)	10 (31.2%)	0.793
Conservative (%)	20 (62.5%)	22 (68.8%)	

Table 2 The trend in changes of ASIA score in 64 patients with acute traumatic spinal cord injury who received progesterone and vitamin D or placebo

Variable	Progesterone + Vitamin D (n = 32)				P-value*	Placebo (n = 32)				P-value*
	Baseline	6 Days	3 Months	6 Months		Baseline	6 Days	3 Months	6 Months	
Motor										
Left Upper	21.4 ± 6.9	23.8 ± 5.9	24.6 ± 8.6	29.6 ± 5.1δ	0.002	22.4 ± 5.3	22.9 ± 4.3	23.3 ± 4.1	25.3 ± 3.7	0.061
Right Upper	21.9 ± 6.1	22.6 ± 7.2	25.9 ± 6.1	29.7 ± 4.9δ	0.031	22.6 ± 5.9	22.8 ± 4.5	23.4 ± 6.3	25.6 ± 3.6	0.053
Left Lower	8.28 ± 2.8	9.43 ± 6.5	10.1 ± 6.4	17.4 ± 6.7δ	<0.001	9.46 ± 3.6	11.3 ± 9.6	11.5 ± 9.6	13.1 ± 10.2	0.001
Right Lower	8.65 ± 3.6	9.37 ± 5.9	10.2 ± 5.9	17.8 ± 8.4δ	<0.001	9.84 ± 5.6	11.4 ± 6.4	11.9 ± 10.7	13.3 ± 10.3	0.001
Sensory										
Left Upper	35.8 ± 13.4	38.3 ± 14.8	38.3 ± 14.8	51.1 ± 14.2	<0.001	36.3 ± 13.3	39.3 ± 12.5	41.3 ± 12.1	48.1 ± 7.6	<0.001
Right Upper	33.8 ± 11.6	38.5 ± 14.6	38.5 ± 14.6	51.3 ± 14.1δ	<0.001	37.1 ± 13.2	39.8 ± 11.9	41.5 ± 11.8	45.6 ± 11.8	0.002
Left Lower	23.3 ± 17.8	30.6 ± 18.3	34.3 ± 17.8	39.8 ± 16.9δ	<0.001	21.8 ± 18.9	27.6 ± 20.1	31.2 ± 20.1	35.6 ± 20.7	<0.001
Right Lower	23.2 ± 17.8	30.8 ± 18.3	34.5 ± 17.9	39.9 ± 16.6δ	<0.001	22.1 ± 17.6	27.1 ± 20.9	31.2 ± 20.2	35.6 ± 20.8	<0.001

*p-value of less than 0.05 is considered statistically significant for trend in changes of ASIA motor and sensory scores in two different study groups.
δ Statistically higher ASIA motor or sensory score in progesterone and vitamin D group when compared to placebo group using independent t-test ($p < 0.05$).

days and 3 months after the treatment. However those who received progesterone and vitamin D had significamtly higher ASIA motor scores of left and right upper extrimities (P = 0.042 and P = 0.047 respectively) as well as left and right lower extrimies (P = 0.041, P = 0.034) when compared to placebo group after 6 months of therapy. The sensory ASIA scores also were higher in progesterone and vitamin D group compared to placebo group in right upper (P = 0.043), left lower (P = 0.048) and right lower (P = 0.039) estrimities after 6 months. We found that the baseline ASIA classification was comparable between two study groups. Although the ASIA motor and sensory scores were significantly higher in study group compared to placebo after 6 months, however the ASIA classification was comparable between two study groups (Table 3).

We compared the ASIA scores between those receving the treatment within 4 hours of injury and those receving it after 4 hours (Table 4). In progesterone and vitamin D group the ASIA motor and sensory scores for all four extrimities at 6 months of treatment was significantly higher in those receiving the therapy within the first 4 hours after injury when compared to those receiving it after 4 hours. This was consistant with placebo group in which the ASIA motor scores of left and right upper extrimities (P = 0.045 and P = 0.043 respectively) and sensory scores of left and right lower extrimities (P = 0.034 and P = 0.034 respectively) were significantly higher in those receving the treatment within 4 hours of injury. Bivarient correlation analysis revealed that the time lag between the injury and starting the treatment was negatively associated with 6-month motor power of left (r = –0.366, P = 0.003) and right (r = –0.336, P = 0.007) upper as well as left (r = –0.259, P = 0.039) and right (r = –0.260, P = 0.038) lower extrimities in progesterone and vitamin D group. In the same way the 6-month sensory ASIA scores of left (r = –0.305, P = 0.014) and right (r = –0.303, P = 0.015) upper as well as left (r = –0.301, P = 0.016) and right (r = –0.309, P = 0.023) lower extrimities in progesterone and vitamin D group were negatively associated with time lag. In the placebo group, we could not find any linear correlation between time lag and the 6-month motor and sensory ASIA scores. We also performed a subgroup analysis in order to determine the differences of ASIA motor score between men and women. We found that there was no significant difference between men and women in ASIA motor and sensory scores after 6 months of therapy in progesterone and vitamin D group. This was consistant in placebo group. The functional outcome and recovery was also compared between those who underwent surgical

Table 3 The ASIA classification at 6 days, 3 and 6 months in 64 patients with traumatic spinal cord injury who received progesterone and vitamin D (n = 32) or placebo (n = 32)

	Progesterone + Vitamin D (n = 32)	Placebo (n = 32)	P-value
ASIA class at 3 days			
A (%)	0 (0.0%)	0 (0.0%)	0.395
B (%)	11 (34.4%)	13 (40.6%)	
C (%)	10 (31.2%)	6 (18.8%)	
D (%)	9 (28.1%)	10 (31.2%)	
E (%)	2 (6.3%)	3 (9.4%)	
ASIA class at 6 days			
A (%)	0 (0.0%)	0 (0.0%)	0.774
B (%)	12 (37.4%)	10 (31.3%)	
C (%)	11 (34.4%)	9 (28.1%)	
D (%)	7 (21.9%)	10 (31.2%)	
E (%)	2 (6.3%)	3 (9.4%)	
ASIA class at 3 months			
A (%)	0 (0.0%)	0 (0.0%)	0.903
B (%)	10 (31.2%)	10 (31.2%)	
C (%)	7 (21.9%)	6 (18.8%)	
D (%)	9 (28.1%)	11 (34.4%)	
E (%)	6 (18.8%)	5 (15.6%)	
ASIA class at 6 months			
A (%)	0 (0.0%)	0 (0.0%)	0.664
B (%)	6 (18.8%)	7 (21.9%)	
C (%)	8 (24.9%)	9 (28.1%)	
D (%)	6 (18.8%)	6 (18.8%)	
E (%)	12 (37.5%)	10 (31.2%)	

intervention and those who were managed conservatively. The results were comparable between these groups in both the intervention group and the placebo group.

Discussion

Several lines of evidence indicate that progesterone has neuroprotective effects for CNS trauma including traumatic brain injury[5,14,16,17] and spinal cord injury.[7] However most of the studies addressing the effects of progesterone on outcome of traumatic spinal cord injury are cell culture or animal studies and clinical information is scarce in the literature. Thus we performed this randomized clinical trial to determine the effects of progesterone and vitamin D on outcome of spinal cord injury. We found that the 6-month ASIA motor and sensory scores was significantly higher in those receving progesterone and vitamin D when compared to those who received placebo. In other words, the synergic administration of progesterone and vitamin D along with methylprednisolone possess an additive value when compared to methylprednisolone alone. The idea to add vitamin D to progesterone came from the previous reports that indicate the synergic neuroprotective effects of these two agents both experimentally [12] and clinically.[14]

Due to the fact that spinal cord injury often results in complete loss of motor and sensory function, many search projects for novel pharmacological therapies are constantly under way. After spinal cord injury, ventral horn motoneurons show early degeneration and chromatolysis, with death occurring by necrosis or apoptosis depending on the severity and/or type of the lesion.[18–20] Several strategies have been developed to preserve neuronal function and repair damage, including transplant of peripheral nerves, olfactory ensheating cells, stem cells or Schwann cells and enhancement of axonal growth using fibronectin conduits.[21] Pharmacological approaches have also been employed, such as delivery of neurotrophic factors, antioxidant compounds, antiglutamatergic drugs and steroids.[3,4,22]

Steroid hormones offer promising therapeutic perspectives during the acute phase of spinal cord injury.[23,24] Glucocorticoids, in this respect, have been the standard treatment for patients with acute spinal cord injury.[4,22] However administration of hight dose methylprednisolone in acute phase of spinal cord injury has been associated with significant immunosuppression, elevated infection risk, and myopathy.[23,24] Early reports have shown that progesterone preserves neurons after section of the hypoglossal and facial motor nuclei,[8] whereas in the spinal cord, treatment of rats with progesterone increases motoneuron survival after axotomy or injury, protects cultured neurons against glutamate toxicity and normalizes defective functional parameters of injured neurons.[25,26] In spite of these evidences, there is no general consensus that progesterone confers protection to the injured spinal

Table 4 The ASIA scores of 32 patients with acute spinal cord injury receiving therapy less than 4 hours or after that in two study groups

	≤4 hours	>4 hours	P-value
Progesterone + Vitamin D Group (n = 32)			
ASIA score at baseline			
Motor			
Left Upper	24.2 ± 3.7	24.3 ± 8.1	0.723
Right Upper	24.2 ± 3.8	23.2 ± 7.3	0.599
Left Lower	7.9 ± 2.8	9.2 ± 6.8	0.727
Right Lower	8.3 ± 2.7	9.5 ± 2.9	0.724
Sensory			
Left Upper	38.6 ± 10.7	34.4 ± 10.8	0.235
Right Upper	38.8 ± 10.8	35.5 ± 9.9	0.302
Left Lower	24.7 ± 11.9	19.5 ± 2.3	0.464
Right Lower	24.6 ± 9.6	19.3 ± 2.4	0.447
ASIA score at 6 months			
Motor			
Left Upper	34.7 ± 6.9	19.5 ± 2.2	0.001
Right Upper	34.8 ± 6.7	19.3 ± 2.4	0.002
Left Lower	19.3 ± 9.4	11.3 ± 1.2	0.048
Right Lower	19.4 ± 9.4	11.3 ± 4.9	0.034
Sensory			
Left Upper	43.4 ± 12.1	35.3 ± 17.9	0.052
Right Upper	43.4 ± 12.2	35.3 ± 17.9	0.052
Left Lower	40.6 ± 14.8	32.1 ± 21.4	0.046
Right Lower	40.7 ± 14.7	32.1 ± 21.1	0.047
Placebo Group (n = 32)			
ASIA score at baseline			
Motor			
Left Upper	22.6 ± 5.2	22.1 ± 5.9	0.762
Right Upper	22.6 ± 5.2	22.1 ± 5.8	0.761
Left Lower	10.1 ± 8.9	7.7 ± 7.8	0.496
Right Lower	10.6 ± 9.8	7.7 ± 7.9	0.441
Sensory			
Left Upper	37.7 ± 13.1	32.5 ± 10.6	0.297
Right Upper	38.7 ± 13.8	32.8 ± 10.6	0.267
Left Lower	23.9 ± 19.9	16.7 ± 15.8	0.338
Right Lower	24.1 ± 19.9	16.6 ± 15.8	0.324
ASIA score at 6 months			
Motor			
Left Upper	29.2 ± 3.9	21.4 ± 3.2	0.045
Right Upper	29.2 ± 3.7	21.6 ± 2.7	0.043
Left Lower	14.3 ± 1.4	12.0 ± 1.1	0.577
Right Lower	14.2 ± 1.4	11.7 ± 1.4	0.546
Sensory			
Left Upper	45.7 ± 12.6	40.7 ± 10.2	0.078
Right Upper	46.0 ± 12.6	40.7 ± 10.2	0.063
Left Lower	37.5 ± 20.2	30.7 ± 22.7	0.034
Right Lower	37.5 ± 20.3	30.7 ± 26.1	0.034

cord.[27,28] The effects of progesterone administration on functional outcome of patients with moderate and severe traumatic brain injury is also controversial.[14,29–31] Although some authors have reportd favorable results by administration of progesterone,[14,29] two recent large multicenter phase III clinical trials have challenged these effects. It has been recently reported that nor early (within 8 hours of injury),[30] neither very early (within 4 hours of injury)[31] intravenous administration of progesterone in patients with moderate and severe traumatic brain injury is associated with improvement of functional recovery. These studies have demonstrated that the mortality rate and the functional outcome was comparable between those who received progesterone and those who received placebo.[30,31] These negative results were further discussed by Stein who found the problem within the definition, appropriate patient selection, precise and quantitative measures of outcomes, how to tailor treatment parameters to suit the patient's condition and several other issues like trial design and execution.[32]

Experimental studies in animal models of SCI have shown that progesterone brings a strong neuroprotection, measured by the response of different neuronal

parameters, including the sodium pump, the cholinergic marker choline acetyltransferase (ChAT), the growth-associated protein GAP-43, the myelin basic protein (MBP) and brain-derived neurotrophic factor (BDNF).[28,32] Labombarda and co-workers demonstrated that administration progestroned in rats with SCI is associated with inhibition of astrocyte proliferation and activation; anti-inflammatory effects by preventing microglial activation and proliferation, and early proliferation of NG2+ progenitors and late remyelination.[6] In injured animals, in vivo progesterone treatment for 72 h restores to normal the reduced levels of the sodium pump mRNA and ChAT, whereas levels of GAP-43 mRNA are further enhanced. These are important effects, because ChAT catalyzes acetylcholine synthesis, the release of which at the neuromuscular junction starts muscle contraction.[33,34] In turn, the Na,K-ATPase maintains the membrane potential, neuronal excitability and entry of metabolites and ions into the soma, whereas GAP-43, due to its location at the growth cone, is involved in axonal regeneration. Therefore, the responses of these markers to progesterone in injured rats are interpreted as protective and regenerative for the damaged tissue.[33]

The exact mechanism of the synergistic effect of vitamin D with progesterone clearly needs to be explored. There is evidence that vitamin D interacts with progesterone and estradiol to stimulate their secretion in human placenta[35] and also acts in maintaining bone health in postmenopausal women. It is important to note that many of the physiological properties of vitamin D are also attributed to progstrone[36]—for example, both are natural hormones present in males and females. Neuroprotective concentrations of progesterone and vitamin D result in the specific activation of MAPK in primary cortical neurons.[12] Progesterone has previously been reported to activate MAPK in unchallenged primary hippocampal neurons after 30 minutes of exposure.[37] Thus upregulation induces expression of antiapoptotic genes like BCl-2, which then protects cells from toxic injury. Vitamin D activates MAPK in primary cortical neurons. There are reports suggesting that vitamin D activates MAPK in different experimental models.[38] The cell death data demonstrated that a lower concentration of vitamin D (20 nmol/L) significantly enhanced the neuroprotective efficacy of progesterone, as demonstrated by a marked MAPK activation in the combination therapy. Higher VDH concentration (100 nmol/L) reduces progesterone mediated neuroprotection but still activated MAPK. There is supporting evidence that MAPK is a necessary but not sufficient condition for neuroprotection by combinatorial treatment. For example, medroxy-progesterone acetate (MPA), despite activating MAPK, does not afford neuroprotection against glutamate insult in hippocampal neurons. Interestingly, MPA in combination with estrogen activates MAPK but blocks the neuroprotective effect of estrogen.[39] Nilsen and Brinton[37] suggested that a possible reason behind this paradox is that nuclear translocation of phosphorylated ERK is necessary to obtain steroid-induced neuroprotection.

We note some limitations to this study. First, we did not measured the serum levels of sex hormones and cytokines and thus we cannot comment on the mechanism of action of progesterone and vitamin D as a neuroprotective agent. Second, since no biomarkers or imaging data are provided, it is not possible to know whether changes in the rate of recovery" over time could have been amenable to treatment effects. Earlier recovery might be a factor in improving general health of the patients, but this cannot be determined here. Third, we did not measure the baseline vitamin D level, thus we cannot comment on the role of vitamin D level on functional outcome and recovery. Forth, we did not include a group of progesterone or vitamin D alone. Thus we cannot claim the synergic effects of these agents based on the results of the current study. According to the previous evidence regarding the synergic effects of these agents [12,14] we only included a combined group. The current study is among the first studies addressing the effects of progesterone on outcome of SCI on human subjects.

In conclusion, results of the current study indicate that synergic administration of progesterone and vitamin D in acute phase of SCI is associated with better functional outcome and recovery when compared to methylprednisolone alone. In other words, progesterone and vitamin D encompass neuroprotection effects which could be determined in clinical setting. Further studies are required to shed light on this issue.

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Disclosure

None of the authors have any commercial and financial conflicts of interest to be declared regarding the manuscript.

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