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Clinical effectiveness of progesterone in acute traumatic spinal cord injury. A randomized single centre placebo-controlled study

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Keywords
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ABSTRACT

Aim: With only limited treatment options in acute spinal cord injury (SCI) currently available, we aimed to investigate the effect of progesterone on neurological recovery after acute SCI.

Material and Methods: Randomised double-blind placebo-controlled single-centre trial on 128 patients of acute SCI (within 24 hours of injury) was conducted at our institute with the approval of the ethics committee. Eligible patients were allocated to the progesterone or placebo arm. Of 133 eligible patients, one from the placebo arm expired in the acute phase while 4 were lost to follow-up, leaving 128 patients in the study.68 patients remained in the progesterone arm and 60 in placebo. Patients in the progesterone arm received intramuscular progesterone while those in the placebo received intramuscular isotonic saline twice daily for five consecutive days. Neurological assessment was done at baseline, day six, first and sixth months using the American Spinal Injury Association (ASIA) score and motor and sensory actual neural recovery (ANR) scores.

Results: Baseline characteristics were comparable between the groups. At the end of six months, significant improvement occurred in motor and sensory ASIA scores in the progesterone arm (p=<0.01). Compared with the placebo, motor scores were significantly higher in the progesterone arm at 6 months while sensory scores were not (p= <0.01 and p= 0.59 respectively). Additionally, at 6 months, motor ANR was significantly higher in the progesterone arm vs placebo (p=<0.01 vs 0.65). Early progesterone administration (within six hours of injury) was associated with significantly higher motor and sensory ASIA scores at 6 months (p= <0.01vs0.04 respectively).

Conclusion: Administration of intramuscular progesterone within 24 hours in acute SCI was associated with better neurological recovery. Further multicentric studies are required to shed more light on the strength and consistency of this improved outcome.

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Introduction

Approximately, 2,50,000-5,00,000 people per year suffer spinal cord injury (SCI) globally. In the aftermath of trauma, spinal cord injuries carry the most devastating outcomes with severe motor deficits and high socioeconomic burden. [1] Traumatic SCI often results in early necrosis of anterior horn cells leading to a partial or complete loss of motor neurons and consequent severe weakness. [2] Sadly, though the pathophysiology behind traumatic SCI is well understood, the treatment options remain limited with no single drug showing robust effectiveness. Transplant of peripheral nerves, enhancement of axonal growth using fibronectin conduits, usage of olfactory ensheathing cells, stem cells or Schwann cells with an aim to minimize the necrosis associated with SCI have been tried in the past with some success.[3] Pharmacologic approaches, such as neurotrophic factors, antioxidant molecules, antiglutamatergic drugs and steroids have also been previously used for the treatment of SCI. [4,5]

The world neurosurgery society, in contrast to the results of a Cochrane review conducted recently deemed the use of methylprednisolone to be more harmful than beneficial in SCI. The role of corticosteroids in SCI thus stands conflicted. [1,6] The gonadal steroids (17β-estradiol and progesterone) on the other hand in experimental trials have shown consistent optimistic results in traumatic brain and spine injuries.[4]Pertinent to our study, progesterone has been shown to prevent neuronal loss following traumatic brain injury with increased survival of motor neuron, protection against glutamate toxicity and oxidative stress after SCI .[4] Thus, many experimental studies using animal model have abundantly displayed the neuroprotective role of progesterone in SCI. Despite its well proven effectiveness in animal trials, its place in clinical trials is murky. Keeping this in mind, we took up this randomized placebo-controlled study to investigate the effect of progesterone on neurological recovery after acute SCI.

Materials and methods

We performed a prospective, randomized double-blind placebo-controlled trial from December 2019 to December 2020 at our institute. We aimed to assess the neurological recovery after acute SCI in treatment (progesterone administered) and placebo arms by monitoring their American Spinal Injury Association (ASIA) score (motor and sensory) and actual neural recovery (ANR) score {final follow-up ASIA score minus ASIA score at the time of admission} at day six (6), one (1) month and after six (6) months of follow-up}. Institutional ethics committee (IEC) clearance was obtained vide IEC no. 38/MC/EC/2020.

Patient selection

All consecutive patients with acute traumatic SCI presenting within 24 hours of the injury between 18 and 65 years of age were included. Patients outside the specified age limit, 24 hours beyond the SCI, those with involvement of only the radicles or nerve roots, with firearm injury, with life-threatening morbidities, history of drug addiction or on steroids, pregnant women and unwilling for a minimum six months regular follow up were excluded from the study.

Randomization and intervention

Patients underwent simple randomization into the two study groups after fulfilling the inclusion criteria (Figure 1). The treatment group received progesterone (AqSusten, Sun Pharma, Uttarkhand, India) via intramuscular injection with a dosage schedule of 0.5 mg/kg of body weight twice daily for five consecutive days. The placebo group received intramuscular 0.9% sodium chloride injections twice daily for five consecutive days.

Study protocol and measurements

A thorough clinical history, including demographic information (age, sex) and detailed physical examination assessing the neurological deficit was recorded. All routine investigations were sent as per the standard protocol including complete blood count (CBC) and biochemistry (random sugar, renal and liver function tests). The time of administering progesterone/placebo injection after injury was recorded.

Patients were neurologically assessed at the time of admission, on day 6 and then at first and six months after the injury using the ASIA score. [7] Motor and sensory ASIA scores and the actual neural recovery score were also calculated at the time of admission, day six, one and six months follow up.

Statistical analysis

A minimum of 60 patients were required to detect any significant differences between the groups as per the ICCP panel for SCI. All relevant data were recorded and GraphPad Prism for Windows, version 9.1.0 (221) was used for data analysis. Per protocol analysis was done. For comparing the serial ASIA scores within the groups, repeated measure one-way ANOVA with Tukey's multiple comparison test was used. Student unpaired t-test was used to compare between the two study groups and $\chi 2$ test was used to compare proportions. We also attempted to compare the ASIA score on the basis of time since injury to administration of progesterone using unpaired t-test. For this, we divided the progesterone group into early (< 6 hours) and late intervention (>6 hours) based on the hours after SCI. Data were expressed as means ± SD or proportions as appropriate. P-value of less than 0.05 was considered statistically significant.

Results

A total of 133 patients met the inclusion criteria, after randomisation 71 and 62 patients were allocated to the case and control group respectively. A team of in-house neurosurgeons blinded to the trial design decided the best treatment plan, surgical or medical management for all patients. Three patients from the treatment arm and one from the placebo arm were lost to follow up and one patient in placebo group expired with sepsis during the acute phase. Thus, at the end of six months, 128 patients (68 cases and 60 controls) were included for the final analysis.

Baseline characteristics of patients in both the study groups were summarized (table 1). Both groups were comparable for age, gender, time between intervention and injury and site of injury. Most common fracture site was the cervical region.76.47% patients in progesterone group and 80% in placebo group underwent surgical management in the form of spinal canal decompression and fixation (anterior or posterior).

Table 1. Baseline characteristics of all patients

	Progesterone (n=68)	Placebo (n=60)	p-value
Age	35.86 ± 13.47	34.97 ± 11.67	**0.99**
Sex			**0.92**
Male	40 (58.8%)	36 (60%)	
Female	28 (41.2%)	24 (40%)	
Time from injury to intervention			**0.74**
< 6 hrs	48	40	
>6 hrs	20	20	
Fracture site			**0.81**
Cervical	**36**	**28**	
C2	2	0	
C3	6	2	
C4	8	8	
C5	10	10	
C6	6	8	
C7	4	0	
Thoracic	26	**22**	
T1-4	6	4	
T5-8	8	12	
T9-12	12	6	
Lumbar	**6**	**10**	
L1	4	6	
L2	2	4	
L3	0	0	
L4	0	0	
L5	0	0	
Treatment			**0.73**
Surgical	52	48	
Conservative	16	12	

ASIA score (motor and sensory for each limb) for both the groups (progesterone and placebo) at the time of admission, 6 days, 1 month and 6 months after injury was recorded (table 2). Mean ASIA score at admission was comparable in both groups (table 2).

At the final (six) months follow up the mean motor ASIA score was significantly higher in the progesterone arm (p=<0.01). Serial improvement in motor ASIA score was noted in both the groups from admission to 6 days, 1 month and 6 months but it reached statistical significance only in the progesterone arm. In this arm, significantly higher motor ASIA score was noted in either of the upper limbs {right and left upper limb (p= <0.01) and in the lower limbs {right and left lower limbs (p=<0.01)}. Further, sub-group analysis showed significant increase in the motor ASIA score of the progesterone group between admission, day 6 and at 1 month without a significant increase between the 1 and 6 month scores. Serial improvement in the motor ASIA score was noted in the placebo group also {right and left upper limb (p=0.07, 0.09 respectively) as well as in right and left lower limb (p=0.07, 0.10 respectively)} but failed to reach statistical significance (table 2).

Table 2. Serial changes in American Spinal Injury Association score in progesterone and placebo group in the individual limbs

Progesterone group (n=68) American Spinal Injury Association score							Placebo group (n=60) American Spinal Injury Association score				
		At admission	6 days	1 month	6 month	p-value	At admission	6 days	1 month	6 month	p-value
Motor	Right UpperLimb	19.52± 6.95	21.32± 5.95	22.56± 5.09	22.79± 5.10	<0.01	20.03± 5.90	20.29± 5.72	20.40± 5.78	20.40± 5.78	0.07
	Left Upper Limb	20 ±6.17	21.32± 5.69	22.41± 5.09	22.64± 5.11	<0.01	19.55± 6.27	19.92± 5.89	20.03± 5.96	20.25± 5.84	0.09
	Right Lower Limb	13.35± 6.17	16.71± 6.59	20.06± 6.75	20.58± 6.82	<0.01	11.66± 4.16	11.92± 4.08	12.22± 3.79	12.22± 3.79	0.07
	Left Lower Limb	12.26± 6.40	15.82± 6.42	19.91± 6.69	20.44± 6.78	<0.01	11.74± 4.14	12.00± 4.06	12.11± 3.90	12.29± 3.72	0.10
Sensory	Right	81.35± 23.45	89.23± 24.64	92±24. 12	92±24. 12	<0.01	83.11± 20.71	84.81± 19.7	86.66± 19.8	86.66± 19.8	<0.0 1
	Left	81.82± 21.37	88.05± 23.41	90.11± 23.47	90.11± 23.47	<0.01	84.59± 19.13	85.48± 18.9	87.03± 19.9	87.33± 20.2	<0.0 1

Table 3. Comparison of total American Spinal Injury Association total score between the two groups

	Motor			Sensory		
	Progesterone	Placebo	p-value	Progesterone	Placebo	p-value
Day 0	65.14±21.01	63.03±15.22	0.65	163.18±44.44	168.67±39.44	0.61
6 months	86.47±22.03	64.80±14.00	<0.01	182.11±47.33	176.2±40.54	0.59

Table 4. Serial trend on actual neural recovery in patients who received progesterone and placebo

Actual neural recovery								
Progesterone group					Placebo Group			
	6 days	1 month	6 months	p-value	6 days	1 month	6 months	p-value
Motor	10.02±6.42	19.79±11.66	21.32±13.44	<0.01	2.33±4.30	2.7±4.94	2.8±5.18	0.19
Sensory	14.11±16.83	18.94±23.86	18.94±23.86	0.03	2.86±5.27	7.26±8.52	7.53±8.49	0.005

Table 5. Comparison of actual neural recovery

Comparison of Actual neural recovery at Day 6 and at 6 months						
	Motor			Sensory		
	Progesterone	Placebo	p-value	Progesterone	placebo	p-value
Day 6	10.02±6.42	2.33±4.30	0.65	14.11±16.83	2.86±5.27	<0.01
6 Months	21.32±13.44	2.8±5.18	<0.01	18.94±23.86	7.53±8.49	0.02

Table 6. American Spinal Injury Association score between early vs late progesterone administration

American Spinal Injury Association (ASIA) score between Early vs late progesterone administration						
	Motor ASIA score			Sensory ASIA score		
	< 6 hours	> 6 hours	p- value	< 6 hours	> 6 hours	p- value
Day 6	80.54±14.14	62.3±30.05	0.02	186.83±39.87	154.4±58.70	0.06
1 Month	91.58±9.31	69.00±33.14	<0.01	192.66±38.82	156.8±57.89	0.04
6 Month	93.75±8.87	69.00±33.14	<0.01	192.66±38.82	156.8±57.89	0.04

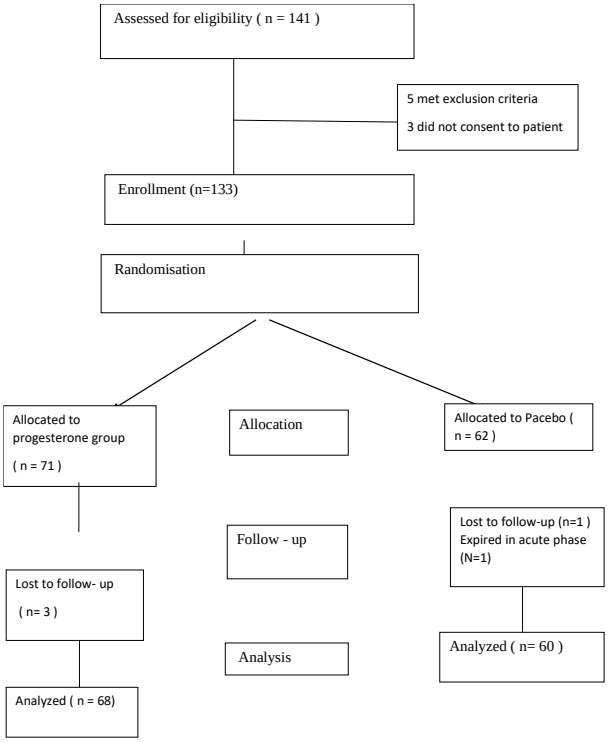

Figure 1. Allocation and analysis

Serial increase in mean sensory ASIA score was also noted from admission to day 6, 1 month and at 6 month follow up in both the arms. Improvement in sensory scores was noted over the time in both groups but a comparison of mean sensory ASIA score on day 0 and at 6 months between the groups did not reach significance (p= 0.59, table 3).

We also compared the serial actual neural recovery score at day 6, 1month and 6 month in both the groups (table 4). Mean motor as well as sensory ANR was found to be higher in the progesterone group. Serial increase in motor ANR at day 6, 1 month and 6 month in both groups was recorded but it was statistically significant only in the progesterone group (p=<0.01). Similarly, sensory ANR also increased in both groups but mean increase in progesterone group was much higher as compared to placebo group. On comparison of motor ANR (table 5) between progesterone and placebo groups at day 6 there was no statistically significant difference (p=0.65) but after 6 months of follow up there was a significant difference (p=<0.01).

On further post hoc analysis, we observed that there was statistically significant increase in mean motor ASIA score at day 6, 1 month and 6 month (80.54±14.14, 91.58±9.31, 93.75±8.87 respectively) if progesterone was administered early (within 6 hours of injury) (table 6). Mean sensory ASIA score also improved at day 6 and 1 month (186.83±39.87, 192.66±38.82) in the early and late progesterone administration groups and there was a significant increase noted with early administration. We also observed, that there was no significant gender difference between men and women in ASIA motor and sensory scores after 6 months of follow up in progesterone group as well as in placebo group. Similarly, neurological recovery was also comparable in surgical vs conservative management groups, in both the progesterone and placebo groups.

Only minor adverse effects such as transient diarrhea in one patient in placebo and nose block with pain at the injection site in the progesterone arm were reported. No major adverse events were recorded.

Discussion

The management of SCI till date remains challenging with no studies showing robust benefit of any drug or surgical intervention. The pathology behind SCI has largely been elucidated in the last decade with primary insult to oligodendrocytes and secondary insult to astrocytes and microglial cells by neuroinflammation.[8] Previous experiments using animal models suggested that SCI leads to increase in oligodendrocytes precursor cell (OPC) numbers

but impairs their differentiation and maturation, thus preventing their remyelination.[9] Jure I et al, demonstrated that SCI leads to down regulation of transcription inhibitors in the first few days following injury but during this period even the transcriptional activators remain downregulated and hence the OPC fail to mature and remyelinate.[10] The interest in progesterone in SCI is due to its ability to act as a differentiation factor and enhance expression of transcription activation factors.

Labombarda F et al in animal studies on rats found progesterone to be beneficial and neuroprotective in central and peripheral nerve injuries. [11] They concluded that SCI results in reduced level of choline acetyltransferase and reduced expression of mRNA for α3 catalytic and β1 regulatory subunits of neuronal Na-K-ATPase and stimulates growth-associated protein (GAP-43). Progesterone administration leads to reversal of this effect by enhanced expression of choline acetyltransferase and restoration of mRNA to normal levels which leads to further enhancement of GAP-43 after a three day course of progesterone therapy.

Various transcription factors responsible for OPC differentiation and maturation were also studied previously (Olig2 and Nkx2.2 involved in OPC differentiation and maturation of myelin producing cells, Mash 1 involved in Nkx2.2 expression, Sox10 responsible for stimulation of premyelinating oligodendrocyte cells into myelinating oligodendrocytes via inducing myelin basic protein expression and MRF involved in myelin and internode formation). [12,13,14,15] Progesterone increased Olig 2 and Nkx2.2 expression and also increased the mRNA expression of Sox 10 and Mash 1.[10,16]

Progesterone is known to stimulate OPCs production and stimulate OPC survival and prevent apoptosis via progesterone receptor dependent mechanism.[16,17,18]

Progesterone administration leads to increased TGFβ1 (transforming growth factor beta 1) mRNA levels which in turn promotes OPC maturation [19] In vivo TGFβ pathway up regulates antimitotic genes p15, p21 and p27 as well stimulates pro-oligodendrogenic gene Olig 1 and Olig 2.[19] Progesterone not only increased the oligodendrocytes maturation but also decreased the pro-inflammatory mediators as well as astrocytes and microglial cells after SCI in experimental models. [20] Further, it also attenuated microglial cells toxicity via fractalkine-CX3CR1 signaling.[21,22]

3α, 5α-tetra-hydro-progesterone, a metabolite of progesterone showed promyelinating activity in central and peripheral nervous system in previously conducted studies.[23] Progesterone also increased brain derived neurotropic factor(BDNF), thereby reducing the neuronal loss by neuro inflammation and oxidative stress which are major mechanisms of cell loss in SCI.[5]

Corticosteroids have often been administered in SCI (NASCIS I, II, III trials) but a statement by the world neurosurgery society suggested them to be a double-edged sword recently.[24] The clinical trials on traumatic brain injury (TBI) patients showed no major adverse effects with use of progesterone (such as increased risk of breast cancer, thrombotic risk, feminization effect in male patients).[25] Given its promising role, in experimental studies and safety profile, progesterone seems to be a safe and effective drug in SCI patients.

The anti-inflammatory beneficial effect of progesterone is well established in experimental animal studies but in clinical trials on humans with brain or spinal cord injury the reports have been conflicting. Several randomized controlled trials previously showed progesterone mediated improvement in neurological outcome after traumatic brain injury (TBI).[25,26] However, multicentric larger trials followed soon after and showed no advantage of progesterone in TBI. Interestingly, these negative trials were questioned for their faulty extrapolation of data from pre-clinical trials and use of subjective measures for defining neurological improvement by some researchers.[27] In a very recently published meta-analysis Begemann M et al concluded that progesterone indeed had a beneficial effect in TBI, especially when given early and by the intramuscular route only.[28]

As the pathophysiology of neural injury is similar in TBI and SCI, we hypothesized that progesterone may have a beneficial effect in patients with SCI. A thorough literature review revealed only one study was conducted previously in this regard. In their randomized, double-blind, placebo controlled trial Aminmansour B et al assessed the effect of progesterone and vitamin D on outcome in patients after acute traumatic SCI and concluded that administration of both the drugs in combination

were associated with better functional recovery and outcome.[29] In this study, motor and sensory ASIA scores improved significantly in the treatment group after 6 months of follow-up. Similar to the only other previously conducted study, we found a beneficial effect of progesterone in SCI with statistically significant improvement in sensory and motor ASIA scores after 6 months of follow up. On comparing the ASIA scores between the progesterone and placebo arm at 6 months, the motor scores showed significant improvement while the sensory scores between the groups did not. The actual neural recovery scores also showed significant improvement in the progesterone motor and sensory arm at 6 months. Additionally, the early administration of progesterone within six hours of the traumatic insult was more beneficial than the late administration (p= <0.01 vs p= 0.04). This time dependent benefit could be explained with the activation of neuroinflammation and destruction progressing relentlessly after the injury.

Limitations and scope

Firstly, we did not measure the baseline and post administration serum progesterone level, therefore a therapeutic range that would predict outcome could not be ascertained. Secondly, if the surgical intervention contributed towards clinical improvement in the progesterone group cannot be ascertained. However, patients undergoing surgical intervention were equally present in the placebo group also. Therefore, any procedural benefit would be similar in both the groups. Despite these limitations, our results remain valid as both the progesterone and placebo group had no significant baseline differences in demographics, level and clinical severity of injury and surgical management.

The areas that can be researched further include the effect of progesterone on expression of serum or cerebrospinal fluid inflammatory (IL-6, IL-8, TNF-α, MCP-1) and structural (GFAP, NSE, S100b, tau) biomarkers following SCI.[30]

Conclusion

No single agent till date has produced remarkable recovery in SCI. In the past few decades, with improved critical and supportive care the mortality after SCI has substantially reduced but the morbidity and caregiver burden remains high. Our study, shows a small but definitely beneficial effect of progesterone in SCI patients. Larger studies are needed to further understand the depth of this therapy. A deeper understanding into the pathophysiology of SCI should also lead to development of more molecules that are effective in reducing the morbidity associated with this condition.

Abbreviations:
SCI – Spinal cord injury
ASIA score – American Spinal Injury Association score
ANR score – Actual Neural Recovery
TBI- Traumatic Brain Injury

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