

**UNIVERSITI TEKNOLOGI MARA**

**TARGETED DELIVERY OF  
BDNF-LOADED POLY  
(LACTIDE-CO-GLYCOLIDE)  
NANOPARTICLE TO THE BRAIN:  
PENETRATION THROUGH  
BLOOD BRAIN BARRIER AND  
NEUROPROTECTIVE  
EFFECT IN STROKE**

**SITI NORSYAFIKA BINTI KAMARUDIN**

Thesis submitted in fulfillment  
of the requirements for the degree of  
**(Pharmacology)**

**Faculty of Medicine**

**September 2019**

## ABSTRACT

Stroke remains a major public health burden in Malaysia due to high morbidity and mortality. Neuroprotection by Brain derived neurotrophic factor (BDNF) has the potential to minimise the ischemic damage and theoretically improve freedom from disability among stroke survivors. There is a need to develop nanoparticle (NP) drug delivery system to deliver BDNF following intravenous route after the ischemic insult due to poor penetration to the brain. In this study we proposed to design BDNF-loaded poly (lactide-co-glycolide) (PLGA) nanoparticles (BDNF-NPs) and to study neuroprotective effect of BDNF on permanent middle cerebral artery occlusion (pMCAO) model of ischemia in rats. BDNF containing PLGA nanoparticles were synthesized using water/oil/water (W/O/W) double emulsion solvent evaporation method. The nanoparticles were characterized for particle size (PS) and zeta potential (ZP) using a dynamic light scattering (DLS) technique. The percentage entrapment efficiency (%EE) was calculated. Penetration of blood brain barrier (BBB) was studied using an in vitro model employing human brain microvascular endothelial cells (HBMECs). The PS and ZP of NPs were found to be 186.6 nm and -18.6 mV respectively with 93% EE. Confocal laser scanning microscopy (CLSM) confirmed penetration and distribution of fluorescent NPs using Coumarin 6 as fluorescent probe into HBMECs and the number of NPs entering the cells was measured. Apolipoproteins (Apo) role in the NP uptake was investigated using ApoE, B100, A1 or C2, incubated with HBMECs. Cell viability was determined using MTS-assay. BDNF release into cells was quantified using ELISA method. No cytotoxicity of Apo-coated/non-coated PLGA NPs was observed. The fluorescent intensity was found to be significantly higher for ApoE-coated NPs compared to ApoA1-coated NPs (1.31 folds,  $p < 0.001$ ) and control, not coated NPs (4.06 folds,  $p < 0.001$ ). The fluorescent intensity of ApoB100-coated NPs was significantly higher compared to control group by 3.88 folds ( $p < 0.001$ ). Significantly higher concentration of BDNF was found in HBMECs after treatment with Apo E- and Apo B100-coated NPs compared to control group by 69.31 ( $p < 0.001$ ) and 57.46 folds ( $p < 0.001$ ) respectively. The final step was to test neuroprotective effect of BDNF-NPs on pMCAO model of ischemia in rats. Sprague-Dawley rats were divided into 4 groups of 7 rats each. Group 1 was subjected to sham operation, group 2, 3 and 4 were subjected to pMCAO. Four hours after pMCAO, group 3 and 4 were intravenously treated with BDNF and BDNF-NPs respectively. Functional outcome was assessed at 2 h and 24 hours after pMCAO using modified Neurologic Severity Score (mNSS), rotarod and grid walking. Rats were sacrificed by terminal cardiac puncture, blood was taken for assessment of neurobiomarkers (NSE and S100 $\beta$ ) level and brain was subjected for infarct area assessment and volume measurement. BDNF-NPs treated group showed significant improvement in mNSS when compared with pMCAO and BDNF treated groups demonstrating decreased mNSS score by 2.0 and 2.0 times. BDNF-NPs treated group showed improved rotarod performance by increasing latency time on rotarod by 2.44 ( $p < 0.001$ ) and 2.76 folds ( $p < 0.001$ ) when compared with pMCAO and BDNF treated groups. The infarct volume in rats treated with BDNF-NPs was significantly smaller by 1.91 and 1.95 folds ( $p < 0.001$ ) when compared with pMCAO and BDNF treated groups. The results were further corroborated by the estimation of neurobiomarkers (NSE and S100 $\beta$ ) level. Overall, BDNF loaded PLGA nanoparticle is a promising drug formulation that act as neuroprotective agent in ischemic stroke model.

# TABLE OF CONTENTS

	<b>Page</b>
<b>CONFIRMATION BY PANEL OF EXAMINERS</b>	<b>ii</b>
<b>AUTHOR'S DECLARATION</b>	<b>iii</b>
<b>ABSTRACT</b>	<b>iv</b>
<b>ACKNOWLEDGEMENT</b>	<b>v</b>
<b>TABLE OF CONTENTS</b>	<b>vi</b>
<b>LIST OF TABLES</b>	<b>xiii</b>
<b>LIST OF FIGURES</b>	<b>xiv</b>
<b>LIST OF SYMBOLS</b>	<b>xviii</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xx</b>
<b>CHAPTER ONE: INTRODUCTION</b>	<b>1</b>
1.1 Background	1
1.2 Problem Statement	7
1.3 Hypothesis	8
1.4 Research Questions	8
1.5 Objectives of the Study	9
<b>CHAPTER TWO: LITERATURE REVIEW</b>	<b>10</b>
2.1 Definition and Classification of Stroke	10
2.1.1 Definition of Stroke	10
2.1.2 Classification of Stroke	11
2.1.3 Classification of Ischemic Stroke	12
2.1.3.1 Global Ischemic Stroke	12
2.1.3.2 Focal Ischemic Stroke	13
2.2 Risk Factors of Stroke	14
2.2.1 Non-Modifiable Risk Factors	14
2.2.1.1 Age	14
2.2.1.2 Gender	15
2.2.1.3 Ethnicity	15

2.2.1.4	Genetics	15
2.2.2	Modifiable Risk Factors	18
2.2.2.1	Hypertension	18
2.2.2.2	Atherosclerosis / Dyslipidaemia	19
2.2.2.3	Diabetes Mellitus	21
2.2.2.4	Smoking	21
2.2.2.5	Alcohol	23
2.2.2.6	Physical Activity	23
2.3	Hemodynamic and Cerebral Circulation	25
2.3.1	Vasculature of the Brain: Structure	25
2.4	Blood Brain Barrier (BBB): Anatomical Structure and Functions of BBB	27
2.4.1	Anatomy	27
2.4.1.1	Development	28
2.4.2	The Functions of Blood Brain Barrier (BBB)	30
2.4.3	<i>In Vitro</i> Blood Brain Barrier Model	35
2.5	Pathophysiology of Cerebral Ischemia	37
2.5.1	Glutamate Excitotoxicity & Ca <sup>2+</sup> Cytoplasmic Overload	39
2.5.2	Oxidative and Nitrosative Stress	41
2.5.3	Inflammatory Mechanisms in Ischemic Stroke	44
2.5.4	Blood Brain Barrier Dysfunction After Stroke	46
2.6	Ischaemic Stroke Treatment and Management: Current Treatment Modalities	49
2.6.1	General Management	49
2.6.2	Reperfusion of Ischaemic Brain	49
2.7	Brain Derived Neurotrophic Factors (BDNF) as Potential Neuroprotective Agent	52
2.7.1	Structures	52
2.7.2	Functions	54
2.7.3	Neuroprotection in Cerebral Ischaemic Events	55
2.8	Challenges in Drug Delivery to Brain Across the Blood Brain Barrier	57
2.8.1	Potential Drug Delivery System Across the Blood–Brain Barrier	58
2.8.1.1	Polymeric Nanoparticles as Drug Delivery System in Brain Drug Delivery	58
2.8.1.2	Liposome-Based Strategies of Drug Delivery to the Brain	61

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background

Stroke is a major public health burden as it is the second leading cause of death and a major cause of physical disability worldwide (Katan & Luft, 2018). Current statistics from the Global Burden of Disease, Injuries, and Risk Factors Study (GBD 2015) revealed a further shift from communicable diseases, maternal, and nutritional causes towards noncommunicable diseases like stroke. This occurrence is likely due to an increment in aging of the world's population as well as a decrease in death rates globally in recent decades (Wang *et al.*, 2016). The most prominent causes of death are vascular in nature, and stroke is currently the second leading cause of death worldwide (Feigin *et al.*, 2017). Ischaemic heart disease and stroke together accounted for 15.2 million deaths (15–15.6 million) in 2015 (Feigin *et al.*, 2017). While ischaemic strokes comprise the highest number of strokes, much of the global burden of stroke measured in proportion to mortality and disability-adjusted life-years (DALYs) are allocated to haemorrhagic stroke (Feigin, Norrving & Mensah, 2017).

A year 2008 study found that stroke consumed about 2-4% of total health care costs worldwide and accounted for more than 4% of direct health care costs in industrialized countries (Donnan, Fisher, Macleod & Davis, 2008).

In developed countries, the incidence and prevalence of stroke are decreasing whereas in the Asia Pacific the number of patients diagnosed with acute ischaemic stroke is on the rise (Aziz *et al.*, 2015). Department of Statistics Malaysia revealed a report in 2017, stated that, in Malaysia, stroke is the number three cause of death after ischaemic heart disease and pneumonia.

Clinically, stroke can be defined as an umbrella of conditions caused by the occlusion or haemorrhage of cerebral blood vessels supplying the brain (Lo, Dalkara & Moskowitz, 2003). In all instances, it will involve death or the dysfunction of neuronal cells and neurological deficits that reflect the location and size of the compromised brain area (Lo, Dalkara & Moskowitz, 2003). Ultimately, it is a leading cause of death and long-lasting physical disability, requiring fast and definite treatment (Keyser, Sulter & Luiten, 1999).