P1.007

The antidepressant effects of electro-acupuncture on the associated protein of hippocampal neurons filtered by biotin label-based antibody array

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**Purpose:** This study aimed on the associated protein of hippocampal neurons in rats with depression, filtering out the possible target of acupuncture by biotin label-based antibody array.

**Methods:** 1. Established model: 30 SD rats were randomly divided into 3 groups: control group, model group, EA group. In addition to the control group, the remaining two groups were modeling 28 days by chronic unpredictable mild stress combined with solitary raising methods to establish the rat depression model. 2. Interventions: EA was given at points Baihui (Du 20), Yintang (Extra) for twenty minutes (intermittent wave, 30 min) 1 hour before the modeling. 3. Behavioral detection: Behavioral tests were given to evaluate if the model is successful. 4. Index detection: Using biotin label-based antibody array to detect the protein expression of hippocampal neurons in rats with depression.

**Results:** 1. Effects on rat behavior: After 28 days, compared with the control group, behaviors were all significantly reduced. Compared with the model group, weight of EA group was significantly increased, sugar intake were significantly higher, crossing number of grid were significantly increased, standing times were also. 2. Expression of proteins in neurons: compare to the control group, the protein expression of RAGE and Activin A in the model group are upregulation (fold change = 1.356,1.240). Compared to the model group, RAGE and Activin A expression are downregulation in EA group (fold change =0.617, 0.624). Expression of proteins in astrocytes: the protein expression of CNTFa and EGFR in model group are upregulation to the control one (fold change=1.503,1.373). But they are downregulation in EA group to the model group (fold change=0.728,0.505).

**Conclusion:** 1. EA may can improve behavioral abnormalities in rats with depression. 2. Expression of RAGE, ActivinA, CNTFRA, EGFR in model group have the increasing trend, and EA may can reduce this trend. 3. This study filtered out some possible targets of acupuncture.

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P1.008

Hippocampal memory enhancing activity of pine needle in scopolamine-induced mouse model

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**Purpose:** We evaluated the neuropharmacological effects of 30% ethanolic pine needle extract (PNE) on memory impairment caused by scopolamine injection in mice hippocampus.

**Methods:** C57BL/6N male mice (12 weeks old) were orally pretreated with PNE (25, 50, or 100 mg/kg) or tacrine (10 mg/kg) for 7 days, and scopolamine (2 mg/kg) was injected intraperitoneally. In order to evaluate memory function, the Morris water maze task was performed for 5 days consecutively. Oxidant-antioxidant balance, acetylcholinesterase (AChE) activity, neurogenesis and their connecting pathway were determined in hippocampal tissues and/or sera.

**Results:** Scopolamine appropriately increased the escape latency and cumulative path-length but decreases the time spent in target quadrant, which were ameliorated by pretreatment with PNE. Pretreatment with PNE attenuated the increased level of reactive oxygen species, malondialdehyde and AChE activity induced by scopolamine injection. Depletion of antioxidant capacities in hippocampus were also recovered by pretreatment with PNE. Consistent with above results, 4HNE (4-Hydroxynonenal)-positive stained cells were ameliorated in mice hippocampus pretreated with PNE. Pretreatment with PNE moderately increased the number of proliferating cells and immature neurons against suppression of neurogenesis by scopolamine in subgranular zone, which was confirmed by ki67- and DCX-positive stained cells. The brain-derived neurotrophic factor (BDNF) and phosphorylated cAMP response element-binding protein (pCREB) were facilitated for improving memory function by PNE pretreatment, which was confirmed by protein and gene expression results.

**Conclusion:** These findings suggest that PNE could be a potent neuropharmacological drug against amnesia, and its possible mechanism might be modulating cholinergic activity via CREB-BDNF pathway.

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P1.009

Anti-adipogenic and Antioxidant Effects of The Traditional Korean Herbal Formula KE-06: An in vitro Study

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**Purpose:** KE-06 has traditionally been used to treat chronic gastritis, gastric ulcers, gastroptosis, indigestion, diarrhea,
and emesis. In the present study, we investigated the anti-obesity effects and antioxidant activity of KE-06.

**Methods:** 3T3-L1 preadipocytes were differentiated into adipocytes with or without KE-06. After differentiation, we measured Oil Red O staining, glycerol-3-phosphate dehydrogenase (GPDH) activity and leptin production in 3T3-L1 adipocytes. In addition, we analyzed its effect on scavenging activities of 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2’-diphenyl-1-picrylhydrazyl (DPPH) radicals in vitro systems. Its effect on low-density lipoprotein (LDL) oxidation was assessed by measuring production of malondialdehyde (MDA).

**Results:** KE-06 significantly inhibited lipid accumulation and triglyceride production, and mediated GPDH, a major enzyme in the process of adipogenesis. Consistent with this, KE-06 stimulation significantly decreased the amount of leptin in 3T3-L1 adipose cells. Furthermore, KE-06 enhanced the scavenging activities on ABTS and DPPH radicals. The generation of MDA during LDL oxidation was significantly reduced by KE-06 treatment.

**Conclusion:** Overall, our findings suggest that KE-06 has the potential for anti-adipogenic activity and antioxidant properties.

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**P1.010**

**Development of Anti-hepatofibrotic Herbal Drug (CGX)**

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**Purpose:** Liver fibrosis is the key pathological change which is arisen by most of chronic hepatic injuries. The progress of hepatic fibrosis determines the clinical outcome of patients, but no therapeutics for the disease exists yet. The objective of the present study is to present the overall status for CGX development regarding its clinical backgrounds, pharmacological studies in animal models, and current process of randomized clinical trial.

**Methods:** CGX has been used for patients suffering various liver diseases, including chronic viral hepatitis and alcoholic liver disorders. The safety study for CGX using rats and beagle dogs, and pharmacological actions in animal models using chemicals (CCl4, DMN, or TAA), chronic alcohol consumption, choline-deficient (MCD) diet, and bile duct ligation (BDL) were presented respectively. The objective of the present study is to present the overall status for CGX development regarding its clinical backgrounds, pharmacological studies in animal models, and current process of randomized clinical trial.

**Results:** CGX is a modification of a traditional Korean herbal medicine, which is under clinical trial phase III for hepatofibrosis therapeutic effect. The main mechanisms of CGX related to anti-hepatofibrotic effects involve the inhibition of hepatic stellate cells producing extracellular matrix (ECM), down-regulation of pro-fibrogenic cytokines (TGF-β, PDGF, CTGF), and modulation of oxidative stressors and enhancements of antioxidant components. In addition, microarray experiment revealed the regulative action of CGX on VEGF gene expression.

**Conclusion:** Various animal data strongly expected that multi-sites clinical trial evidences the fibro-therapeutic effects in patients with chronic viral or alcoholic liver diseases.

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**P1.011**

**Anti-tumor activity of Gleditsia sinensis thorns targeting angiogenesis**

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**Purpose:** Gleditsia sinensis thorns have been used in Korean medicine to treat diverse diseases including thrombosis and relieve symptoms similar to cancer. The present study aims to (1) determine the anti-angiogenic effects of the ethanol extract of Gleditsia sinensis thorns (EEGS) in vitro and in vivo, (2) evaluate anti-tumor potential in vivo, (3) identify the active constituent of EEGS, and (4) understand its underlining mechanism.

**Methods:** EEGS was prepared by maceration of dried powder of Gleditsia sinensis thorns in 80% EtOH. Anti-angiogenic effects of EEGS were determined in vitro by quantifying HUVEC-mediated cell migration and tube formation, and in vivo by measuring new blood vessel formation into the pro-angiogenic factors-imbedded matrigel. Anti-tumor potential of EEGS was evaluated using a tumor-xenografted mouse model. Isolation and identification of active constituent from EEGS were carried out by activity-guided fractionation and NMR-Mass spectroscopy, respectively. Alteration of gene expression following drug treatment was determined by conventional molecular biological methods.

**Results:** EEGS inhibited proliferation of HUVEC without affecting cell viability. Angiogenic properties of HUVEC, such as cell migration and tube formation, were significantly inhibited by EEGS. Formation of new blood vessels induced by pro-angiogenic factors and growth of xenografted tumors were suppressed by EEGS as determined by in vivo animal models. HPLC-NMR-Mass spectrometric analyses revealed that cytochalasin H is an active anti-angiogenic constituent of EEGS. Anti-angiogenic potential of EEGS and cytochalasin H was related with the reduced expression of pro-angiogenic factors, such as EDN1 and MMP2.

**Conclusion:** Taken together, our findings suggest that EEGS can inhibit angiogenesis as well as tumor growth by down-regulating expression of pro-angiogenic factors. Therefore, EEGS can be considered as a good starting material to develop a novel anti-cancer drug targeting angiogenesis. This study