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S1.1

Artemisinin protected neuronal cells against oxidative stress and its underlying mechanisms

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Artemisinin, also known as Qinghaosu (Chinese: 青蒿素), isolated from artemisia annua, is a drug that possess the most rapid action of all current drugs against *Plasmodium falciparum* malaria. It is safe and effective in clinic. In the present study, we found that artemisinin promoted the survial of various cell types such as PC12 cells and primary cortical cultured neurons from oxidative insults. For example, pretreatment of PC12 cells with artemisinin significantly suppressed SNP/H_2O_2-induced cell death by decreasing the production of intracellular reactive oxygen species (ROS), preventing the decline of mitochondrial membrane potential, restoring abnormal changes in nuclear morphology and reducing LDH and caspase 3/7 activities. Western blotting analysis showed that artemisinin was able to stimulate the phosphorylation/activation of extracellular regulated protein kinases (ERK) kinase, AMPK and CREB while had no effect on the Akt pathway. In addition, ERK and AMPK signaling pathway inhibitors attenuated the protective effect of artemisinin whereas the PI3K inhibitor LY294002 had no effect. Taken together, these results suggest that artemisinin as a potential neuroprotectant is able to suppress varous oxdative stress-induced neuronal cell death via the activation of ERK/AMPK signaling. Our results offer support for the potential development of artemisinin to prevent neuronal degenerative disorders.

Keywords: artemisinin; oxidative insults; neuronal cells; AMPK

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S1.2

Xyloketal B reduces cerebral infarction and neurologic deficits in a mouse transient middle cerebral artery occlusion model of stroke by suppressing the ROS/TLR4/NF-κB inflammatory signaling pathway

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The present study aimed to observe the effect of xyloketal B [Xyl-B, novel marine compound isolated from mangrove fungus *Xylaria sp.* (no. 2508)] in a mouse model of stroke. Our previous studies have demonstrated that a pretreatment with Xyl-B exerted neuroprotective effects and attenuated hypoxic-ischemic brain injury in neonatal mice. The present study investigated the pretreatment and post-treatment effects and the underlying mechanisms of Xyl-B on ischemic stroke in adult mice using a transient middle cerebral artery occlusion (tMCAO) model. The tMCAO stroke model was established in adult male C57 mice. The preventive effects of Xyl-B were examined by multiple intraperitoneal injections before ischemia. The post-treatment effects of Xyl-B were determined following the administration of a single-dose of Xyl-B at 0, 1 or 2 h after the onset of ischemia. The regional cerebral perfusion was monitored using a laser-Doppler flowmeter. TTC (2, 3, 5-triphenyltetrazolium chloride) staining was performed to determine the brain infarction volume. We found that both pretreatment with Xyl-B and a single dose of Xyl-B at 50 mg/kg administered at 0, 1 or 2 h after the onset of ischemia reduced the infarct volume, while no significant hemodynamic effects were observed. Moreover, Xyl-B *in vivo* attenuated ROS overproduction; increased the MnSOD protein level; inhibited TLR4, NF-κB and iNOS protein expression; and down-regulated pro-inflammatory cytokine mRNA expression levels, including IL-1β, TNF-α, IL-6 and IFN-γ. Xyl-B also protected blood-brain barrier integrity. Xyl-B protects against focal cerebral ischemia when administered within 2 h after the onset of stroke, and the underlying mechanism may be related to the ROS/TLR4/NF-κB inflammatory signaling pathway.

Keywords: stroke; ischemic brain injury; xyloketal B; middle cerebral artery occlusion; TLR4; NF-κB

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S1.3

Heat-sensitive moxibustion protects against cerebral ischemia/reperfusion injury via anti-inflammation

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Ischemic stroke is a common cause of long-term disability or even death. Suspended moxibustion, an indirect form of moxibustion, is when moxibustion is placed superficially over the skin without physically contact. Suspended moxibustion has been used to treat stroke patients, while the effect of traditional suspended moxibustion has recently been improved with the development of heat-sensitive moxibustion, but no strong evidence of its therapeutic effectiveness is documented to date. The theoretical basis for heat-sensitive moxibustion is that the acupoints are activated from the resting state to the sensitized state during pathological processes thus become heat sensitive. Moxibustion placed above a heat-sensitive acupoint can induce not only internal heat-sensation but also physically detectable elevated temperature at distant locations away from the suspended moxibustion acupoint. In this study, we compared the effects and investigated mechanism of moxibustion at acupoint Dazhui (DU14) with (heat-sensitive moxibustion, HSM) or without tail temperature increase (non-heat-sensitive moxibustion, NHSM). We found that HSM decreased tail-flick latency, increased neurological function score, decreased infarct volume, reduced inflammatory cells infiltration, and decreased inflammatory mediators and the expression of NF-κB signaling pathway in rats with focal cerebral ischemia/reperfusion injury. Our experimental findings indicated that HSM can significantly attenuate inflammation and promote repair after focal cerebral ischemia/reperfusion injury.

Keywords: suspended moxibustion; middle cerebral artery occlusion; cerebral ischemia/reperfusion injury; infarct volume; inflammatory cells; inflammatory mediators ; NF-κB p65

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S1.4

PD149163 induces hypothermia to protect against brain injury in acute cerebral ischemic rats

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Therapeutic hypothermia is a promising strategy for acute cerebral ischemia via physical or pharmacological methods. In this study, we found that PD149163 could prevent neuronal damage, and inhibit proliferation and activation of astrocytes induced by ischemia, without altering local cerebral blood flow. Simultaneously, we observed PD149163 down-regulated caspase-3 and Bax, but elevated Bcl-2, indicating apoptosis was ameliorated. PD149163 could increase p62 expression, decrease the ratio of LC3-II to LC3-I and Beclin expression, suggesting autophagy was inhibited. Furthermore, PD149163 dramatically reduced JNK and AMPK/mTOR signaling pathway activation, which contributed to autophagy inhibition. Taken together, our results indicate that PD149163-induced hypothermia protects against ischemia-induced brain damage, due to down-regulation of autophagy.

Keywords: PD149163; neurotensin; hypothermia; ischemia

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S1.5

Fingolimod depending on S1PR3 protects astrocytes against ischemia-induced neuroinflammative injury via TLR2,4-PI3K/NF-κB signaling pathway

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Sphingosine-1-phosphate receptors (S_1PRs) regulate multiple cellular functions such as proliferation, immunomodulation and apoptosis. Fingolimod (FTY720), a S_1P receptor (S_1PR_1) agonist, is used as immunomodulatory therapy for multiple sclerosis. In the present study, we found that FTY720 protected against oxygen-glucose deprivation/reoxygenation (OGD)-induced cellular injury and inhibited the up-regulation of S_1PR_3 in astrocytes. Further study revealed that FTY720 prevented OGD-induced the elevations of Toll-like receptor 2 and 4 (TLR2, TLR4) and advanced glycation end-products receptor (RAGE), and inhibited phosphatidyl-inositol 3 kinase (PI3K) phosphorylation and nuclear p65 activation. Subsequently, FTY720 significantly decreased inflammatory cytokines secretion including high mobility group box 1(HMGB1), tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) from astrocytes. Transfecting *S1pr3* antisense oligonucleotide into astocytes could abolish all of the above-mentioned effects of FTY720, suggesting that S_1PR_3 was the target for FTY720. Moreover, not RAGE monoclonal antibody but TLR2,4 antagonist could block the protective effects of FTY720. The results from ischemic stroke model of rats confirmed that FTY720 reduced the brain infarct volumes, inhibited astrocyte activations in peri-infarct area and thereby decreased the levels of inflammatory cytokines in serum. Taken together, the present study reveals that FTY720 acting on S_1PR_3 protected against brain ischemia-induced astrocytic activation and injury via TLR2,4/PI3K/NF-κB signaling pathway. Our findings reveal a novel astrocyte-mediated neuroinflammation mechanism depending on S_1PRs, and suggest that S_1PRs might be a potential target for ischemia stroke.

Keywords: sphingosine-1-phosphate receptors; FTY720; oxygen-glucose deprivation/re-oxygenation; astrocyte; stroke

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S1.6

Ginsenoside Rg1 protects against corticosterone-induced injury in primary astrocytes

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The present study aimed to observe the protective effect of ginsenoside Rg1 on corticosterone (CORT)-induced injury in primary astrocytes and its underlying mechanism. Primary astroglial cultures in the hippocampus and prefrontal cortex were established. Corticosterone (CORT) was used to simulate the stress in primary astrocytes. Cx43 phosphorylation level was detected using Western blot. The effect of Rg1 on cell viability was determined by using CCK8 method. Ginsenoside Rg1 significantly reduced the Cx43 phosphorylation level. In addition, ginsenoside Rg1 significantly increased the astrocytes viability in hippocampus and prefrontal cortex compared to CORT-alone treatment group. The protective effects of ginsenoside Rg1 on hippocampal astrocytes can be reversed by Src inhibitor (PP2), p38 inhibitor (SB203580) and Akt inhibitors (BAY1125976). The protective effects of ginsenoside Rg1 on astrocytes in pretrontal cortex can only be reversed by Src inhibitor (PP2) and Akt inhibitor (BAY1125976). In conclusion, ginsenoside Rg1 significantly alleviates CORT-induced the decrease in the activity of protein Cx43 via activating Src, p38 and Akt protein kinases. The effect is different between hippocampus and prefrontal cortex.

Keywords: ginsenosides Rg1; astrocytes; connexins; protein kinase

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S1.7

Elevated plasma levels of homocysteine and neurologic disorders

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Homocysteine (Hcy) is a sulfur-containing amino acid that is generated during methionine metabolism. It has a physiologic role in DNA metabolism via methylation, a process governed by the presentation of folate, and vitamins B6 and B12. Physiologic Hcy levels are determined primarily by dietary intake and vitamin status. Elevated plasma levels of Hcy (eHcy) can be caused by deficiency of either vitamin B12 or folate, or a combination thereof. Certain genetic factors also cause eHcy, such as C667T substitution of the gene encoding methylenetetrahydrofolate reductase. eHcy has been observed in several medical conditions, such as cardiovascular disorders, atherosclerosis, myocardial infarction, stroke, minimal cognitive impairment, dementia, Parkinson's disease, multiple sclerosis, epilepsy, eclampsia, and peripheral neuropathy. There is evidence from laboratory and clinical studies that Hcy, and especially eHcy, exerts direct toxic effects on both the vascular and nervous systems. My talk will be focused on the possible roles of eHcy relevant to various neurologic disorders.

S1.8

TRPM2 channel function requires tyrosine phosphorylation by Fyn kinase

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TRPM2 is a Ca^{2+} permeable non-selective cation channel gated by oxidative/nitrosative stress via production of ADP-ribose, an intracellular TRPM2 agonist. Our past findings have shown that TRPM2 is functionally coupled to voltage-gated Ca^{2+} channels and NMDA receptors, regulates GSK3β and the expression of AMPA receptor subunits and is required for the induction of hippocampal NMDAR-dependent LTD. More recently, we established that genetic deletion of TRPM2 prevents Alzheimer's disease pathology and cognitive deficits in Alzheimer model mice. Although TRPM2 function is potentiated by treatment of cultured neurons with Aβ, the mechanism responsible has not been identified. One candidate mechanism is via tyrosine phosphorylation. Indeed, TRPM2 is regulated by tyrosine phosphatases. Moreover, Aβ is known to promote Fyn activity. We now demonstrate that TRPM2 function is not only regulated, but requires Fyn. To begin with, intracellular application of Fyn (1 U/mL) potentiated TRPM2 currents in both HEK293 cells expressing TRPM2 and in primary hippocampal neurons. Interestingly, treatment with a Src family inhibitor (PP2) and more specifically with a specific Fyn inhibitory peptide, completely abrogated ADPR-evoked TRPM2 currents. This inhibition was not reversed with intracellular application of high concentrations of ADPR (10 mmol/L), suggesting that phosphorylation does not alter agonist binding. Immunoprecipitation of TRPM2 confirmed its tyrosine phosphorylation and association with Fyn. In light of evidence suggesting that Aβ can promote Fyn activity, linked to AD pathology, our data suggests that TRPM2 could represent a connecting point between Aβ and the downstream deleterious consequence of Fyn activity.

Keywords: TRPM2; Fyn; NMDA receptors; whole-cell recording

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S1.9

Rapid screening and predicting permeability of pinocembrin at blood-brain barrier *in vitro* transport model

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Pinocembrin (5,7-dihydroxyflavanone) is one of the flavonoids at the highest content in honeybee propolis. Recently, the accumulating evidences indicate that pinocembrin may be a promising candidate drug for ischemic stroke therapy by its neuroprotective effects. One of the prerequisite for treating central nervous system diseases is that the candidate drug is suitable for passing through blood-brain barrier (BBB). In present study, pinocembrin transport characteristics and influence of P-glycoprotein at BBB is further described. The transendothelial polarized transports of pinocembrin in bilateral directions either with or without CsA were investigated in cultured rat brain microvascular endothelial cells model. The transports of pinocembrin (50 μmol/L) in the presence or absence of CsA (50 μmol/L) were measured at 37°C periodically for 120 min. The P_{app} value of pinocembrin was calculated. The results showed that pinocembrin transport in the B→A direction was similar to that in the A→B direction. The P_{app} value of pinocembrin across the *in-vitro* BBB model was (25.39±4.02)×10^{-6} cm/s in the A→B direction. Moreover, in present of CsA, the P_{app} value of pinocembrin was increased to (29.19±4.45)×10^{-6} cm/s in the A→B direction (P<0.05). These data indicated that pinocembrin could easily pass through the BBB and P-glycoprotein might not be involved in the transport of pinocembrin at the BBB. The entrance into brain of pinocembrin might not be limited by BBB. Overall, the findings demonstrate passive transport manner may be the main manner for pinocembrin to pass through BBB. Furthermore, P-glycoprotein is likely to have little effect on pinocembrin transport process at BBB.

Keywords: pinocembrin; permeability; blood-brain barrier; transport model; *in vitro*
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S1.10

Regulation of mTORC1 signaling network underlying the pathophysiology and treatment of depression: new insights for rapid-acting antidepressants

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Depression is a devastating mental disorder and major depressive disorder afflicts about 16% of the world population at some point in their lives. Although the molecular mechanisms underlying depression are still largely unclear, previous studies suggested that modulators of mTORC1 signaling network would have beneficial neuroprotective and antidepressant effects. This article aims to (1) review research actuality of depression and antidepressants. (2) provide an overview of the architecture and biology of mTORC1 in brain. (3) summarize the recent advances of the role of mTORC1 signaling network in depression and the potential therapeutic strategies resulting from the modulation of this process. The upstream regulators of the mTORC1 signaling network for antidepressants include pathways involved in activation of AMPA receptor, PI3K-Akt, ERK-MAPKs, and CREB-BDNF; dysregulation of downstream targets of mTORC1 signaling network, such as synaptic protein synthesis, the phosphorylation of eEF2, and autophagy would be associated with stress and depression. (4) point towards future research directions. Faster-acting antidepressants are needed, particularly for suicide-risk patients for current therapies for depression. It has recently been shown that treatment with new agents, for example, ketamine or (2*R*, 6*R*)-HNK (the metabolism of ketamine) results in an improvement in depressive behaviors within hours and lasts up to two weeks of dosing patients who are resistant to typical antidepressants, which have limited efficacy and delayed response times of weeks to months. The prospects for next-generation rapid-acting antidepressants would focus on developing more selective AMPA receptor agonists that activate the mTORC1 signaling pathway free of ketamine's adverse effects.

Keywords: depression; antidepressant; mammalian Target of Rapamycin Complex 1 (mTORC1) signaling network; α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor; ketamine; brain-derived neurotrophic factor (BDNF); synaptic protein synthesis; eukaryotic elongation factor 2 (eEF2); autophagy

S1.11

Effects of chronic mild stress on behavioral and neurobiological parameters–role of glucocorticoid

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Major depression is thought to originate from maladaptation to adverse events, particularly when impairments occur in mood-related brain regions. Hypothalamus-pituitary-adrenal (HPA) axis is one of the major systems involved in physiological stress response. HPA axis dysfunction and high glucocorticoid concentrations play an important role in the pathogenesis of depression. In addition, astrocytic disability and dysfunction of neurotrophin brain-derived neurotrophin factor (BDNF) greatly influence the development of depression and anxiety disorders. Therefore, we investigated whether depressive-like and anxiety-like behaviors manifest in the absence of glucocorticoid production and circulation in adrenalectomized (ADX) rats after chronic mild stress (CMS) exposure and its potential molecular mechanisms. The results demonstrate that glucocorticoid-controlled rats showed anxiety-like behaviors but not depression-like behaviors after CMS. Molecular and cellular changes included the decreased BDNF in the hippocampus, astrocytic dysfunction with connexin43 (cx43) decreasing and abnormality in gap junction in prefrontal cortex (PFC). Interestingly, we did not find any changes in glucocorticoid receptor (GR) or its chaperone protein FK506 binding protein 51 (FKBP5) expression levels in the hippocampus or PFC in ADX rats subjected to CMS. In conclusion, the production and circulation of glucocorticoids are one of the contributing factors in the development of depression-like behaviors in response to CMS. In contrast, the effects of CMS on anxiety-like behaviors are independent of the presence of circulating glucocorticoids. Meanwhile, stress decreased GR expression and enhanced FKBP5 expression via higher glucocorticoids exposure. Gap junction dysfunction and changes in BDNF may be associated with anxiety-like behaviors.

Keywords: depression; chronic mild stress; GR; FKBP5; BDNF; Gap junction
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S1.12

Interventional effect of prostaglandin E1 on the expression of Apaf-1 and TLR4 in the rats with cerebral ischemia-reperfusion injury

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The present study aimed to observe the interventional effect of prostaglandin E1 on the expression of Apaf-1 and TLR4 in the rats with cerebral ischemia-reperfusion (CIR) injury. Thirty-two healthy adult male Wistar rats were randomly divided into sham operated group, CIR model group, and PGE1 pretreated groups (12 μg/kg; 24 μg/kg). Rat model of cerebral ischemia/reperfusion was established by bilateral common carotid artery ligation. The expression of Apaf-1 and TLR4 in hippocampus and epencephalon was detected by immunohistochemical staining method. After 20 min of ischemia and reperfusion for 6 h, the number of positive cells of Apaf-1 (hippocampus: 11.30±2.54 *vs* 0.87±0.78; epencephalon: 5.13±1.53 *vs* 0.67±0.43) and TLR4 (hippocampus: 11.70±1.60 *vs* 2.43±1.17; epencephalon: 9.83±2.82 *vs* 1.97±1.03) increased in CIR model group compared with sham operated group (P<0.05). Compared with CIR model group, the positive cell numbers of Apaf-1 were reduced dose-dependently in PGE1 pretreatment all group [hippocampus: 8.20±1.90 (12 μg/kg) and 4.07±1.39 (24 μg/kg); epencephalon: 4.30±1.53 (12 μg/kg) and 1.67±0.80 (24 μg/kg) and TLR4 (hippocampus: 8.93±2.86 (12 μg/kg) and 7.30±1.62 (24 μg/kg); epencephalon: 5.83±3.16 (12 μg/kg) and 3.97±1.33 (24 μg/kg), all P<0.05]. PGE1 had inhibitory effects on the expression of Apaf-1 and TLR4 in hippocampus and epencephalon of the rat with cerebral ischemia-reperfusion injury.

Keywords: prostaglandin E_1; rats; cerebral ischemia-reperfusion; apoptotic protease activating factor-1; Toll-like receptor 4

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S1.13

Rosuvastatin preconditioning inhibits inflammatory cytokines release in VSMCs of MCA after cerebral ischemia-reperfusion

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The present study aimed to observe the effect of rosuvastatin preconditioning on inflammatory cytokines- IL-1β and IL-6 release in vascular smooth muscle cells (VSMCs) after ischemia reperfusion. Thirty-six healthy SD rats were randomly assigned into three groups: sham operation group (sham group), focal cerebral ischemia-reperfusion group (model group) and rosuvastatin preconditioning group (test group). There were 12 rats in each group. At 24 h after middle cerebral artery occlusion (MCAO) for 2 h, the mRNA and protein expression of IL-1β and IL-6 release in VSMCs of middle cerebral artery were detected by real-time PCR and Western blot, respectively. At 24 h after MCAO for 2 h, the mRNA and protein expression of IL-1β and IL-6 were markedly up-regulated in rats of model group; rosuvastatin significantly inhibited over-expression of IL-lβ and IL-6 at the mRNA and protein levels. Rosuvastatin preconditioning can decrease the release of inflammatory cytokines IL-1 β and IL-6 in VSMCs of MCA, which is beneficial to reduce the ischemia-reperfusion injury in brain.

Keywords: rosuvastatin; inflammatory cytokines; IL-1β; IL-6; vascular smooth muscle cells; ischemia-reperfusion

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S1.14

Enhanced scratching elicited by a pruritogen and an algogen in a mouse model of contact hypersensitivity

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Chemical pruritogens and algogens evoke primarily itch and pain, respectively, when administered to the skin of healthy human subjects. However, the dominant sensory quality elicited by an algesic chemical stimulus may change in patients with chronic itch where bradykinin, elicits itch in addition to pain. Here we tested whether normally pruritic and algesic chemicals evoked abnormal itch- or pain-like behaviors in the mouse after the development of contact hypersensitivity (CHS), an animal model of allergic contact dermatitis. Mice previously sensitized to a hapten (squaric acid dibutylester) applied to the abdomen, exhibited spontaneous itch-like scratching and pain-like wiping directed to the site on the cheek of the CHS elicited by a subsequent challenge with the same hapten. In comparison with responses of control mice, CHS mice exhibited a significant increase in the scratching evoked by bovine adrenal medulla 8-22, a peptide that elicits a histamine-independent itch, but did not alter the scratching to histamine. Bradykinin, an algogen that elicited only wiping in control mice, additionally evoked significant scratching in CHS mice. Thus, within an area of CHS, histamine-independent itch is enhanced and chemically evoked pain is accompanied by itch.

Keywords: contact hypersensitivity; itch; pain; pruritogen; algogen

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S1.15

Protective effects of astaxanthin against oxygen and glucose deprivation damage via the PI3K/Akt/GSK3β/Nrf2 signaling pathway in SH-SY5Y cells

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The study aimed to observe the protective effects of astaxanthin (ATX) and its molecular mechanism in SH-SY5Y cells. The SH-SY5Y cells were pre-treated with ATX (5, 10, 20 and 40 μmol/L) for 24 h and were co-treated with OGD for 3 h. The cell viability was measured by MTT assay. ROS and mitochondrial membrane potential (Δψm) were measured. The levels of SOD and MDA were investigated. Cells different apoptosis states were observed with Annexin V-FITC/PI double-staining assay kit by flow cytometry. The expression of Bax, Bcl-2, caspase-3, Akt, p-Akt, GSK3β, p-GSK3β, Nrf2 and HO-1 were detected by Western blot. Pre-treatment with PI3K/Akt inhibitor LY294002 (10 μmol/L) for 1 h and GSK3β inhibitor LiCl (10 μmol/L) for 1 h was employed before ATX (20 μmol/L, 24 h) and OGD (3 h), and protein expressions of Akt, p-Akt, GSK3β, p-GSK3β, Nrf2 and HO-1 were analyzed by Western blot. ATX significantly decreased apoptotic rate, and attenuated OGD-mediated reactive oxygen species (ROS) production. In addition, ATX inhibited OGD-induced mitochondrial membrane potential and increased Bcl-2/Bax ratio. PI3K/Akt/GSK3β/Nrf2 signaling pathway in SH-SY5Y cells was activated. ATX and LiCl treatment increased the protein levels of p-Akt, p-GSK3β, nucleus Nrf2, and heme oxygenase 1 (HO-1), whereas these protein expression levels decreased by treatment with LY294002. We suggest that ATX confers neuroprotection against cerebral ischemia-induced apoptosis via the PI3K/Akt/GSK3β/Nrf2 signaling pathway *in vitro*.

Keywords: astaxanthin; SH-SY5Y; OGD; PI3K/Akt; GSK3β; Nrf2

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S1.16

In the dorsal raphe neucleus, the role of Ca^{2+}, PKC and CaMKII in sleep-wake regulation

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Dorsal raphe nucleus (DRN) is the largest single collection of neurons containing 5-HT in the entire brain and particularly attractive in a wide variety of complex physiological and behavioral processes, such as sleep–wake regulation. Calmodulin dependent kinase II (CaMKII) and PKC are important signal-transducing molecules activated by Ca^{2+}. Since the Ca^{2+} modulation in DRN plays an important role in sleep–wake regulation, it should be presumed that the intracellular CaMKII/PKC signaling in DRN may be involved in the regulation of sleep-wake. The polysomnogram consisting of EEG and EMG was recorded for analyzing sleep architecture. Immunohistochemisrty and Western-blotting methods were used in this study to investigate the roles of Ca^{2+}, CaMKII and PKC in sleep–wake regulation in rat DRN. Ca^{2+} in the DRN exert arousal effects by reducing the NREMs, SWS and REMs via up-regulating serotonergic functions and activating CaMKII-PKC. However, inhibition of PKC leads to significant promotion of total sleep time especially the NREM sleep, but there were no changes in sleep parameters after the inhibition of CaMKII by its inhibitor KN-93 in DRN. The molecular, pharmacological, and behavioral findings of this study demonstrate a novel wake promoting and sleep-suppressing role for the Ca^{2+}/CaMKII/PKC signaling pathway in DRN. Abnormalities in CaMKII are found in patients with several neurological disorders that are associated with disturbed sleep, such as schizophrenia, depression, and Alzheimer's disease. Several psychotropic drugs modulate CaMKII activity. In addition, PKC is a cellular target of most current mood stabilizing and anti-manic agents and involved in bipolar disorder. The data of the present study raise the question whether PKC or CaMKII modulations may also be effective on the sleep disorders or the mood disorders associated with sleep disorders.

Keywords: dorsal raphe nucleus; Ca^{2+}; PKC; CaMKII; sleep

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S1.17

Trehalose, an autophagy-enhancing disaccharide, has potential to be translated into a therapeutic for the treatment of Parkinson's disease

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Using an alpha-synuclein (aSYN) rat model of Parkinson's disease (PD), we evaluated three regimens of trehalose administration to identify an efficacious dosing regimen that can be used to clinically evaluate trehalose.

Thirty-four female, Sprague-Dawley rats (280–325 g) split into 5 groups received unilateral injections of AAV1/2 delivering A53T aSYN or empty vector (EV) into the substantia nigra (SN). Commencing on day of surgery and continuing for 6 weeks, rats received vehicle (sterile drinking water) or trehalose (2.67 $g \cdot kg^{-1} \cdot d^{-1}$) administered in the drinking water (2%, *w/v*), as three separate administrations 8 h apart (0.89 g/kg tid, *po*) or as a single administration (2.67 $g \cdot kg^{-1} \cdot d^{-1}$, *po*). Behaviour was assessed pre-surgery, 3 and 6 weeks post-surgery using the cylinder test of forelimb asymmetry. After 6 weeks the rats were killed and striatal tissue analysed for dopamine (HPLC) and aSYN (Western blotting) levels. The number of TH+ve cells in the SN was assessed stereologically.

Rats receiving A53T aSYN and vehicle exhibited increased forelimb asymmetry, reduced striatal dopamine and increased A53T aSYN load per TH+ve neuron cf. rats receiving EV. Administration of trehalose as a single oral administration (2.67 $g \cdot kg^{-1} \cdot d^{-1}$, *po*) reduced asymmetry to a level similar to rats receiving EV, increased striatal dopamine levels (by 54%) and reduced the A53T aSYN load per TH+ve neuron (by 41%) cf. rats receiving A53T aSYN alone. The same daily amount of trehalose administered as either 3 doses, 8 h apart or in the drinking water did not alter behaviour, striatal dopamine or A53T aSYN load per TH+ve neuron.

The pharmacokinetic (PK) profile of once-daily trehalose administration (2.67 $g \cdot kg^{-1} \cdot d^{-1}$, *po*) was then assessed. One hundred Sprague-Dawley rats (200–260 g) received trehalose (2.67 $g \cdot kg^{-1} \cdot d^{-1}$, oral gavage in sterile water) for 7 days. Groups of 5 rats were sacrificed at pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h post-dose on days 1 and 7. Plasma and brain samples were analysed for trehalose levels using a validated LC-MS/MS method and PK parameters calculated. On Day 1, the plasma T_{max}, C_{max}, $AUC_{0\text{-inf}}$, and $t_{1/2}$ were 0.25 h, 8.9 μg/mL, 11.1 h·μg/mL and 0.76 h, respectively. Brain levels of trehalose were approximately 1% of plasma levels. No accumulation of trehalose occurred between Day 1 and Day 7.

We demonstrated that once daily administration of trehalose was efficacious and defined the PK associated with efficacy. These PK parameters can be targeted in the clinical development of trehalose.

Keywords: Trehalose; Parkinson's disease; disease modification

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S1.18

Genetic knock-out of transient receptor potential melastatin 2 (TRPM2) channels is neuroprotective against hypoxic-ischemic brain injury in the neonatal mouse

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Hypoxic-ischemic encephalopathy (HIE) is a commonly occurring condition in neonates and a serious cause of neonatal deaths worldwide. Survivors of HIE face life-long neurological consequences, including motor challenges, cognitive and learning disabilities and seizures. Transient receptor potential melastatin 2 (TRPM2), a calcium-permeable ion channel, has been implicated in mediating ischemic brain damage in adult rodents. In this study, we investigated the role of TRPM2 channels in the mouse model of neonatal HIE. To assess morphological brain changes following injury, we used 2,3,5-triphenyltetrazolium chloride (TTC) staining, as well as whole brain imaging and Nissl staining. To assess functional sensorimotor recovery following injury, we performed a battery of strain- and sex-independent neurobehavioural tests. Finally, we evaluated the biochemical changes following injury using Western blot and immunohistochemistry. We found the infarct volumes to be significantly reduced and behavioral outcomes to be improved in $TRPM2^{+/-}$ and $TRPM2^{-/-}$ mice compared to wildtype littermates. As well, TRPM2-null mice showed reduced dephosphorylation of GSK-3β and reduced activation of astrocytes and microglia in the brain hemisphere ipsilateral to the occluded artery, unlike wildtype mice. Our results suggest that genetic knock-out of TRPM2 channels is neuroprotective in a mouse model of neonatal HIE.

Keywords: TRPM2; hypoxic-ischemic brain injury; GSK-3β; neuroprotection

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S1.19

Protective effect of glibenclamide in experimental ischemic stroke

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Brain edema plays an important role in cerebral ischemic pathogenesis and indicates a worse result of ischemic stroke. Glibenclamide has gained attention for its prominent anti-edema property, but up to now little is known about how glibenclamide treatment alleviated brain edema in acute ischemic stroke. The aim of this study was to evaluate the potential neuroprotective role of glibenclamide in blood brain barrier (BBB) protection in cerebral ischemic injury, and investigate whether glibenclamide modulated inflammatory responses after stroke. In the OGD endothelial cells model, glibenclamide (6 μmol/L) was administered 4.5 h after OGD onset. Cell viability, apoptosis, claudin 5 expression was evaluated. Focal cerebral ischemia in male SD rats was induced by transient middle cerebral artery occlusion. Glibenclamide (10 μg/kg) was injected intraperitoneally 5 h after ischemia onset. Neurological deficits, infarct volume, brain edema, blood–brain barrier (BBB) extravasation were evaluated at 24 h after stroke. Inflammatory mediators were also detected 8 h after stroke. Compared with vehicle group, 6 μmol/L glibenclamide significantly improved endothelial cell viability, alleviated cell apoptosis and increased claudin 5 expression level. Compared with vehicle group, 10 μg/kg glibenclamide significantly improved neurological deficits, decreased infarct and edema volume at 24 h after transient ischemic stroke. Our data also showed that glibenclamide effectively alleviated leakage of Evans blue and IgG, up-regulated tight junction's protein, such as claudin 5, occludin expression in ischemic brain, and inhibited the over-expressed MMP-9 in plasma. In addition, glibenclamide reduced the activation of microglia, decreased the pro-inflammatory mediators' expression, including phosphorylation NF-κB, iNOS, Cox-2 and TNF-α in the acute stage in ischemic cortex which may be detrimental to BBB. These results suggested that glibenclamide effectively protected brain BBB against ischemic damage by decreasing endothelial cells apoptosis, ameliorating tight junction proteins loss, which might partly due to its function on mitigating inflammatory responses in the acute stage of ischemic stroke.