Abstract

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THERAPEUTIC POTENTIALS OF HYPOXIC- AND BAICALEIN-ENRICHED FRACTION-PRECONDITIONED HUMAN NEURAL STEM CELLS (NSC) FOR IN VITRO ISCHEMIC STROKE MODEL

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Introduction: Recently, human neural stem cells (NSC) grafting has emerged as encouraging approach for treatment of stroke. Nonetheless, the therapeutic potential of NSC-based treatment is limited, mainly due to a large number of implanted cells died after grafting into the injury site.

Objectives: To circumvent this problem, this study aimed to enhance therapeutic potentials of human NSCs prior to transplantation through hypoxic and baicalein-enriched fraction (F5) preconditioning.

Methods: Hypoxic condition was maintained at physiological hypoxia (2% O_2) for 24 h whilst normoxic condition was maintained at atmospheric oxygen tension (21% O_2) for 24 h. F5 was used as the pharmacological agent to precondition H9-hNSCs. NSCs were preconditioned with 1.56 µg/mL of F5 for 24 h. Then, the cellular adaptation upon hypoxic and F5 preconditioning was determined based on NSC proliferation and differentiation capacities. The effects of hypoxic and F5 preconditioning on the expression profiles of eight candidate genes encompassing hypoxia-driven neuroprotective signaling, NSC marker, neuronal lineage marker, as well as cellular redox homeostasis were investigated using qPCR. The therapeutic potentials of both hypoxic- and F5-preconditioned NSCs for in vitro ischemic stroke (IVIS) model was visualised over 72 h.

Results: Hypoxic preconditioning enhanced NSC self-renewal, survival and multipotency. 60S ribosomal protein large P1 (RPLP1) and ribosomal protein L13A (RPL13A) were the most reliable reference genes for qPCR normalisation of normoxic- and hypoxic-preconditioned NSCs. Hypoxic preconditioning induced innate neuroprotective signaling through transcriptional activation of hypoxia-inducible factor-1 alpha (HIF-1α), vascular endothelial growth factor A (VEGFA), angiopoietin 1 (ANGPT1), neurogenic locus notch homolog protein 1 (Notch 1), nuclear factor erythroid 2-related factor 2 (Nrf2) and sodium dismutase 1 (SOD1). Based on the HIF-1α stabilisation potential of baicalein at ambient conditions, F5 was postulated to trigger effects mimic hypoxic preconditioning under normoxia. Interestingly, F5 preconditioning increased NSC proliferation, viability and lineage specific differentiation. Hypoxanthine phosphoribosyl transferase 1 (HPRT1) and RPL13A were the most stably expressed reference genes for qPCR normalisation of control (0.1% DMSO) and F5-preconditioned NSCs. Moreover, F5 preconditioning stimulated hypoxia-mimetic signaling intrinsically via HIF-1α, VEGFA, ANGPT1, Notch 1, Nrf2 and SOD1 upregulation. Both hypoxic- and F5-preconditioned NSCs were applied to IVIS model on wound-healing based culture slide for 72 h of live imaging. F5-preconditioned NSCs accelerated migration and homing towards IVIS model over an experimental period of 72 h compared to hypoxic-preconditioned NSCs. The neuroprotective factors induced by hypoxic preconditioning are postulated to degrade rapidly when exposed to oxygen. Contrarily, F5-preconditioned NSCs attained intrinsic neuroprotective mechanisms without compromising their stability under normoxia.

Conclusions: In conclusion, both the hypoxic and F5 preconditioning had successfully enhanced therapeutic potentials of NSCs for ischemic stroke. F5-preconditioned NSCs with enhanced therapeutic efficacy was more likely to be applicable in clinical setting and thus could be a promising therapeutic tool for ischemic stroke in the future.

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UNDERSTANDING THE GENETIC HISTORY OF THE MALAY AND ORANG ASLI POPULATIONS IN PENINSULAR MALAYSIA BY KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR)

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Introduction: Extensive diversity of killer cell immunoglobulin-like receptor (KIR) genes among diverse populations has made these immune genes become a potential anthropological marker. The variations of KIR

allotypes between two randomly selected individuals are also useful in individual identification.

Objectives: To characterise 16 KIR genes content and determine the allelic variations within exons of seven most polymorphic KIR genes among OA sub-groups and Malay sub-ethnic groups in Peninsular Malaysia using PCR-SSP technique and NGS technique.

Materials and methods: Here, we genotyped KIR genes content and allelic polymorphisms for the first time for Malay and Orang Asli (OA) populations in Peninsular Malaysia using polymerase chain reaction sequence specific primers (PCR-SSP), next-generation sequencing (NGS) and finally optimised by Sanger typing method. A total of 333 individuals representing 10 Malay sub-ethnic groups (Aceh, Banjar, Bugis, Jawa, Champa, Kedah, Kelantan, Mandailing, Minangkabau and Patani) and 167 individuals from six OA sub-groups (Batek, Kensiu, Lanoh, Che Wong, Semai and Orang Kanaq) have participated in this research.

Results: Fourty KIR genotypes were observed among Malays and 25 genotypes in OA populations. Generally, the Malays having homogenous haplotype profile with slightly high frequencies of Haplotype A, whereas OA are heterogeneous with various genotypes and wide range of haplotypes. Based on the principal component analysis, the Malay sub-ethnic groups are located between the Chinese, Indian and Orang Asli populations. The Semang sub-groups show genetic affinity toward the Australian Aborigines, Papuan, Indian and African populations. They shared several unique genotypes (AB6, BB71, BB73 and BB159) and have high frequencies of Haplotype B, which could be speculated as general features of ancient populations. The Senoi demonstrated high frequency of Haplotype A, and expressing hybrid phenotypes between Indo-China tribes and Semang population. In contrast, Orang Kanaq, the only Proto-Malay subgroup studied is significantly different from both related Taiwanese/Chinese and neighboring Malayo-Polynesian speaking populations and show evidence of becoming a distinct population. The NGS has successfully characterised 302 different allotypes of seven studied KIR genes among Malay and OA populations. KIR2DL1, KIR2DL4, and KIR3DL3 genes show less allelic variations and in favour on particular allele, which possibly being affected by natural selection and creates selective sweep. In addition, KIR2DS4, KIR3DL1, KIR3DL2 and KIR2DL3 are highly polymorphic genes with considerable number of alleles discovered, suggesting that these genes evolved on balanced selection. The KIR3DL2 gene could be the best marker for population studies, as it comprises signature alleles (KIR3DL2*001 and *002) that support the theory of modern human migration.

Conclusion: Comprehensive datasets generated from this research demonstrate the value of KIR genes content and allelic polymorphisms in elucidating genetic relationships between the Malays and OA with other world populations.

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CHANGES OF DREAM AND BDNF PROTEINS EXPRESSIONS, PRO-INFLAMMATORY AND OXIDATIVE STRESS LEVELS IN SPINAL CORD OF STREPTOZOTOCIN-INDUCED PAINFUL DIABETIC NEUROPATHY RATS UPON MINOCYCLINE AND IFENPRODIL TREATMENTS

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Introduction: Diabetic neuropathy (DN) is a long-term complication of diabetes mellitus (DM) which could be painful (PDN) or non-painful (non-PDN).

Objectives: To explore the effect of minocycline and ifenprodil on the (i) proteins expressions of NR2B sub-unit (NR2B) and phosphorylated NR2B sub-unit (phospho-NR2B) of N-methyl-D-aspartate (NMDA) receptors, microglial activation, brain-derived neurotrophin factor (BDNF) and Downstream Regulatory Element Antagonist Modulator (DREAM) proteins), (ii) pro-inflammatory cytokines (interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α)) and (iii) oxidative stress markers (malondialdehyde (MDA), superoxide dismutase (SOD) and catalase) in the pathogenesis of DN in the spinal cord of streptozotocin-induced diabetic rats.

Materials and methods: One hundred and sixty-eight Sprague-Dawley male rats were assigned into seven groups (n = 24) consisting of non-diabetic control (S+CB), diabetic PDN control (S+STZ), diabetic non-PDN control (non-PDN), minocycline-treated PDN groups (M 80 and M 160) and ifenprodil-treated PDN groups (I 0.5 and I 1.0). DM was induced with a single streptozotocin injection at 60 mg/kg. Nociceptive behavioural tests such as Von Frey, hot-plate and formalin tests were conducted to assess tactile allodynia, thermal hyperalgesia and chemical hyperalgesia, respectively. Treatment of either saline, minocycline (80 µg/day or 160 µg/day) or ifenprodil (0.5 µg/day or 1.0 µg/day) was administered intrathecally for seven days. Chronic inflammatory pain was induced with formalin injection before being sacrificed three days later. The spinal cord lumbar enlargement region was collected for immunohistochemistry, Western Blot (WB) and enzyme-linked immunoabsorbent assay (ELISA) analyses.

Results: PDN rats developed tactile allodynia and chemical hyperalgesia but not thermal hyperalgesia, in which were prevented by minocycline and ifenprodil at both lower and higher doses used. Meanwhile, non-PDN group showed lower tactile allodynia, thermal and chemical hyperalgesia. There was significant higher NR2B, activated microglia, BDNF and DREAM proteins ipsilaterally and contralaterally by immunohistochemistry and WB analyses in (S+STZ) group, in which the results were reduced in non-PDN group. Minocycline and ifenprodil at both lower and higher doses significantly attenuated the expressions and mean relative NR2B, phospho-NR2B, BDNF, DREAM proteins levels and activated microglial positive neurons in a dose-dependent

manner. Furthermore, (S+STZ) and non-PDN groups showed a significant higher TNF-α level. Minocycline inhibited both cytokines. Moreover, MDA level was significantly higher in (S+STZ) and non-PDN groups. Significant lower catalase enzyme activity with insignificant SOD enzyme activity was detected in (S+STZ) group whilst marked higher catalase activity with lower SOD enzyme activity were detected in non-PDN group. Minocycline and ifenprodil attenuated MDA level and lead to higher catalase and SOD activities in the spinal cord.

Conclusion: Minocycline and ifenprodil is effective to combat PDN through their strong anti-nociceptive, anti-oxidant and anti-inflammatory activities as has been shown in this study.

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THE FEEDING PROCESS OF *PLASMODIUM FALCIPARUM* IN RESEALED ERYTHROCYTES CONTAINING ENDOCYTIC MARKERS AND ITS ROLE IN ARTEMISININ ACTION

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Introduction: Haemoglobin metabolism during the intraerythrocytic stage of the malaria parasite, *Plasmodium falciparum* is a preferable target for artemisinin, which is widely used for treatment and control of malaria. It has previously been reported that the parasite ingests haemoglobin via mouth-like structures named cytostomes. Haemoglobin-containing vesicles that bud off from cytostomes are transported to the digestive vacuole (DV) where the haemoglobin is degraded. However, the details of endocytic process as well as how artemisinin affects this process are still debatable.

Objectives: In this study we re-examined the endocytic process of the parasite and observed the effect of artemisinin on this process by using an endocytic marker, tetramethylrhodamine-dextran (TMR-dextran) and a pH indicator, Lysosensor Blue (LB) and SNARF-1-dextran.

Materials and methods: Resealed erythrocytes containing TMR-dextran were prepared at an optimised ratio of 1:3 of erythrocytes to haemolysis buffer volume. The presences of acidic structures were identified by the LB and SNARF-1-dextran. The endocytic process of the parasite and the effect of artemisinin on this process were observed under a fluorescence microscope.

Results: The resealed erythrocytes permitted retention of 33.56 ± 7.84% of the original haemoglobin contents and showed comparable parasite's invasion efficiency to normal erythrocytes. An early endocytic event of the parasites was initiated at mid ring stage with appearance of typical small endocytic compartments and a large spherical structure termed as a "big gulp". An acidic DV concentrated with TMR-dextran and labelled with LB, appeared at the later stages of trophozoite and schizont. Artemisinin treatments on this process showed no modification on the haemoglobin intake by the parasite and no alterations of the LB label and SNARF-1-dextran of the DV indicating no major pH changes of the vacuole.

Conclusion: In conclusion, haemoglobin is ingested by the parasite at early intraerythrocytic stage and accumulated in the acidic DV where the vacuole showed no significant pH alterations upon the artemisinin treatments.

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DETERMINATION OF THE HEALTH ECONOMIC FACTORS DEMANDED BY THE MALAYSIAN HAJJ PILGRIMS IN MAKKAH IN 2013

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Introduction: Desire among Muslim around the world to perform Hajj is tremendously high as it is the fifth pillar in Islam and compulsory for Muslim who is mentally, physically and financially capable. Hajj is an annual Muslim pilgrimage to Makkah that involved 2.5 million pilgrims from all around the world. It caused huge congestion that could lead to potential diseases outcomes and numerous problems during its performance.

Objectives: The objectives of this study are generally to study the demand for healthcare services among the Malaysian Hajj pilgrims in Makkah in 2013 and specifically (1) to determine the type of health problems faced by the Malaysian pilgrims during Hajj, (2) to identify healthcare services demand by the Malaysian pilgrims in Makkah during Hajj and (3) to determine the health economics factors associated to demand for health provision among Malaysian pilgrims during Hajj.

Materials and methods: A cross-sectional study was conducted that involved 379 Malaysian pilgrims in 2013/1434H. The survey was conducted after the pilgrims completed their Hajj ritual. Earlier, a qualitative study that was a Focus Group Discussion (FGD) was conducted as a baseline data collection for questionnaire construction. Six groups of participants were involved, where 36 participants were selected based on inclusion and exclusion criteria. A total of 400 sets of questionnaires were distributed at Abraj Janadriyah Hotel, which was occupied by more than 3,000 Malaysian pilgrims.

Results: The response rate for this survey was 93%. Male respondents were constituted of 49.6% and female respondents were 50.4% with the mean age 52 years old. The underlying disease among Malaysian pilgrims during Hajj was respiratory disease (77.5%). Various problems were encountered during Hajj, where healthcare services (52%) were substantially highlighted by the pilgrims, followed by the public services (15%), Hajj management (12%), Hajj activity (8%), hygiene issues (7%), and food quality (6%). The health personnel (36.1%) and quality medication (34.7%) are among the important healthcare services demanded by the Malaysian pilgrims in Makkah. The health economic factors related to facilities during the ritual, such as public services (bus, or/and car), healthcare services and the food management were found significantly ($P < 0.05$) associated with the confronted problems and type of diseases (communicable and non-communicable diseases) commonly affected population within the congestion.

Conclusion: In conclusion, this research provides a fundamental input to be reference for the health care providers, and the Hajj management authority to improve the quality and improve efficiency of Hajj management in for Malaysian pilgrims in year ahead.

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FACTORS ASSOCIATED WITH BREAST CANCER AND ROLE OF NUTRITIONAL STATUS ON SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN

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Introduction: Breast cancer is the leading killer of women in Malaysia. Nutritional status and adiponectin are modifiable risk factors for breast cancer occurrence that can be efficiently targeted.

Objectives: The purpose of this study was to determine the associated factor of breast cancer and relationship between nutritional status and high molecular weight (HMW) adiponectin.

Materials and methods: This was a case-control study, conducted from January 2014 until August 2015 at Hospital Universiti Sains Malaysia (HUSM) and Universiti Sains Malaysia (USM). Untreated breast cancer cases ($n = 55$) were assigned as cases, and healthy controls ($n = 58$) who were staff at HUSM and USM acted as controls. Sociodemographic and reproductive data were obtained with a standard questionnaire and dietary data was obtained from validated diet history questionnaire (DHQ). Anthropometric assessments (weight, height, hip, waist circumference (WC) and body fat composition) were measured and overnight fasting venous blood samples were analysed for lipid profiles, plasma glucose, insulin, high sensitivity C reactive protein (hs-CRP) and HMW adiponectin. Physical activity was measured using accelerometer for two weekdays and one weekend.

Results: Simple logistic regression found these factors associated with breast cancer risk: sociodemographic (age, lower education level, exposed to secondhand smoke, lower monthly household income), physical examination (systolic blood pressure, diastolic blood pressure, pulse, more than 1 year of traditional medicine) and nutritional status (WC, HDL cholesterol, TG, blood glucose and sugar intake). Multiple logistic regression was performed to determine factors associated with breast cancer risk. The predictors of breast cancer development were exposed to secondary smoke (OR = 10.00, [95% CI: 2.42, 41.30]), monthly household income of less than RM2,300 (OR = 18.05, [95% CI: 2.56, 127.10]) and systolic blood pressure (OR = 1.08, [95% CI: 1.04, 1.12]). Multiple linear regression analysis revealed that there was a significant linear negative relationship between WC and HMW adiponectin ($\beta = -0.05$; $P = 0.005$) among breast cancer cases. Besides, HDL cholesterol was positively associated with HMW adiponectin ($\beta = 1.83$; $P = 0.010$) among breast cancer cases. BMI was negatively associated with HMW adiponectin ($\beta = -0.02$; $P = 0.001$) among healthy controls.

Conclusion: This study indicated the potential role of modifiable risk factor in the occurrence of breast cancer.

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