UNIVERSITI TEKNOLOGI MARA

THE EFFECT OF HYPOTHERMIA AND PROGESTERONE (P4) AGAINST GLUTAMATE CHALLENGED PRIMARY CORTICAL ASTROCYTES ON S100B, GLUTAMATE UPTAKE, GLT-1 AND P62

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MSc

2018

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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Date	:	May 2018

ABSTRACT

Glutamate excitotoxicity is a concept that underpinned the massive release of glutamate to extracellular space, thereby inducing neuronal and glial injuries. Hypothermia has been proposed to offer neuroprotection even though the mechanism underlying it is poorly understood. Furthermore, prolonged time is required for hypothermia to exert its effects as well as inconsistencies and variation in the outcomes patients. An adjuvant therapy with hypothermia may be an alternative to reduce exposure time and obtain consistent outcomes. Progesterone (P4) is a neurosteroid which has been shown to elicit neuroprotection in neuronal cells with ischemic injury. This study investigates the neuroprotective effects of hypothermia and P4 on astrocytes following glutamate-induced toxicity. The cultured primary cortical astrocyte cells were exposed to 50 µM of glutamate for 15 minutes followed by incubation under hypothermia conditions with and without P4 for 24 hours. After 24 hours, the viability of cells was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The intracellular concentration of glutamate in astrocyte cells was estimated by the glutamate uptake assay. The levels of p62 and S100β were measured using ELISA. The membrane protein was extracted and estimated for GLT-1 by Western Blot. There were significant increases in the percentage of viable cells as well as the concentration of glutamate uptake by the astrocyte cells in mild hypothermia with P4 (88.27 ± 3.96%) and moderate hypothermia with P4 (80.94 ± 4.12%) as compared to normothermia after glutamate induced toxicity (51.05 ± 4.10%). Further analysis revealed that there was a significant effect of moderate hypothermia with P4 (142.78 ± 13.85 pg/ml) in increasing the S100β level in comparison to normothermia across the glutamate-induced toxicity groups (74.34 ± 4.42 pg/ml) and significantly increasing in glutamate uptake ($p \leq 0.01$) after treated with both mild and moderate hypothermia and P4. There was a significant increase of membrane GLT-1 in both mild hypothermia (1.07 ± 0.01 FD) and moderate hypothermia (0.87 ± 0.08 FD) group when compared to the normothermia (0.53 ± 0.05 FD) group. The p62 level was shown significant reduced in both mild and moderate hypothermia and P4 ($p \leq 0.01$) in comparison to normothermia across the glutamate-induced toxicity group. In conclusion, hypothermia and P4 reduced the glutamate-induced toxicity in the astrocyte cells by increasing glutamate uptake via GLT-1.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my master and for completing this long and challenging journey successfully. My special gratitude and thanks go to my lovely supervisor Dr. Rosfaiizah Siran, and co-supervisors, Madam Mazatulikhma Mat Zain, Dr Noor Azean Anis Abd Aziz and Dr. Andrean Husin. Thank you for the support, patience, guidance and ideas in assisting me with this project. I also would like to express my gratitude to the staff of the Culture Tissue Lab Tisu, Institute of Science and also to the staff of the Institute of Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA Sg. Buloh campus for giving me the opportunity to work in their laboratories and the facilities provided, knowledge and assistance.

I also would like to express my appreciations to my beloved parents, Abd Talib Jusoh and Salwa Che Wil. Thank you so much for their prayers, patience, love and support. My gratitude also goes to my siblings, thank you for the support and understanding.

Not to forget, to all my friends who helped me a lot in motivating and giving opinions in this study. Last but not least, thanks to those who supported me directly and indirectly in contributing to the completion of this study. Alhamdulillah and thank you.

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