شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم باللّه العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة

شبكة المعلومات الجامعية

بالرسالة صفحات لم ترد بالأصل

Study the Therapeutic Role of Mesenchymal Stem Cells in a Model of Temporal Lobe Epilepsy

A Thesis

Submitted for the degree of Ph.D. of Science in Biochemistry As fulfillment for requirements of the Ph.D. of Science

By

Rania Said Salah Mohamed zedan
Assistant researcher- National Research Centre
(M.Sc. Biochemistry, Faculty of Science,
Tanta University, 2010)

Under the Supervision of

Prof. Gilane Mohamed Sabry
Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Hanaa Hamdy Ahmed
Professor of Hormones
Head of Hormones Department
Medical Research Division
National Research Centre

Prof. Somia Hassan Abd-Allah
Professor of Biochemistry
Medical Biochemistry and
Molecular Biology Department
Faculty of Medicine
Zagazig University

Dr. Rasha El-Sherif Hassan
Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Biochemistry Department
Faculty of Science - Ain Shams University
(2020)

The Acknowledgement

My deepest heartfelt gratefulness to ***Prof. Hanaa Hamdy Ahmed****, Professor of Hormones, Head of Hormones Department, Medical Research Division, National Research Centre for suggesting the point of this thesis, building up the hypothesis related to the results. Also, I thank her kind supervision, continuous support, and valuable guidance in all of the theoretical and practical aspects of this work.*

I thank ***Prof. Gilane Mohamed Sabry****, professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for kindly supervising the present work, reading and criticizing the thesis. Her valuable guidance and ultimate support are greatly appreciated.*

I express my appreciation to ***Prof. Somia Hassan Abd-Allah****, Professor of Biochemistry, Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University for her great help and valuable advices to accomplish this work in the part of stem cells of the current work.*

Sincere thanks and gratitude are to ***Dr. Rasha El-Sherif Hassan****, Assistant Professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for kindly supervising this work, her valuable guidance and ultimate support are greatly appreciated.*

I wish to express my deepest feeling of gratitude of ***Prof. Wagdy Khalil Bassaly Khalil****, Professor of Molecular Genetic, Cell Biology Department, Genetic Engineering and Biotechnology Division, National Research Centre for his scientific help and providing all facilities throughout this work in regarding the molecular study of the current work.*

I wish to thank ***Dr. Ahmed A. Abd-Rabou****, Researcher of Biochemistry, Hormones Department, Medical Research Division, National Research Centre for his scientific help and continuous support.*

List of contents

Title	Page
Abstract	**xxi**
Introduction	**1**
Aim of the Work	**4**
Review of literature	**5**
Epilepsy	**5**
Epileptic historical perspective	**5**
Prevalence of epilepsy	**6**
Classification of epilepsy	**6**
Temporal Lobe Epilepsy	**9**
Hippocampal sclerosis	**12**
Mossy Fiber Sprouting	**15**
Symptoms of epilepsy	**17**
Etiology of epilepsy	**18**
Pathogenesis of epilepsy	**19**
Pathophysiology of TLE	**20**
Circuit	**21**
Oxidative stress	**25**
Neuroinflammation	**28**
Apoptosis	**31**
Diagnosis of epilepsy	**34**
Current therapeutic approaches for epilepsy	**37**
Medications	**37**
Surgery	**39**
Vagus nerve stimulation	**39**

Title	Page
Ketogenic diet	**40**
Animal models for TLE	**40**
Pilocarpine model for TLE	**41**
Stem cells	**46**
Stem cell properties	**46**
Self-renewal	**46**
Potency	**47**
Types of stem cells	**48**
Embryonic stem cells	**50**
Umbilical cord blood stem cells	**51**
Induced pluripotent stem cells	**52**
Adult stem cells	**52**
Neural stem cells	**53**
Hematopoietic stem cells	**53**
Mesenchymal stem cells	**54**
Bone marrow-derived mesenchymal stem cells	**57**
Adipose tissue-derived mesenchymal stem cells	**57**
MSCs identity	**59**
MSCs homing efficiency	**60**
Paracrine action of MSCs	**64**
Mesenchymal stem cells in neurodegenerative diseases	**65**
MSCs crossing the blood brain barrier	**67**
Neuronal differentiation of MSCs	**68**
Induction of endogenous neurogenesis	**69**
Immunomodulation and neuroinflammation activity	**71**
Protein aggregate clearance	**75**

Title	Page
Anti-apoptotic activity	**75**
Neoangiogenesis activity	**76**
Antioxidative activity	**77**
MSCs for cellular replacement via direct reprogramming	**78**
Challenges for MSCs applications	**79**
Material and Methods	**81**
Rat mesenchymal stem cells (Rat MSCs)	**81**
Bone marrow mesenchymal stem cells derivation and expansion	**81**
Adipose mesenchymal stem cells derivation and expansion	**82**
Characterization of mesenchymal stem cells	**83**
Morphological characterization of MSCs	**83**
Identification of MSCs surface markers CD29, CD73, CD34 and CD45 gene expression by conventional polymerase chain reaction	**83**
Total RNA extraction from MSCs	**83**
First-strand cDNA synthesis	**85**
Reverse transcription Polymerase Chain Reaction (RT-PCR)	**86**
Labeling of MSCs	**87**
MSCs infusion	**90**
Biological experimental protocol	**90**
I) Materials	**90**
Chemicals and drugs	**90**
Animal handling	**90**
Induction of status epilepticus	**91**
Animal grouping	**92**

Title	Page
II) Methods	**93**
Brain tissue sampling	**93**
Biochemical determinations	**94**
1-Detection of heat shock protein-70 in brain homogenate	**94**
2-Assessment of S100 calcium binding protein β in brain homogenate	**99**
3-Quantification of Caspase-8 in brain homogenate	**102**
4-Estimation of gamma-aminobutyric acid in brain homogenate	**105**
5-Evaluation of semaphorin4D in brain homogenate	**108**
6-Determination of galanin neuropeptide in brain homogenate	**112**
7-Quantitative evaluation of brain total protein	**116**
Molecular genetic analyses	**117**
Gene expression analysis by RT-PCR	**117**
Total RNA extraction	**117**
RT-PCR	**120**
Agarose gel electrophoresis	**122**
Histopathological procedure	**122**
Statistical analyses	**123**
Results	**124**
Mesenchymal stem cells identification	**124**
Verification of MSCs homing	**125**
Observational changes after pilocarpine administration	**127**
Biochemical findings	**127**
Molecular genetic data	**136**
Histopathological description	**145**

Title	Page
Discussion	**156**
Summary	**180**
Conclusion	**184**
References	**185**
Arabic abstract	
Arabic summary	
Arabic conclusion	

List of abbreviations

ACh	Acetylcholine
AChR	Acetylcholine receptor
AD	Alzheimer's disease
AD-MSCs	Mesenchymal stem cells derived from adipose tissue
AEDs	Anti-epileptic drugs
AIF	Apoptosis-inducing factor
Akt	Serine/threonine-protein kinase
ALS	Amyotrophic lateral sclerosis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMV	Avian myeloblastosis virus
Ang-1,2	Angiopoietin-1,2
ANOVA	One way analysis of variance
ATP	Adenosine triphosphate
Aβ	Amyloid-beta
Bad	Bcl-2 associated agonist of cell death
Bax	Bcl-2-associated x protein
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma-2
Bcl-Xl	B-cell lymphoma-extra large
BDNF	Brain derived neurotropic factor
Bim	Bcl-2-like protein 11
BM-MSCs	Mesenchymal stem cells derived from bone marrow
$[Ca^{++}]m$	Mitochondrial calcium concentration
CAD	Caspase actuated DNase
CAMP	Cyclic adenosine monophosphate
Casp-8	Caspase-8

CBZ	Carbamazepine
CCR	CC chemokine receptor
CD	Cluster of differentiation
cDNA	Complementary DNA
CI	Complex I
$[Cl^-]I$	Intracellular chloride concentration
CNS	Central nervous system
CNTFs	Cilliary neurotrophic factors
COX-2	Cyclooxygenase-2
CREB	Cyclic adenosine monophosphate response element binding protein
CSF	Cerebrospinal fluid
CT	Computed tomography
CXCR	CXC chemokine receptor
DAMP	Damage associated molecular patterns
DEDs	Death effector domains
DFF	DNA fragmentation factor
DISC	Death induces signaling complex
DMEM	Dulbecco's modified eagle's medium
dNTP	Deoxynucleoside triphosphate
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix.
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalography
E_{GABA}	$GABA_AR$ currents
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
ESCs	Embryonic stem cells

EtOH	Ethanol
FADD	Fas associated death domain protein
FAK	Focal adhesion kinase
FasL	Fas ligand
FasR	Fas receptor
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
FGF-1	Fibroblast growth factor-1
FGF-2	Fibroblast growth factor-2
FKHR	Fork head in rhabdomyosarcoma
FKHRL-1	Fork head in rhabdomyosarcoma like-1
FLAIR	Fluid-attenuated inversion recovery
GABA	Gama-aminobutyric acid
GABA-R	Gama-aminobutyric acid receptor
GABA-RA	Gama-aminobutyric acid receptor-A
GABA-RB	Gama-aminobutyric acid receptor-B
GABA-T	Gama-aminobutyric acid aminotransferase
GAD67	Glutamic acid decarboxylase 67
GAL	Galanin
GALR2	Galanin receptor 2
GAT-1	Gama-aminobutyric acid transporter-1
GAT-3	Gama-aminobutyric acid transporter-3
GDNF	Glial-derived neurotrophic factor
GJIC	Gap junctional intercellular communication
GM-CSF	Granulocyte macrophage-colony stimulating factor
GSH	Glutathione
GSH-PX	Glutathione peroxidase
GSH-RX	Glutathione reductase