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Original Article

# Spinal cord regeneration by modulating bone marrow with neurotransmitters and Citicholine: Analysis at micromolecular level


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ABSTRACT

*Background:* Spinal cord injury results in disruption of brain-spinal cord fibre connectivity, leading to progressive tissue damage at the site of injury and resultant paralysis of varying degrees. The current study investigated the role of autologous bone marrow modulated with neurotransmitters and neurotransmitter stimulating agent, Citicholine, in spinal cord of spinal cord injured rats.

*Methods:* Radioreceptor assay using [3H] ligand was carried out to quantify muscarinic receptor. Gene expression studies were done using Real Time PCR analysis.

*Results:* Scatchard analysis of muscarinic M1 receptor showed significantly decreased $B_{max}$ ($p < 0.001$) and $K_d$ ($p < 0.01$) compared to control and significant reversal ($p < 0.001$) in both the treatment groups (spinal cord injury treated with 5HT and GABA, and spinal cord injury treated with Citicholine). Muscarinic M1 receptor gene expression in spinal cord injured group showed significant down regulation ($p < 0.001$) compared to control, and both the treatment groups significantly reversed ($p < 0.001$) these changes to near control when compared to spinal cord injured group. The confocal microscopic study using specific antibody of muscarinic M1 confirmed the gene expression studies.

*Conclusion:* Thus our results suggest that the neurotransmitters combination along with bone marrow or Citicholine with bone marrow can reverse the muscarinic receptor alterations in the spinal cord of spinal cord injured rats, which is a promising step towards a better therapeutic intervention for spinal cord injury because of the positive role of cholinergic system in regulation of both locomotor activity and synaptic plasticity.

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## At a glance commentary

### *Scientific background on the subject*

Spinal cord injury (SCI) affects many pathways in brain and spinal cord. A proper understanding of alterations in central signalling will help in devising better treatment options. The present study investigated the role of autologous bone marrow modulated with neurotransmitters and Citicholine in spinal cord injured rats for functional recovery.

### *What this study adds to the field*

Promising results in our animal study showed that treatment with 5HT and GABA with bone marrow aspirate, and Citicholine with bone marrow aspirate can facilitate functional recovery of altered muscarinic receptor function. The modulation of bone marrow aspirate by neurotransmitters or citicholine have opened a new treatment strategy in SCI.

Spinal cord injury (SCI) has permanent and devastating neurological deficits and disability. SCI is a progressively debilitating condition with poor prognosis and remote possibility of repair of the damaged neurons in the Central Nervous System [1]. The spinal cord acts as the primary information pathway between the brain and peripheral nervous systems of the body. SCI causes tissue damage through both primary and secondary mechanisms. The primary mechanical injury results in damage to neuronal and vascular tissue. Neurological dysfunction results more from the secondary changes than the primary neuronal damage [2]. Axons are damaged beyond repair and neural cell membranes are broken in spinal cord injury. During spinal shock, even undamaged portions of the spinal cord become temporarily disabled and cannot communicate normally with the brain. Therefore, effort has been undertaken to develop *in vivo* models of SCI and to study the cellular and molecular mechanisms of synaptic connections and information processing in the spinal cord.

Cholinergic motor neurons stimulate muscle contraction. The imbalance in the excitation and inhibition within the motor circuit disrupts coordinated body muscle contraction [3]. Acetylcholinesterase, the enzyme that catalyses a reaction of hydrolysis of acetylcholine to choline and acetate [4], is regarded as a specific marker for cholinergic function [5] and is used as a differentiation marker. Receptors activate a multitude of signalling pathways important for modulating neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine (ACh) release [5].

Stem/progenitor cells derived from the ependymal region of the spinal cord have the ability to self-renew and are multipotential for neurons and glia. These cells have the ability to regenerate the injured mammalian spinal cord [6]. Treatments using human embryonic stem cells have shown improved mobility in rats with spinal cord injuries, providing the first physical evidence that the therapeutic use of these cells can help restore motor skills lost from acute spinal cord tissue damage. Haematopoietic system is used as a source of progenitor cells for the CNS and it also has the property to differentiate into both microglia and macrophage when injected directly to the brain of adult mice. Although bone marrow cells (BMC) normally give rise to mesenchymal derivatives, such as osteoblasts, adipocytes, myoblasts and chondrocytes, recent studies indicated that these cells were capable of remarkable phenotypic plasticity. BMC is induced to differentiate into cells with surface markers characteristic of neurons [7].

The bulbospinal monoamine transmitters, released from serotonergic, noradrenergic, and dopaminergic systems, exert modulatory control over spinal sensory systems as acetylcholine, an intrinsic spinal cord biogenic amine transmitter. Generally, the monoamines facilitate motor activity [8]. The administration of Serotonin (5-HT) and Gamma aminobutyric acid (GABA) as therapeutic agents for cell proliferation and differentiation is a novel approach. Our earlier studies showed that 5-HT and GABA acting through specific receptor subtypes $5HT_2$ (+) and GABA [9] respectively, control cell proliferation and act as co-mitogens. Serotonin and GABA along with bone marrow cells in combination showed reversal of glutamate receptors and motor abnormality shown in the Parkinson's rat model [9].

Citicholine is an exogenous source for acetylcholine synthesis, a key neurotransmitter [10]. Supplementation with Citicholine can increase the amount of choline available for acetylcholine synthesis and aid in rebuilding membrane phospholipid stores after depletion [11].

The main objective of the present study was to investigate role of autologous bone marrow modulated with neurotransmitters and neurotransmitter stimulating agent, Citicholine, in spinal cord of spinal cord injured rats for the functional recovery.

## Materials and method

### *Animals*

Male adult Wistar rats of 200–250 g body weight were used for the experiments. Each group consisted of 8–10 rats. They were housed in separate cages under 12-h light and 12-h dark periods and were maintained on standard food pellets and water *ad libitum*. All animal care and procedures were in accordance with Institutional, CPCSEA and National Institute of Health guidelines.

### *Experimental design*

Under all aseptic precautions and ether anaesthesia, monoplegia was induced by shearing between the T9 and T12 vertebra of the experimental rats. A specially designed rubber chamber with silastic catheter [12] was inserted subcutaneously and the tip of silastic tube inserted to the injury site and fixed with sutures. Spinal cord injury was confirmed by monoplegia. Those rats that developed monoplegia after 3 h of the surgery were selected for further experiments. These rats were randomly divided into the following groups as (i) Control (C), (ii) Spinal cord injured (SCI), (iii) Spinal cord injured + Bone