UNIVERSITI PUTRA MALAYSIA

EVALUATION OF THE EFFECTIVE TIME FOR GSK-3 INHIBITION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL BY TIDEGLUSIB (NP-12) IN PREVENTING NEUROINFLAMMATION

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EVALUATION OF THE EFFECTIVE TIME FOR GSK-3 INHIBITION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL BY TIDEGLUSIB (NP-12) IN PREVENTING NEUROINFLAMMATION

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Glycogen synthase kinase-3 (GSK-3) is an enzyme involved in various neurodegenerative and neuro-inflammatory diseases, including multiple sclerosis (MS) and its animal model of experimental autoimmune encephalomyelitis (EAE). The severity of the disease could be characterized by the degree of cellular invasion to the central nervous system (CNS) and demyelination that partly mediated by Th1 and Th17 effector cells. The present study is to investigate the efficacy of GSK-3 inhibition at a different course of treatment in ameliorating disease progression. This study also aims to evaluate the effects of GSK-3 inhibition to central neuro-invasion and demyelination. Female C57BL/6 mice were induced with myelin oligodendrocyte glycoprotein (MOG35-55) in conjunction with complete freund's adjuvant (CFA) and pertussis toxin. Inhibition of GSK-3 is performed by the administration of NP-12, a small heterocyclic Thiadiazolidinones (TDZD), intraperitoneally during pre-EAE induction, on the same day, and post-EAE. Data revealed that NP-12 delivery during pre-EAE induction greatly protected the mice from EAE, delayed the onset of EAE symptoms by 7 days from day-14.3 ± 0.5 (in EAE mice) until day-21.3 ± 3.2 (in the treated mice). Furthermore, NP-12 treated-EAE mice had notably reduced the inflammatory cells infiltration and axonal damage (demyelination) in the spinal cord. Inhibition of GSK-3 also abrogated the production of Th1, Th9 and Th17 associated cytokines but, increases the production of IL-4 level to 1790 ± 95.0 pg/mL compare to EAE 480 ± 38.0 pg/mL and in IL-10 780 ± 22.0 pg/mL compared to EAE 410 ± 16.0 pg/mL. These data demonstrate the effectiveness of NP-12 at a different time course of administration to reduce the severity of EAE disease and protect the CNS environment from potential cellular invasion or demyelination, thus, suggests the potential use of GSK-3 inhibitor in the treatment of EAE.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN MASA EFEKTIF BAGI PERENCATAN GSK-3 DI DALAM EAE OLEH TIDEGLUSIB UNTUK PENCEGAHAN NEUROINFLAMASI

Oleh

AHMED SALAM AL-ZAIDI

Disember 2018

Pengerusi : Masriana Hassan, PhD
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Glycogen synthase kinase-3 (GSK-3) adalah enzim yang terlibat dalam pelbagai penyakit neurodegeneratif dan neuro-inflamatori, seperti multiple sclerosis (MS) dan model heliks eksperimental autoimmune encephalomyelitis (EAE). Keparahan penyakit tersebut boleh dicirikan oleh tahap pencerobohan selular kepada sistem saraf pusat (CNS) dan nyah-myelinasi yang melibatkan effektor-effektor sel Th1 dan Th17. Kajian ini dijalankan untuk mengkaji keberkesanan perencatan GSK-3 pada waktu rawatan yang berbeza di dalam membaikpulih kondisi penyakit. Kami juga bertujuan untuk menentukan kesan perencatan GSK-3 kepada pencerobohan neuro pusat dan nyah-myelinasi. Model tikus betina C57BL/6 dirangsang oleh glikoprotin myelin oligodendrocyte (MOG35-55) bersama toksin pertusis lengkap (CFA) dan toksin pertusis. Inhibisi GSK-3 dilakukan melalui Tideglusib, heterosiklik kecil Thiadiazolidinones (TDZD), dengan cara ‘intraperitoneal’ semasa induksi pra-EAE, pada hari yang sama, dan selepas induksi EAE. Data menunjukkan bahawa penghantaran Tideglusib semasa induksi pra-EAE sangat melindungi tikus dari kondisi EAE, hal ini telah menunda permulaan simptom EAE sebanyak 7 hari dari hari-14.3 ± 0.5 (dalam tikus EAE) hingga hari-21.3 ± 3.2 (dalam tikus yang dirawat). Tambahan pula, tikus EAE yang dirawat Tideglusib telah mengurangkan pencerobohan sel-sel radang dan kerosakan aksonal (nyah-myelinasi) dalam kord rahim. Inhibisi GSK-3 juga membatalkan penghasilan sitokin Th1, Th9 dan Th17 yang berkaitan, tetapi meningkatkan tahap IL-4 kepada 1790 ± 95.0 pg / mL berbanding EAE 480 ± 38.0 pg / mL dan IL-10 780 ± 22.0 pg / mL berbanding EAE 410 ± 16.0 pg / mL.. Data telah menunjukkan keberkesanan Tideglusib pada masa yang berlainan dapat mempengaruh keparahan penyakit EAE, dan dapat melindungi persekitaran kanser daripada pencerobohan atau potensi nyah-myelinasi selular. Oleh itu, kajian dengan bukti telah mencadangkan potensi penggunaan GSK-3 di dalam rawatan EAE.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Multiple sclerosis (MS) is a progressive chronic autoimmune inflammatory and demyelinating disease of the central nervous system (CNS) affecting over 2.5 million people worldwide (Milo & Kahana, 2010). The etiology of MS is uncertain, however, both cellular and environmental factors have an influence on disease demonstration (Comabella & Khoury, 2012). MS is characterized pathologically by multiple areas of CNS white matter demyelination of brain stem (disruption of myelin that insulates and protects nerve cells), axonal injury/loss, inflammation, cerebellum and blood-brain barrier (BBB)0disruption (Dhib-Jalbut, 2007).

In order to closely study the pathogenesis and pathophysiology of MS, different in vivo models have been developed using experimental animals. Experimental Autoimmune Encephalomyelitis (EAE) is one of the most used animal models to investigate neuro-inflammatory pathways and pathophysiology of MS. It is the most common model because it mimics the features of MS, such as inflammation, demyelination, and axonal injury, although it may not demonstrate all pathophysiological characteristics of the human disease. The pathophysiology of EAE is based on the reaction of the adaptive immune system against brain-specific antigens. This reaction promotes inflammation and demolition of the antigen carrying structures, ending with neurological and pathological characteristics comparable which those noticed in MS patients (Stromnes & Goverman, 2006). Central to the pathophysiology of MS, and by extension the EAE model, is glycogen synthase kinase-30(GSK3), a serine/threonine kinase which is an essential factor in the inflammatory process (Beurel, Grieco, & Jope, 2015). For this reason, several drugs have been developed targeting GSK-3, as the inhibition of this enzyme results in an impressive anti-inflammatory protection that ameliorates the disease severity and symptoms (Klein & Melton, 1996). One such drugs targeting GSK3 is NP-12 which is a synthetic derivative of Thiadiazolidine (TDZD) with potent neuroprotective and anti-inflammatory effects in vivo (Luna-Medina et al., 2007). However, treatments with GSK-3 in EAE models with diff erent degrees of inflammatory conditions did not always translate into a decrease of the disease severity as expected. In some cases, no beneficial eff ects were observed during the disease, whereas others demonstrated an exacerbation of clinical symptoms (Beurel et al., 2013).

In line with this, the present study aimed at evaluating the effect of NP-12 injections associated with diff erent degrees of inflammatory conditions; pre, same time, and after the C57BL/6 mice being induced with EAE. Our data indicate that early treatment with NP-12 is highly eff ective in reducing the clinical score and delay the onset of EAE. we postulate that the eff ect of NP-12 in pre and same time of injections were able to diminish the lymphocyte infiltration into the spinal cord and reduce

demyelination in brain and spinal cord. Finally, our study demonstrates that the increase in serum cytokine levels via the NP-12 injection would increase their immunosuppressive capacity by increase the production of anti-inflammatory cytokines.

1.2 Problem statement

Recent studies have only demonstrated the effect of GSK-3 inhibitor, NP-12, before EAE induction. Furthermore, the effect of NP-12 on cell infiltration and demyelination during EAE still unclear. Moreover, cytokine levels after the administration of NP-12 to EAE is not elucidated yet. For these reasons, there is the need for experimental evidence identifying the ideal time to administrate NP-12 in EAE-induced mice, to obtain the most effective treatment result.

1.3 Objectives

1. To optimize the induction of EAE in C57BL/6 mice and determine the effect of GSK-3 inhibition on the severity of clinical symptoms.
2. To evaluate the effects of NP-12 on EAE-induced mice in vivo, and in the brain and spinal cord by histopathological analysis.
3. To compare the serum level of cytokines produced in NP-12 treated and control EAE-induced mice.

1.4 Research question

What is the effect of GSK3 inhibition on the in vivo EAE mouse model at different time point?

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