EFFECT OF REPEATED DOSE OF ISOPRENALINE ADMINISTRATION ON RAT'S KIDNEY

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Kidney expresses beta (β)- receptors for physiological regulation of renin secretion, blood pressure and blood volume. Some studies had shown that over stimulation of beta receptor may trigger oxidative stress and inflammation in kidney tissue. However, it is unclear how repeated over-stimulation of β receptors would affect kidney structure and function. Thus, this study assessed the effect of repeated β- adrenergic stimulation using isoprenaline (ISO) on rat kidneys. Male Wistar rats (n=24) were randomly allotted and given 5 mg/kg or 10 mg/kg of ISO or normal saline as the vehicle (control) subcutaneously for 14 days. Rat's blood pressure was recorded weekly using non-invasive tail-cuff blood pressure method. The body weight gain was monitored daily throughout the experiment. The rats were sacrificed and the renal and serum were collected for further analysis. The results showed that the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean atrial pressure (MAP) in treated groups were significantly higher than the control group at the end of the experiment ($p<0.05$). Heart rate in treated groups were significantly decreased compared to control group ($p<0.05$). As for the renal profile, blood urea nitrogen (BUN) using Diacetyl Monoxime method showed no significant difference between groups ($p>0.05$). Whereas, serum creatinine by Jaffe's method also showed no significant difference between groups, however there was an increment trend between control vs. ISO 10mg/kg group ($p=0.06$). For the morphology studies using Hematoxylin and Eosin staining, vacuolation was observed more in rats treated with ISO 10 mg/kg group, however there were no signs of mesangial expansion, infiltration of leukocytes and tubular damage observed. In conclusion, these findings showed that repeated of low dose ISO leads to early stage of renal injury however longer time of stimulation might be needed to cause any overt structural and functional changes.

Keywords: isoprenaline, beta-overstimulation, renal injury

DO GENES DICTATE OUR HAPPINESS, SLEEP PATTERN, AND ANGER?

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Behavioural genetics is broadly defined as the study of the inheritance of behavioural phenotypes. This field provides the understanding of behavioural variation by nature and nurture and their interaction. Emotions can differ from one person to another and nowadays, sleep deprivation is common, but not everyone has to experience its consequences. This study aimed to investigate the association of *OXTR* with happiness, *5-HTTLPR* with unhappiness, *BHLHE41* with short sleep, *PER3* with morningness-eveningness and *DARPP-32* with anger among Biomedical Science undergraduates in the University of Malaya. In this study, a total of 170 students were recruited. Three questionnaires were distributed, namely Oxford Happiness Questionnaire (OHQ), Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) and Affective Neuroscience Personality Scales (ANPS). Genomic DNA extracted from saliva was subjected to the examination of the five targeted polymorphisms (*OXTR* rs53576, *5-HTTLPR* rs4795541, *BHLHE41* rs121912617, *PER3* rs57875989, and *DARPP-32* rs907094) using polymerase chain reaction approaches. Phenotypic and genotypic frequencies and the associations between them were analysed using Fisher's exact tests. The genotypes were validated by DNA sequencing. Results showed no association of *5-HTTLPR* rs4795541, *BHLHE41* rs121912617, *PER3* rs57875989, and *DARPP-32* rs907094 with their respective phenotypes. However, homozygous GG of *OXTR* rs53576 demonstrated significant correlation with unhappiness ($p = 0.0340$), which opposed what had been suggested by the initial studies. Among the Year 4 students, the number of those who were happy, identified as regular sleepers, and had lower anger levels, were significantly higher (all $p < 0.05$). The intermediate type of sleepers was significantly higher ($p < 0.05$) than both morning and evening types among the students as a whole, and also in separate years. This study concluded that happiness, anger, short sleep and morningness-eveningness are shaped from the gene-environment interaction, rather than from the genes alone.

Keywords: gene polymorphisms, emotions, sleep patterns

PHENOTYPIC CHARACTERISATION OF HYPOXIA-INDUCED MDA-MB-231 BREAST CANCER CELL LINE

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Hypoxia, a common characteristic of locally advanced solid tumours has been associated with diminished therapeutic response. The hypoxic environment surrounding cancer cells causes the upregulation of hypoxia-inducible factor-1α (HIF-1α) and is critically involved in cell invasion and metastasis. Cancer cells form actin-rich protrusions called invadopodia, which degrade the extracellular matrix (ECM) and potentially allow tumours to invade surrounding tissue. Tumour cells are often deprived of oxygen, and hypoxia can promote invadopodia formation in vitro. But how this pathway is regulated is still unclear. The transcription factor HIF-1α, which is stabilized in low oxygen conditions, was required for hypoxia-induced invadopodia formation. In this study, phenotypic characterisation was employed to explain the alteration of the cellular metabolism of breast cancer cells due to hypoxia. However, there is limited information available on the changes of cellular metabolism of breast cancer cell due to hypoxia. This study generally aims to identify the metabolic changes under hypoxic and normoxic conditions in breast cancer cells as its energy will increase invasiveness under hypoxic conditions. MDA-MB-231 breast cancer cell line was cultured. Hypoxia chamber and a hypoxia mimetic called dimethyloxaloylglycine (DMOG) was used to mimic the hypoxia condition in cells. Then, phenotype microarray for mammalian cells (PMM) was applied to elucidate the cellular metabolism by determining the carbon sources utilization of the cells in hypoxia. The result of PMM showed that 11 types of biochemical substrates were significantly utilised in hypoxia compared to normoxia such as dextrin. From the PMM findings, we suggest these substrate are potential energy for targeting hypoxia region because it is known that hypoxic solid tumours are resistant to chemotherapy and radiotherapy. Therefore, the result of this study proposed that these 11 biochemical substrates can be used as carrier target in drug delivery.

Keywords*:* Hypoxia, HIF-1α, Phenotype Microarray

EFFECT OF REPEATED DOSE OF ISOPRENALINE ADMINISTRATION ON RAT'S AORTA

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Heart failure incidence has shown a global increasing trend, hence becomes our major health concern. Isoprenaline (ISO), a non-selective β-agonist has been frequently used as an experimental drug-induced heart failure rat model. Vascular dysfunction is known to be part of the pathophysiology of heart failure. However, limited evidence is known on the vascular effects in such experimental model. Thus, this study was aimed to assess the effect of repeated administration of ISO on the rat's aorta. Three groups of male Wistar rats (n=24) were given saline, 5 mg/kg or 10 mg/kg of ISO subcutaneously for 14 days respectively. On the 15th day, the rats were sacrificed, the thoracic aorta and serum were collected for further analysis. The present findings showed that the systolic blood pressure (SBP) via tail-cuff method in both ISO treated groups were all significantly increased vs. control group ($p<0.05$). Meanwhile, rats heart rate (HR) was significantly decreased vs. control group ($p<0.05$). Alpha–adrenergic vasoconstrictor, phenylephrine (PE, 10^{-7}M), and endothelium-dependent vasorelaxant, acetylcholine (ACh, 10^{-9}~10^{-4}M), was used to test the vascular functionality (PowerLab System). A significant reduction in ACh-induced relaxation was found in both ISO treated groups vs. control group ($p<0.05$), indicating loss of the endothelial functions. Furthermore, the bioavailability of nitric oxide in the blood from the Griess assay was significantly reduced in ISO 10 mg/kg group ($p<0.05$), while ISO 5 mg/kg group showed a trend of reduced function vs. control group ($p=0.055$). Gelatin zymography method was used to study the matrix metalloproteinases–9 enzyme activities, and it was found that there was no significance detected between all the groups ($p>0.05$). However, ISO 10 mg/kg group did show trend of increased activity vs. control group. In conclusion, this study showed repeated administration of low dose ISO for 2 weeks could lead to vascular dysfunction.

Keywords: Isoprenaline, aorta, endothelial dysfunction

AWARENESS, KNOWLEDGE AND ATTITUDE (AKA) ON URINARY TRACT INFECTION (UTI) AMONG GOVERNMENT SECONDARY SCHOOL STUDENTS IN SHAH ALAM, MALAYSIA

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This cross-sectional study was conducted to provide preliminary insight of *Awareness, Knowledge* and *Attitude* (AKA) assessment on UTI among adolescents which includes the general level of AKA and correlation between the domains as well as the relationship between total AKA score and the sociodemographic factors**.** A cross-sectional study was conducted on 136 government secondary school students in Shah Alam utilizing an adapted and modified questionnaire. The instrument used in this study consist of socio-demographic questions and AKA domains employing descriptive statistics, linear regression and MRA via SPSS Version 23.0. In general, AKA level was reported as moderate (0.5 ± 0.11). Among the three domains, *Knowledge* (0.70 ± 0.12) risen with the most astounding mean, took after *Awareness* (0.36 ± 0.22) and *Attitude* (0.65 ± 0.11). A positive but weak correlation was found between the domains; *Awareness* and *Knowledge* is significant at p = 0.034, r = 0.157; R^2 = 0.02 and similarly, a weak correlation was also found between *Knowledge* and *Attitude* which is significant at p = 0.000, r = 0.411; R^2 = 0.17. After covariates adjustment, female gender was found to have the strongest relationship with total AKA score. The general level of total AKA score on UTI is moderate. The AKA domains in this study were found to be positively correlated and female was found to be the best predictor for a better total AKA score on UTI. Thus, these findings provide important information to formulate an effective education intervention to improve the AKA on UTI among adolescents.

Keywords: Urinary tract infection, Adolescents, Awareness, Knowledge, Attitude

PARKIN (*PRKN*) GENE HOMOZYGOUS EXON DELETION IN A MALAYSIAN FAMILY WITH YOUNG-ONSET PARKINSON'S DISEASE

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Young-onset Parkinson's Disease (YOPD) is a rarer form of PD as compared to late-onset PD. Here we present a family with two female siblings who develop YOPD and have a paternal grandmother who is also suspected to have had PD. Both parents are normal. The disease pattern of this family suggests an autosomal recessive mode of inheritance. To genetically diagnose the affected individuals, we screened for mutations in the *PARKIN* (*PRKN*), *PTEN induced kinase 1* (*PINK1*) and *GTP cyclohydrolase 1* (*GCH1*) genes. Variants were assessed for their likely pathogenicity by *in silico* analysis. 26 pairs of primers were designed to amplify coding regions of the three genes through conventional PCR. Mutation analysis was performed initially in the proband by Sanger sequencing and sequences were assessed for variants by comparing the patient sequences against a reference sequence using NCBI-BLAST. The mutations identified in the proband was then screened in the other sister and in controls to rule out non-pathogenic variants. Another 26 sets of primers were designed to determine the breakpoint of the exon deletions in *PRKN*. The impact on the protein sequence was predicted using the online tool ExPASy. Based on the result, *PINK1* and *GCH1* were normal. A homozygous deletion involving exons 8 and 9 was found in *PRKN* for both the patients. The deletion breakpoint was determined to be within ~45 kbp away from exon 8 and ~3.5 kbp from exon 9. This large ~69 kbp deletion was postulated to cause a frameshift and a premature stop codon, truncating the mutant protein to 297 amino acids compared to the normal protein which is 465 amino acids. In conclusion, we have identified a large homozygous deletion of exons 8 and 9 in *PRKN* as the likely cause for YOPD in this Malaysian family.

Keywords: Parkinson's Disease, PRKN, Homozygous deletion

ASSOCIATION BETWEEN POLYMORPHISM OF *GSTT1* AND *GSTM1* AMONG TYPE-2 DIABETES MELLITUS IN MALAYSIAN PATIENTS WITH NEPROPATHY

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Diabetic nephropathy (DN) is one of the severe microvascular complications of diabetes mellitus. DN may link with oxidative stress that stimulated by hyperglycaemia. Deletion of *GSTT1* and *GSTM1* genes may associate with DN due to their function on detoxification of oxidative stress products. Previous studies have shown an association between deletion of *GSTM1* and *GSTT1* gene with increased risk of T2DM and DN. However, limited study has been carried out among Malaysian on the polymorphism of *GSTM1* and *GSTT1* among T2DM and DN. The aim of this study is to investigate the association between deletion of *GSTT1* and *GSTM1* genes with susceptibility of T2DM and DN in Malaysian. 885 extracted DNA samples which consist of 192 T2DM/DN patients, 175 T2DM patients and 518 controls are obtained. *GSTT1* and *GSTM1* genes are then amplified using multiplex polymerase chain reaction. The genotype of all individual are recorded, and the results are analysed using Chi-square test. Significant correlation is shown between null genotype *GSTM1* and T2DM, while no significant difference of both null genotype *GSTT1* and *GSTM1* shown between T2DM patients and T2DM/DN patients (p=0.717and p=0.549 respectively). There is no significant difference between main ethnics (Malay, Chinese and Indian) in Malaysia with diabetic status (p=0.186), although there is a significant difference of *GSTM1* and *GSTT1* gene distribution. *GSTM1* gene involve more remarkable in lower the risk of T2DM among Malaysian compared to *GSTT1* gene. However, *GSTT1* and *GSTM1* null genotype do not significantly associated with the susceptibility of DN in T2DM Malaysian patient.

Keywords: Glutathione S-transferase, Type 2 Diabetes Mellitus, Diabetic Nephropathy

ASSOCIATION STUDY OF *PTPN2* GENE POLYMORPHISMS IN MALAYSIAN PATIENTS WITH CROHN'S DISEASE

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Crohn's disease (CD) is one of the sub-entities of inflammatory bowel disease (IBD) which causes chronic inflammation in any part of the gastrointestinal tract. It is known to have low incidence and prevalence in Asian countries, but multiple studies have reported increasing trend of CD cases in Asia. The development of CD has been shown to have strong genetic association. Through Genome Wide Association Studies, *Protein Tyrosine Phosphatase, non-receptor type 2 (PTPN2)* gene was identified as one of the susceptible loci of CD. PTPN2 acts as a crucial negative regulator of immune response where it protects the function of intestinal epithelial barrier, controls cytokine signalling pathway, regulates differentiation of T helper cell and involves in autophagosome formation. Hence, our study aimed to investigate the association of *PTPN2* gene polymorphisms with the onset of CD in the Malaysian population. A total of 137 CD patients and 274 matched healthy controls were recruited in this study. Genomic DNA was extracted from venous blood using a conventional phenol-chloroform extraction method. The examination of five targeted Single Nucleotide Polymorphisms (SNPs) (rs1893217, rs7234029, rs2542152, rs487273 and rs16939895) were carried out via conventional Polymerase Chain Reaction (PCR) using tetra primer amplification refractory mutation system (ARMS) approach. Genotyping data was analyzed using Fisher's exact test, odds ratio, 95% confidence interval and Hardy Weinberg equilibrium. The validation of genotyping assay was performed on selective samples by DNA sequencing. All five selected SNPs were not significantly associated to the onset of CD in the Malaysian cohorts. In ethnic stratification analysis, the rs487273 heterozygous G/T genotype was found to reduce the risk of getting CD in the Chinese population (P = 0.0253; OR = 0.4396). In conclusion, there was no significant association of *PTPN2* gene polymorphisms with the onset of CD in the Malaysian cohort.

Keywords: *PTPN2* gene, Crohn's disease, Single nucleotide polymorphism

ANTI-INFLAMMATORY EFFECT OF *ZINGIBER ZERUMBET* ETHYL ACETATE EXTRACT IN ESTROGEN DEPLETED RATS OF ALUMINIUM-INDUCED ALZHEIMERISM

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Alzheimer's disease (AD) is a progressive disorder in which brain cells deteriorate leading to the loss of cognitive functions, primarily memory. The loss of estrogen in aging women increases the brain vulnerability to neurodegenerative diseases. This study embarks on the evaluation of *Zingiber zerumbet* (*Z. zerumbet*) administration on the neurological changes of estrogen-depleted (ovariectomized) rats intoxicated with aluminium. Behavioural study and specific biochemical analysis on Alzheimer-induced animals were performed. The whole duration of the experiments was 8 weeks. A total of forty-eight adult female *Wistar* rats were divided into 6 main groups (8 animals/group); group 1 (OVX), group 2 (OVX + AlCl3), group 3 (OVX + AlCl3 + NAC), group 4 (OVX + AlCl3 + E2), group 5 (OVX + AlCl3 + 200mg/kg *Z. zerumbet*) and group 6 (OVX + AlCl3 + 400mg/kg *Z. zerumbet*). Ovariectomy (OVX) in all groups was performed after 1 week of habituation. Estradiol (E2), 10µg/kg (subcutaneous), *Z. zerumbet* extract, 200mg/kg and 400mg/kg (oral gavage) and N-acetylcysteine (NAC), 30mg/kg (oral gavage) in the designated groups were given from week-2 until week-8. Aluminium chloride (AlCl3) (oral gavage) were given to the respective groups for 5 weeks starting from week-3. The behaviour of the rats was observed prior to AlCl3 induction and prior to killing on week-8. Y-maze spontaneous alternation task was employed to determine the cognitive impairment status of the rats. *Z. zerumbet*-treated rats were found to be less sensitive to hippocampal damage as shown by Y-maze test. There were significantly low levels of β-amyloid and phosphorylated-Tau protein in *Z. zerumbet*-treated rats. There were significantly low levels of TNF-α, IL-1β and IL-8, in *Z. zerumbet*-treated rats compared to negative control rats. In conclusion, *Z. zerumbet* ethyl acetate extract has anti-inflammatory properties which able to protect the brain in estrogen-depleted rats from detrimental effect of aluminium induced Alzheimerism.

Keywords: anti-inflammatory, *Zingiber zerumbet*, estrogen depleted, Alzheimer's disease

GENETIC ASSOCIATION OF *ZNF365* POLYMORPHISMS WITH CROHN'S DISEASE PATIENTS IN MALAYSIA

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Crohn's disease (CD) is one of the prominent types of inflammatory bowel disease (IBD). CD is characterized by transmural inflammation at any part of the gastrointestinal tract and causes abdominal pain and fever. CD is currently without cure. The exact etiology of CD remains unknown, however studies suggested that a complex interaction of environmental, immune response and genetic component leads to the onset of CD. Single nucleotide polymorphism (SNP) can alter the gene expression and affect the gastrointestinal homeostasis. Genome-wide association studies (GWAS) revealed 140 susceptible loci for CD, including *Zinc Finger Protein 365 (ZNF365).* This study aimed to assess the frequency and distribution of *ZNF365* genetic polymorphisms and their association with CD in the Malaysian cohort. Genotype-phenotype relationship of *ZNF365* polymorphisms in CD patients was also investigated. A total of 137 CD patients and 274 healthy controls were recruited in this study. Genomic DNA was extracted using a conventional phenol-chloroform extraction method. DNA quality and quantity were assessed using nanophotometer. Five SNPs of *ZNF365* were genotyped using amplification-refractory mutation system PCR (rs10995271, rs10822044 and rs10761659) and quantitative real-time PCR (rs7076156 and rs7915131). The genotyping results were validated by DNA sequencing approach. Statistical analysis i.e., Fisher's exact test, odds ratio, 95% confidence interval (CI) and Hardy-Weinberg equilibrium were computed. Three *ZNF365* SNPs showed significant association to the onset of CD in the Malaysian cohorts. The rs10995271 C allele (P=0.0023; OR=1.5884) and rs10761659 G allele (P=0.0002; OR=1.8929) were found to increase risk of CD. For stratification analysis, rs7915131 homozygous C genotype was found to significantly increase CD risk in Indian (P=0.0106; OR=3.5581). For genotype-phenotype analysis, rs10822044 T allele (P=0.0257; OR=0.5234) was found to reduce the risk of colon inflammation in CD patients. In conclusion, *ZNF365* gene was found to be significantly associated with CD in the Malaysian population.

Keywords: Crohn's disease, gene polymorphism, *ZNF365* gene

ANTI-OXIDANT EFFECT OF *ZINGIBER ZERUMBET* ETHYL ACETATE EXTRACT IN ESTROGEN-DEPLETED RATS OF ALUMINIUM-INDUCED ALZHEIMERISM

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Aluminium can cause Alzheimer's disease (AD) with oxidative stress as one of the possible mechanisms mediating the event. AD is worsened in ageing women due to the loss of hormone-based neuroprotective and antioxidant agent in the form of estrogen. In this study, the effect of ethyl acetate extract of *Zingiber zerumbet* (*Z.zerumbet*) rhizome (200 and 400 mg/kg) on estrogen-depleted rats of aluminium-induced Alzheimerism were examined. Wistar rats were divided into six groups containing 8 per group. After 1 week of habituation, all animals were ovariectomized (OVX). The sham group received PBS and sesame oil (vehicle) while other groups were treated with either NAC (30 mg/kg, oral gavage), estradiol (14µg/100g, s.c.) and *Zingiber zerumbet* extract of the respective dosage were given via oral gavage for 6 weeks ; group1 (OVX), group2 (OVX+$AlCl_3$), group3 (OVX+$AlCl_3$+NAC), group4 (OVX+$AlCl_3$+E_2), group5 (OVX+$AlCl_3$+200mg/kg *Z.zerumbet*) and group6 (OVX+$ALCL_3$+400mg/kg *Z.zerumbet*). Aluminium chloride ($AlCl_3$) was given starting from week-3 to week-5 through oral gavage. Behavioral assessments were done at two time points; prior to $AlCl_3$ induction and prior to killing. At the end of the experiment, the animals were killed for biochemical analysis. The cognitive impairment status was assessed via Y-maze spontaneous alternation task. Biochemical analysis of brain homogenate revealed that *Z. zerumbet* ethyl acetate extract at 200 mg/kg and 400 mg/kg significantly reduced the level of protein carbonyl, 8-hydroxydeoxyguanosine(8-oHDG), Isoprostane, β-amyloid and phosphorylated Tau. Both doses of extracts also showed significant increase of Superoxide dismutase (SOD). However, administration of ethyl acetate *Z.zerumbet* extract at 400 mg/kg showed better protective effects in estrogen-depleted rats of aluminium-induced Alzheimerism as shown with higher level of SOD in the brain homogenate as compared to 200 mg/kg dose. Ethyl-acetate extract of *Z.zerumbet* has protective effects against estrogen-depleted rats of Aluminium-induced Alzheimerism and this is mediated through its antioxidant properties.

Keywords: *Zingiber zerumbet*, Aluminium, Anti-oxidant, Estrogen-depleted, Alzheimer's disease

EFFECTS OF LYSOSOMAL INHIBITION ON HUMAN BRAIN ENDOTHELIAL CELLS DAMAGE

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Lysosome is an organelle that contains hydrolases which are responsible for degradation of macromolecules into simpler molecules which are then recycled as energy sources or as a building blocks to synthesise new proteins and organelles. The substrates that were ready to be degraded are delivered to lysosome through autophagic pathway. Autophagy system will collect the degradable substrate into a double membrane vesicle called autophagosome which is then delivered to lysosome for degradation. Defects in the autophagy-lysosomal system can lead to the accumulation of damaged organelles and aggregated proteins that will increase the risk of cellular damage. Recently, the disruption of this process in human brain endothelial cells (HBEC) is likely to be linked with development of neurodegenerative diseases. In order to understand the effects of lysosome inhibition to the cells, an in-vitro model was established in this study. Ammonium chloride (NH_4Cl) was used as lysosome inhibitor. Its optimal concentration and duration of exposure were firstly evaluated by MTT assay. Next, Western blotting was conducted to investigate the inhibition of autophagy-lysosomal process of the selected MTT concentration by detecting the expression of autophagy protein markers such as LC3-II and p62. Lastly, the morphological change was observed by using an inverted microscope. Result from MTT assay shows that the optimum concentration of NH_4Cl to inhibit autophagy-lysosomal system in 24 hours is 72 mM. The autophagy protein marker LC3-II and p62 expression are expected to be higher in NH_4Cl treatment group in comparison to control group, indicating that the autophagy-lysosomal process is inhibited. Meanwhile the morphology of the cells in treatment group is expected to be different in comparison to control group. In conclusion, this study is expected to give some preliminary results of the effects of lysosomal inhibition on HBEC.

Keywords: autophagy; lysosome inhibitor; brain endothelial cells, neurodegenerative

EFFECTS OF AUTOPHAGY INHIBITION ON CELLULAR CHANGES OF HUMAN BRAIN ENDOTHELIAL CELLS

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Autophagy is an evolutionarily conserved and strictly regulated lysosomal pathway that degrades cytoplasmic material and organelles. Accumulating evidence indicates that lysosomal and autophagy dysfunction are the main mechanisms underlying common neurodegenerative diseases such as Parkinson, Alzheimer, and Huntington diseases. Human brain endothelial cells (HBEC) are crucial for brain vascular repair and maintenance, but their physiological functions may be impaired in neurodegenerative or neurovascular diseases through endothelial dysfunction. The main changes that have been observed in endothelial dysfunction are decreased in nitric oxide, increased in inflammation marker and reduced of tight junction protein. Thus, in this study, we investigated the effects of autophagy inhibition on cultured HBEC exposed to a lysosomal inhibitor, ammonium chloride. MTT assay was conducted to evaluate the cells viability. Western blot analysis was conducted to investigate the expression of inflammation marker Intercellular Adhesion Molecule 1 (ICAM-1), endothelial Nitric Oxide Synthase (eNOS), and tight junction protein (Claudin-5). This study is expected to show that ammonium chloride could inhibit HBEC viability in a concentration and time-dependent manner. Ammonium chloride-induced lysosome inhibition may promote endothelial dysfunction in human brain endothelial cells through the decrease of eNOS expression, increase of ICAM-1, and reduction of Claudin-5 expression. Thus, this study is expected to investigate if lysosomal-autophagy system inhibition could lead to cellular changes in HBEC.

Keywords: autophagy, human brain endothelial cells, lysosome

EFFECTS OF AMYLOID PRECURSOR PROTEIN OVEREXPRESSION ON RHO-GTPASE PATHWAY IN NEURONAL CELLS

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that causes deficit cognitive functions and it is commonly seen in the elderly. According to the Amyloid Cascade Hypothesis, it is proposed that amyloid-beta (Aβ) accumulation causes formation of senile plaque in AD and Aβ is derived from amyloid precursor protein (APP). Rho-GTPases are guanine nucleotide binding proteins which are well-known to contribute in numerous aspects of neuronal growth. Dysregulation of Rho-GTPase has been implicated to be involved in pathophysiology of AD. Here we present a study to investigate the effects of APP overexpression on Rho-GTPase pathway in neuronal cell. SH-SY5Y cells were transduced with APP, followed by validation of its overexpression using western blot. The expression of Rho-GTPase pathway proteins were then analysed using western blot. Based on the data collected, APP overexpression caused an increase in RhoA and RhoC proteins expression level. However, the increment in RhoA protein is not statistically significant. Furthermore, APP overexpression decreased the expression of p-Rac1/cdc42, Rac1/2/3 and Cdc42, where the changes were not statistically significant. In conclusion, APP overexpression regulates Rho-GTPases pathway in neuronal cells by up-regulating RhoC proteins expression. Further studies are required to validate the roles of APP in modulating the Rho-GTPases family proteins and the findings may suggest potential therapeutic options for AD.

Keywords: Amyloid precursor protein, Rho-GTPase, Alzheimer's disease