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Faculty of Veterinary Medicine
Surgery Anesthesiology and Radiology

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Evaluation of Adipose Derived Stem Cell Therapy in a Canine Model of Multiple Sclerosis

Thesis presented by

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(B. V. Sc., 2011)
(M.V.Sc., 2015)

For fulfilling the degree of PhD.
(Surgery, Anesthesiology and Radiology)

Under the Supervision of

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Supervision Sheet

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Abstract:

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system, whose etiology remains unknown. MS destroys oligodendrocytes, which are the cells responsible for creating and maintaining the myelin sheath, which helps the neurons carry electrical signals. This work aimed to evaluate the treatment of experimentally induced MS in dogs using laser activated adipose derived stem cells. Twenty-four animals were used in study divided into 2 main groups. All animals received bilateral intraspinal injection of 20 μl of 0.1 % Ethidium bromide in the lateral columns using a microneedle syringe attached to a capillary tube through a drilled hole at the first lumbar vertebra. Treatment using prepared Stem cell preparation was applied by injection of (10 $X10^6$ nucleated cells) directly in the CSF at the day 14 from the induction of the MS. Results showed amelioration of the clinical signs over time confirmed by the resolution of the previous lesions on MRI. Histopathology showed that injecting stem cells directly into the CSF lead to positive migration of the cells and homing into the site of the lesion as confirmed by PKH26 stain. Therapy lead to marked inhibition of apoptic activity, decreasing inflammation and glial scar formation, regeneration of the destructed axons, remyelination of the lost myelin as detected by increasing of Myelin Basic Proteins and differentiated into oligodendrocyte progenitors expressing Olig2 marker. The electron microscopy showed the remyelination sequence till forming a dense myelin sheath around the axons. From the forementioned results we concluded that treatment using laser activated stem cells holds a promising therapeutic option for treatment of MS.

Keywords: Multiple sclerosis; demyelination; spinal cord; Dog model; Adipose derived stem cells; low level Laser irradiation.

I dedicate this thesis:

To my family, especially my dear Mother for her unconditioned endless love, great support and encouragement in every step of my life.

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List of abbreviations

ADEM	acute disseminated encephalomyelitis
ADSCs	Adipose Derived Stem Cells
BBB	blood-brain barrier
BDNF	Brain derived neurotrophic factor
BMSCs	Bone marrow-derived stromal cells
CDV	Canine Distemper Virus
CNS	Central Nervous System
CNTF	Ciliary neurotrophic factor
CSF	cerebrospinal fluid
EAE	Experimental autoimmune/allergic encephalomyelitis
EGF	Epidermal growth factor
EM	Electron microscopy
ESCs	Embryonic Stem Cells
FGF	basic fibroblast growth factor
GFAP	Glial Fibrillary Acidic Proteins
HGF	Hepatocyte growth factor
HSCs	Hematopoietic Stem Cells
iPSCs	Pluripotent Stem Cells
LPC	lysophosphatidylcholine
MBP	Myelin Basic Protein
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
MS	Multiple sclerosis
MSCs	Mesenchymal Stem Cells
NGF	Nerve growth factor
NSCs	Neural Stem Cells
OPCs	Oligodendrocyte Progenitor Cells
PNS	peripheral nervous system
PPMS	Primary Progressive Multiple sclerosis
PRMS	Progressive Relapsing Multiple sclerosis
RRMS	Relapsing-Remitting Multiple sclerosis
SCI	Spinal Cord Injury
SPMS	Secondary Progressive Multiple sclerosis
SVF	stromal vascular fraction
TGF-b	Transforming growth factor beta
TMEV	Theiler’s murine encephalomyelitis virus
VEGF	vascular-endothelial growth factor

Introduction

Multiple sclerosis (MS) is a multifocal demyelinating disease of the central nervous system (CNS) which can lead to sever physical and cognitive disability and neurological defects. Damage of this myelin sheath protecting the nerve cells in the brain and spinal cord progresses to damage or destruction of the axons (nerve fibers) over time leading to irreversible neurodegeneration explaining the progression of the disease and the increase in disability (**Compans and Cooper**, **2008**).

Although the etiology of MS is yet obscure, some researches demonstrated that the cause of MS is multifactorial and includes the genetic predisposition, infectious agents and environmental influences (**Barnett and Sutton**, **2006**). Over the past several decades, a number of animal models have been developed in order to understand different aspects of MS (**Denic et al.**, **2011**). Many breeds of Dogs are hereditarily subjected to demyelination of the CNS and the Canine Distemper encephalitis was initially described as “acute MS of the dog” by human neuropathologists making them an ideal model for studying MS yet very little studies were conducted on dogs (**Pachner**, **2011**).

Unfortunately, there is no definite cure for MS and current remedies only help in alleviating the symptoms and halting the immune attack (**Uccelli et al.**, **2013**). But Stem cell therapies can offer a new hope for the treatment of such neurological diseases, by differentiating into oligodendrocytes and astrocytes (**Pittenger et al.**, **1999**) and secreting neurotrophic factors with immunomodulatory effects that could prevent further cellular damage and providing a regenerative microenvironment for remyelination (**Caplan and Dennis**, **2006 and Cohen**, **2013**)Adipose Derived Stem Cells (ADSCs) can be harvested by minimally invasive procedures that should facilitate their use in cell transplantation (**Tsuji et al.**, **2014**). These cells are capable to differentiate to other cells outside their lineage, such as neural progenitors and oligodendrocytes. Many of the neurotrophic factors have been identified as secretome of ADSCs (**Ghasemi et al.**, **2014**).