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

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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 S100β) 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 S100β) level. Overall, BDNF loaded PLGA nanoparticle is a promising drug formulation that act as neuroprotective agent in ischemic stroke model.

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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 occurance 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).