Introduction

Spinal cord injury (SCI) is a major public health issue worldwide. It causes changes in all physical systems and functional abilities (Krause & Crewe, 1991). It is a devastating neurological injury, resulting in varying degrees of paralysis and sensory loss which are permanent and irreversible. In India, approximately fifteen lakh people are affected with SCI. Majority of them (82%) are males in the age group of 16-30 years (Gupta et al., 2008). A recent survey reported that the prevalence of SCI ranges from 236 per million in India to 1800 per million in the USA (Hagen et al., 2012). SCI is caused mainly from motor vehicle accidents, fall from heights, sports injuries (Dunn et al., 2000). It also results from a gunshot or knife wound that penetrates and damages spinal cord. When the spinal cord is injured, the nerves above the level of the injury continue to work. However, below the level of the injury communication is disrupted which can result in loss of movement, sensation, bowel and bladder control. The spinal cord relay messages between the brain and various parts of the body. Disruption of the spinal cord leads to diminished transmission of descending control from the brain to motor neurons and ascending sensory information.

In medical terms, SCI is defined as “the occurrence of an acute, traumatic lesion of neural elements in the spinal canal resulting in temporary or permanent sensory deficit” (Thurman et al., 1995). An injury to the spinal cord occurs when pressure is applied to the spinal cord or the blood supply, which carries oxygen to the spinal cord is disrupted. The consequence of injury depends on the site of injury and completeness of injury. The higher the level of lesion, the greater is the injury. The major conditions that result from injury to the spinal cord are quadriplegia, paraplegia and monoplegia. Quadriplegia is the paralysis of all four limbs, hands and the trunk. Paraplegia involves paralysis from the chest or waist downwards. Monoplegia is the paralysis of one limb or hand. A complete injury
results in no function below the level of injury, no sensation and no movement. An incomplete injury results in some functional disability below the level of injury. The spinal cord nerve tissue is like brain tissue in that it usually does not fully recover when damaged.

The pathophysiology of SCI is characterized by an initial primary injury followed by secondary deterioration. SCI causes destruction of sensory nerve fibres and also lead to loss of sensation such as touch, pressure and temperature. SCI often leads to a mutilation of the respiratory system (Beth et al., 2007), secondary musculoskeletal deterioration (Shields & Dudley-Javoroski, 2003) and sexual function. The neurologic symptoms include pain, numbness, paresthesias, muscle spasm, weakness and bowel/bladder changes. Other effects of SCI also includes low postural blood pressure (postural hypotension), inability to regulate blood pressure effectively, reduced control of body temperature (poikilothermic) and inability to sweat below the level of injury.

Since SCI affects CNS (central nervous system), its understanding lead to new strategies to reverse the damage caused by SCI. Gamma Amino Butyric Acid (GABA) (Todd et al., 1992), glycine (Todd & Sullivan, 1990), serotonin (5-HT) (Basbaum et al., 1978), norepinephrine (Dahlström & Fuxe, 1965), dopamine (Fleetwood-Walker & Coote, 1981), choline acetyl transferase (ChAT) (Todd, 1991), acetyl choline esterase (AChE) (Kása, 1986) are distributed throughout the spinal cord. There are reports suggesting that neurotransmitter release from intra spinal grafts is a highly relevant parameter to evaluate the functional ability of transplanted cells (Leanza et al., 1993b; Cenci et al., 1994; Leanza et al., 1999; Cenci & Kalén, 2000).

Acetylcholine (ACh) is the key neurotransmitter for para sympathetic nervous system. It modulates spinal sensory processing in the dorsal horn (Myśliński & Randic, 1977; Urban et al., 1989) through the intrinsic cholinergic inter neurons found in the dorsal horn (Barber et al., 1984; Todd, 1991). ACh is also found in the motor neurons. (Villégier et al., 2010). Depletion in the motor
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neurons causes a decrease in ACh concentration (Rosario et al., 2007). In mammals rhythmic limb movement, such as walking is controlled by pattern-generating neurons within the spinal cord. During early development, motor neurons seem to become spontaneously active and they release ACh, which excites neighbouring cells as a form of cell-cell communication. Motor neurons thus mediate locomotion via ACh. Knock off model of mice that lacked the enzyme necessary for synthesising ACh resulted in development of defective spinal circuit that lacked the control of leg movements. This demonstrates the relevance of ACh in control of leg movements. Thus ACh is necessary for a proper neural circuit.

Necrosis or cell death is a pathophysiological process that occurs as a result of secondary damage after SCI. Cell death continues to occur over several days and weeks following SCI. In the secondary phase, lipid peroxidation and free-radical production also occurs. The invading inflammatory cells increase the local concentrations of cytokines and chemokines. SCI triggers apoptosis, which kills oligodendrocytes in injured areas of the spinal cord days to weeks after the injury. Oligodendrocytes are the cells that form the myelin sheath around axons and speeds the conduction of nerve impulses. Apoptosis strips myelin from intact axons in adjacent ascending and descending pathways, which further impairs the spinal cord's ability to communicate with the brain. Thus free radicals and apoptosis increase the damage in SCI. Both neurons and glia die by apoptosis; the response of oligodendrocytes in long tracts undergoing Wallerian degeneration is particularly long lived and is responsible for chronic demyelination and some of the dysfunction in chronic SCI. After SCI in the rat, posttraumatic necrosis occurred and apoptotic cells were found from 6 hours to 3 weeks after injury (Maria et al., 1997).

In the present scenario, basic research on SCI focuses on several areas that target functional restitution and regeneration of the injured neurons within the spinal cord. Stimulating the rejuvenation of axons is a key factor of spinal cord
repair because every axon in the injured spinal cord that can be reconnected and increases the chances for improvement of function. Previously, CNS neurons were thought to be incapable of regeneration. But, Liu and Chambers (1958) indicated that central projections of primary afferent fibres can develop in the spinal cord after injury. Subsequent work by Richardson et al., (1980) demonstrated axonal elongation. Axonal growth alone is not sufficient for functional recovery. Axons have to make the proper connections and re-establish functioning synapses. Therefore, SCI research should focus on preventing the loss of function and on restoring lost functions, including sensory and motor functions with the ultimate goal of fully restored to the individual levels of activity and function that a person had before injury. Targets for intervention for improving functional outcome in SCI include free radical reduction, prevention of neuronal populations from apoptosis and promotion of neurite outgrowth.

With recent molecular strategies and techniques, research in the understanding of neuronal injury and neural regeneration provide new promises for reversal of SCI that was thought to be permanent and irrevocable (Carlson & Gorden, 2002). A variety of tissues and cells have been implanted in the damaged spinal cord to restore function. These include bone marrow cells (BMCs), olfactory ensheathing cells, dorsal root ganglia, adrenal tissue, hybridomas, peripheral nerves or transplanted conduits of schwann cells. It is hypothesised that these cells would rescue, replace or provide a regenerative pathway for injured neurons, which would then integrate or promote the regeneration of the spinal cord and restore function after injury (Zompa et al., 1997). Thus the promising treatment of SCI is cell-based therapy (Stanworth & Newland, 2001; Hipp & Atala, 2004) due to the limited success of pharmacological treatment. Cellular transplantation strategies have been used in various models of SCI (Eftekharpour et al., 2008). The cell replacement approach has the advantage that it promotes regeneration and repair. Regeneration involves replacement of lost or damaged neurons and induces axonal regeneration. Repair involves replacement of
supportive cells such as oligodendrocytes in order to prevent progressive demyelination and induce remyelination (Totoiu & Keirstead, 2005). In addition, BMC transplantation promotes protection of endogenous cells from further cell damage by attenuation of secondary injury process. BMC can also generate endoderm and ectoderm derivates including neural cells (Jiang et al., 2002; Kim et al., 2002). Non-embryonic sources of adult stem cells, which are not of ethical and legal concerns usually associated with embryonic stem cell research, offer great promise for the advancement of spinal cord treatment (Moore et al., 2006). BMCs support the repair of damaged tissues. Under specific experimental condition, BMCs differentiate into mature neurons or glial cells (Munoz et al., 2003). Transplanted BMCs improvises neurological deficits in the CNS injury models by producing neural cells or myelin producing cells (Chopp et al., 2000; Akiyama et al., 2002). BMCs actively remyelinate spinal cord once administered directly or intravenously (Dezawa et al., 2005).

Consequences of SCI are devastating and any strategy to alleviate neurological loss is attractive (Treherne et al., 1992). Neurotransmitters relay, amplify and modulate signals between the neurons. 5-HT is known to play a facilitory role in locomotor circuit by increasing motoneuron excitability, modulating spinal central pattern generators (CPG) (Rossignol et al., 1988) and improving locomotor behaviour following SCI (Kim et al., 1999; Ribotta et al., 2000). A small amount of 5-HT can activate super-sensitive motor neurons (Li et al., 2007). Reports suggests that the cell-specific effect of 5-HT on regenerating neurons within the adult CNS by increasing the calcium concentration of the cells (Murrain et al., 1990). GABA receptors are known to be involved in during neuronal development. The presence of GABA receptors in developing oligodendrocytes provides a new mechanism for neuronal–glial interactions during development and offers a novel target for promoting remyelination following white matter injury (Luyt et al., 2007). GABA increases synaptic plasticity. Soltani et al., (2011) reported that GABA promotes proliferation of β-
cells in pancreas. GABAergic inputs to hippocampal progenitor cells promote neuronal differentiation (Tozuka et al., 2005). Continuous application of GABA could promote dendritic growth in vivo, influence ganglion sensitivity to ACh and alter development of pre synaptic specialisation (Wolf et al., 1987). 5-HT and GABA can be also used as agents for cell proliferation and differentiation. Earlier reports from our lab showed that 5HT acting through specific receptor subtypes 5HT2 (Sudha & Paulose, 1998) and GABA acting through specific receptor subtypes GABA_B (Biju et al., 2002) control cell proliferation and act as co-mitogens. These reports have paved way to study of the effect of 5-HT and GABA in after SCI.

SCI is a major cause of concern and the role of cholinergic neurotransmitter system in SCI has not been widely studied. In the present study, we have chosen Wistar rats as our model for SCI. Rats have been chosen to study not only because they are readily available but also because the morphological, biochemical and functional changes that occur are similar to those seen in humans (McTigue et al., 2000; Metz et al., 2000; Norenberg et al., 2004; Fleming et al., 2006). Since ACh is the major neurotransmitter in the motor neurons, the study of cholinergic alteration during SCI will enlighten the signalling pathways that is involved in the SCI mediated motor deficits. This study further investigated the effect of regenerative cell proliferation and differentiation in SCI, when BMCs, 5-HT and GABA are supplemented individually and in combination. The present study also investigated the second messenger alterations by studying inositol triphosphate (IP3), 3′-5′-cyclic adenosine monophosphate (cAMP) and 3′-5′-cyclic guanosine monophosphate (cGMP) functional regulation and gene expression of Phospholipase C (PLC) and cAMP regulatory element binding protein (CREB). The changes in gene expression of anti oxidant enzymes like Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) were investigated. Gene expression studies of apoptotic factors like Bax, Caspase-8, Tumour Necrosis factor α (TNFα) and Nuclear factor kappa-light-chain-enhancer of activated B
cells (NF-κB) were studied. The gene expression of neuronal survival factors Brain Derived Neurotrophic Factor (BDNF), Glial Derived Neurotrophic Factor (GDNF), insulin like growth factors (IGF-1), Akt-1 and Cyclin D2 were also studied. We also demonstrated the autologous differentiation of BMC to neurons using comitogenic 5-HT and GABA by confocal studies with Bromodeoxyuridine (BrdU) labelling and Neuronal-specific nuclear protein (NeuN) expression. Behavioural studies were planned to evaluate the locomotor function in control and experimental rats. Our present study on 5-HT, GABA and BMC dependent regulation of muscarinic receptors in the spinal cord and brain will certainly enlighten novel therapeutic possibilities for the treatment of SCI.