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http://dx.doi.org/10.1016/j.imr.2015.04.072

P1.066

Acupuncture suppresses stress-induced neuroinflammation in the rat hypothalamus

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Purpose: Acupuncture has been used to relieve stress. Although recent studies have shown that acupuncture can reduce stress, its mechanism remains unclear. The present study investigated the effect of acupuncture on immobilization stress- induced neuroinflammation in the rat hypothalamus.

Methods: Rats were immobilized for 60 min. Manual acupuncture was performed at HT7 during the immobilization period. Gene expression of several pro-inflammatory factors and serum level of stress hormone and PGE2 were evaluated using real-time PCR and ELISA, respectively.

Results: The stress-alleviating effect of acupuncture was confirmed by inhibiting hypothalamic CRF mRNA expressions as well as serum corticosterone levels in response to immobilization stress. The mRNA expression of pro-inflammatory mediators including TNF-α and IL-1β in the hypothalamus increased significantly after immobilization stress. Acupuncture treatment at HT7 during the immobilization significantly suppressed the immobilization stress-induced increase of pro-inflammatory mediators in the hypothalamus. Also, acupuncture inhibited significant increases in hypothalamic COX-2 mRNA as well as serum PGE2 levels in response to immobilization stress.

Conclusion: These data clearly suggest a stress-relieving effect of acupuncture and the inhibition of neuroinflammation as a possible action mechanism for its anti-stress effect.

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http://dx.doi.org/10.1016/j.imr.2015.04.073

P1.067

Effect of acupuncture stimulation on scopolamine-induced impairment of spatial working memory via activation of cholinergic system and regulation of BDNF and CREB expressions in rats

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Purpose: Acupuncture is an alternative therapy that is widely used to treat various neurodegenerative diseases and effectively improve cognitive and memory impairment. The aim of this study was to examine whether acupuncture stimulation at the Baihui (GV20) acupoint improves memory defects caused by scopolamine (SCO) administration in rats. We also investigated the effects of acupuncture stimulation at GV20 on the cholinergic system as well as the expression of brain- derived neurotrophic factor (BDNF) and cAMP-response element-binding protein (CREB) in the hippocampus.

Methods: SCO (2 mg/kg, i.p.) was administered to male rats once daily for 14 days. Acupuncture stimulation at GV20 was performed for 5 min before SCO injection. After inducing cognitive impairment via SCO administration, we conducted a passive avoidance test (PAT) and the Morris water maze (MWM) test to assess behavior.

Results: Acupuncture stimulation at GV20 improved memory impairment as measured by the PAT and reduced the escape latency for finding the platform in the MWM test. Acupuncture stimulation at GV20 significantly alleviated memory- associated decreases in the levels of choline acetyltransferase (ChAT), BDNF and CREB proteins in the hippocampus. Additionally, acupuncture stimulation at GV20 significantly restored the expression of choline transporter 1 (CHT1), vesicular acetylcholine transporter (VAChT), BDNF and CREB mRNA in the hippocampus. These results demonstrate that acupuncture stimulation at GV20 exerts significant neuroprotective effects against SCO-induced neuronal impairment and memory dysfunction in rats.

Conclusion: These findings suggest that acupuncture stimulation at GV20 might be useful in various neurodegenerative diseases to improve cognitive functioning via stimulating cholinergic enzyme activities and regulating BDNF and CREB expression in the brain.

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http://dx.doi.org/10.1016/j.imr.2015.04.074

P1.068

Do acupuncture needle size and needling depth matter? A laser Doppler imaging study

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Purpose: Acupuncture needle size and needling depth are considered important factors which may influence the specific treatment effects of acupuncture, but few studies have investigated related physiological changes. We investigated the impact of acupuncture needle size and needling depth on microperfusion.

Methods: A randomized, crossover experiment was performed on 44 healthy volunteers after ethics committee approval in KyungHee University, Seoul, Korea. They were randomly allocated to 4 acupuncture interventions: Deep needling with Thick needle (DT, 0.40X40 mm, 1.5 cm depth), Deep needling with Piliform needle (DP, 0.12X40 mm, 1.5 cm depth), Superficial needling with Piliform needle (SP, 0.12X40 mm, 2 mm depth), and Superficial needling with Thick needle (ST, 0.40X40 mm, 2 mm depth). Each participant received all 4 interventions, with each acupuncture

intervention followed by 3 to 7 days of washout period. Acupuncture in all groups was implemented at left LI10 with manipulation after insertion, 5 min, 10 min after insertion followed by needle removal. Microcirculation by laser Doppler perfusion imaging (LDPI) was measured 5 minutes before acupuncture till 10 minutes after removal of acupuncture. A repeated measures ANOVA was used for statistical analysis.

Results: In each group, microperfusion increased by needle insertion was maintained till after needle removal, and different needle size induced different blood perfusion changes, with the increase in ST group significantly higher than SP, DT and DP groups (repeated measures ANOVA, $P < 0.0001$), but there was no significant difference by needling depth.

Conclusion: ST intervention induced the greatest blood perfusion changes compared with other interventions, but the impact of different needling depths was not clear with blood perfusion changes. Studies investigating other physiological changes than blood perfusion by different acupuncture needle sizes and needling depths are needed.

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http://dx.doi.org/10.1016/j.imr.2015.04.075

P1.069

Indirubin-3'-monoxime inhibits platelet activation through suppression of glycoprotein VI-mediated signal transduction and a possible role for ERK

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Purpose: Indirubin-3'-monoxime (I3O), an active ingredient of Danggui Longhui Wan, exhibit antithrombotic activity through antiplatelet activity. Hence, we investigated the antiplatelet activity of I3O and the underlying mechanisms, focusing on the possible involvement of phospholipase Cγ2 (PLCγ2) and extracellular signal-regulated kinase (ERK) 1/2.

Methods: To identify the antithrombotic activity of I3O, we investigated using FeCl3-induced thrombus formation model, and platelet aggregation and coagulation ex vivo. In addition, the mechanism by which I3O is mediated the antithrombotic activity was assessed by determining platelet aggregation, immunoblotting, adenylyl cyclase activity, arachidonic acid (AA) liberation, and AA-metabolites conversion.

Results: In a rat carotid artery injury model, oral administration (20 mg/kg/day) of I3O for 3 days significantly prolonged occlusion time, and ADP- and collagen-induced platelet aggregation, comparable with aspirin. In washed platelets in vitro, I3O potently inhibited collagen-induced platelet aggregation by suppression of PLCγ2 phosphorylation, consistent with the blockade of diacylglycerol (DAG) and AA formation, P-selectin secretion and the production of thromboxane B2 (TXB2). As expected, platelet aggregation induced by phorbol-12-myristate 13-acetate (PMA), a protein kinase C (PKC) activator, was inhibited by I3O. Both I3O and U0126 markedly reduced collagen-induced phosphorylation of ERK1/2 and p47. Besides, I3O generally suppressed phosphorylation of JNK, p38, GSK-3β, and AKT. Hence, we investigated to identify the effect of I3O in collagen receptor level; as a result, I3O concentration-dependently showed the inhibition pattern in immunofluorescence assay of glycoprotein VI (GPVI), as a collagen receptor. Moreover, I3O not only inhibited the phosphorylation of the tyrosine kinase Syk of GPVI but also suppressed the phosphorylation of PLCγ2 and ERK1/2 stimulated by convulxin, as a specific stimulator.

Conclusion: Our results indicate that an antiplatelet effect of I3O is due to the suppression of GPVI-mediated signal transduction. In collagen-stimulated platelet activation, ERK1/2 phosphorylation is an adenylyl cyclase-dependent pathway through modulation of PKC-p47 signaling and COX-1-mediated AA metabolic pathways.

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http://dx.doi.org/10.1016/j.imr.2015.04.076

P1.071

Inhibitory effects of berberine on osteoclast differentiation

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Purpose: Osteoporosis is a bone desease that results from imbalance between new bone formation and bone resorption leading to bone loss and is especially troublesome for postmenopausal females who suffer from estrogen deficiency. Thus, the purpose of this study was to investigate new treatment for osteoporosis.

Methods: We evaluate the effects of berberine on receptor activator of nuclear factor-kB ligand (RANKL)-induced osteoclast differentiation from bone marrow-derived macrophages(BMMs) and performed cytotoxicity assay and western blot analysis. The mRNA expression levels of the indicated genes were analyzed by real-time PCR.

Results: We found that Berberine inhibits RANKL-induced osteoclast differentiation in a dose-dependent manner without affecting cytotoxicity. The mRNA expression of c-Fos, nuclear factor of activated T cells cytoplasmic 1 (NFATc1), tartrate-resistant acid phosphatase (TRAP), and osteoclast-associated receptor (OSCAR) was considerably inhibited by berberine treatment. berberine inhibited RANKL-mediated c-Fos and NFATc1 expression in a dose- dependent manner.

Conclusion: In this study, we identified that berberine was the efficient inhibitor of RANKL-induced osteoclast differentiation. Our results suggest that berberine may be useful in the prevention of osteoporosis.

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http://dx.doi.org/10.1016/j.imr.2015.04.078