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IL-01 Significance of double-blind, placebo-controlled clinical studies on herbal (Kampo) medicines

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To elucidate the efficacy of Choto-san (Diao-Teng-San) in comparison with a placebo, a double-blind study was carried out in 139 patients suffering from vascular dementia. Choto-san extract glanules (7.5 g/d) and a placebo, were given 3 times a day for 12 weeks, respectively. Informed consent was obtained from the patients and/or their families. Choto-san was statistically superior to the placebo in global improvement rating, utility rating, and global improvement ratings of subjective symptoms, psychiatric symptoms and disturbance in daily living activities. Such items as spontaneity of conversation, lack of facial expression, decline in simple mathematical ability, nocturnal delirium, sleep disturbance, hallucination or delusion, and putting on and taking off clothes were significantly improved at one or more evaluation points in those taking Choto-san compared to those taking the placebo. However, no significant difference between the Choto-san and placebo groups was observed in the global improvement rating of neurological symptoms at any of the evaluation points. There was no significant difference between the two groups in terms of the overall safety rating. These results suggest that Choto-san has a favorable effect on vascular dementia and can be recommended as a treatment for this condition. Double-blind, placebo-controlled studies are significant to evaluate the efficacy and safety of herbal medicines.

IL-02 Molecular physiology of volume-regulated chloride channels in heart

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Changes in cell volume are known to regulate chloride (Cl^-) channels in many cell types. To date at least two types of volume-regulated chloride channels have been described in cardiovascular system: the outward rectifier Cl^- channel (ORCC or Clvol) and the Cl^- inward rectifier (Clir). Clvol in the heart is characterized by activation by hypotonic cell swelling (Clswell), outward rectification, greater permeability to iodide than chloride, slow inactivation at positive potentials, and an intermediate (about 50 pS) unitary conductance. Basally active Cl^- channels (Clb), which share many properties of Clvol, have also been observed under isotonic conditions in cardiac and endothelial cells. The single channel underlying both Clb and Clswell is the same ORCC. The gene encoding Clvol in these cells may be ClC-3. However, the differences in pharmacology and modulation of Clvol in various cell types and species also point to the possibility of a heterogeneous family of multiple volume-regulated Cl^- channels. Clir has been recently discovered in mammalian cardiac cells in our laboratory. Clir may be encoded by ClC-2, another member of the ClC family. In hypertrophied heart Clvol is persistently activated. The molecular expression and functional distribution of volume-regulated Cl^- channels (ClC-2 and ClC-3) in cardiac cells, vascular smooth

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The drug was provided by Tianjin Zhong wei Pharmaceutical Company Ltd. Blood samples for pharmacokinetic analyses were collected pre-dose and then at 0.17, 0.33, 0.67, 1, 2, 3, 4, 6, 8, 12, 24, 30, 36, and 48 h after dosing for single dosage groups. For multiple dosage group, on d 5, d 6, and d 7 pre-dose and at 4 h after dosing and on d 8 the times for blood collection were the same as that of single dosage groups. HPLC was used to determine the blood concentration of ibudilast. **RESULTS:** The pharmacokinetic parameters of 5 mg and 10 mg groups were: T_{max} (1.6±1.2) h and (1.6±0.8) h, C_{max} (17±5) μg/L and (25±7) μg/L, AUC (103±24) μg· L^{-1}· h and (144±65) μg· L^{-1}· h, $T_{1/2}$ (5.4±2.1) h and (4.4±2.2) h, respectively. For 20 mg group: T_{max} (3.3±1.6) h, C_{max} (56±22) μg/L, AUC (623±185) μg· L^{-1}· h, $T_{1/2}$ (31±15) h. Following multiple dosing of 10 mg, steady state C_{max} was (45±18) μg/L and steady state C_{min} was (19±9) μg/L, the peak to trough fluctuation index (DF) was 85 %±9 %, and AUC_{ss} (768±340) μg· L^{-1}· h. Following the last dosing of 10 mg, the pharmacokinetic parameters were: T_{max} (2.7±1.6) h, C_{max} (48±21) μg/L, AUC (658±278) μg· L^{-1}· h, and $T_{1/2}$ (33±13) h. **CONCLUSION:** After multiple dosing of 10 mg ibudilast sustained release capsule, the peak to trough blood concentration fluctuation keeps stable, and the recommended dosage is 10 mg twice daily.

S-04 Gastric oxidative stress and hemorrhagic ulcer in *Salmonella typhimurium*-infected rats

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AIM: The infection of *Salmonella typhimurium* (*S typhimurium*) may lead to various organ diseases. This research was to propose that the infection of *S typhimurium* caused gastric oxidative stress and disruption of gastric mucosal barriers, and thus resulted in gastric hemorrhagic ulcer in rats. **METHODS:** Male pathogen-free Wistar rats were deprived of food for 24 h. Single injection of live culture of *S typhimurium* (1×10^6-1×10^9 CFU/rat in 1.0 mL of sterilized phosphate buffer saline (PBS) was challenged iv to rat 5 h after withdrawal of food. Age-matched control rats received PBS only. After gastric surgery and vagotomy, rat stomachs were irrigated for 3 h with either normal saline or a simulated rat gastric juice containing HCl 100 mmol/L, pepsin 600 mg/L, and NaCl 54 mmol/L. Gastric parameters, including mucosal glutathione and lipid peroxide generation as well as luminal hemoglobin content and mucosal damage were determined. **RESULTS:** The production of gastric hemorrhagic ulcer and various gatric parmeters was dependent on the concentration of *S typhimurium* injected. The maximal response was obtained at the concentration of 1×10^9 CFU/rat. The presence of luminal gastric juice produced gastric hemorrhagic damage. High relation of ulcer formation to mucosal lipid peroxide generation and glutathione levels was also observed in those *S typhimurium*-infected rats. This exacerbation of ulcerogenic parameters was dose-dependently inhibited by pretreatment of ofloxacin. Pretreatment of antioxidants, such as reduced glutathione, allopurinol and dimethylsulfoxide, also caused amelioration of gastric hemorrhagic damage in *S typhimurium*-infected rats. **CONCLUSION:** Infection of *S typhimurium* may produce gastric hemorrhage and mucosal ulceration via aggravation of gastric oxidative stress that can be effectively ameliorated by antioxidants or antibiotics.

S-05 Effects of prousion on scavenging free radicals

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AIM: To detect the effects of prousion on hydroxyl free radicals and superoxide free radicals *in vitro*. **METHODS:** Prousion, natural porous ore material with some biological functions found in Japan, Japan Patent Application Number: BFC4j (B2002-52397). Prousion-A, B, C (P-A, P-B, P-C), offered by Dr M Hashimoto, were the powders mixed of these porous ore in different proportions (grey powder, d ≤3 μm). The effects of prousion on hydroxyl free radical and superoxide free radicals were determined by the assays of deoxyribose and xanthine oxidase, respectively. Their direct effects on these free radicals were also performed with the Electron spin resonance (ESR). **RESULTS:** 1) IC_{50} for P-A, P-B, and P-C on scaveng-

ing hydroxyl free radicals by the assay of deoxyribose was 0.2209, 0.2962, and 0.2183 g/L, respectively, the order for their actions was C>A>B. The 95 % confidence limit was 0.0909-0.5367, 0.1302-0.6741 g/L, and 0.0895-0.5322 g/L, respectively. 2) The inhibition produced by P-A, P-B, and P-C at the concentration of 1.4 mg/L on hydroxide free radicals detected by ESR was 35.24 %, 36.92 %, and 55.14 %, respectively. This action was in a dose-dependent manner and the order for their actions was C>B≈A, confirming the results obtained by the assay of deoxyribose. 3) IC_{50} for P-A, P-B, and P-C on scavenging superoxide free radicals by the assay of xanthine oxidase was 0.4025, 0.3854, and 0.3626 g/L, respectively, the order was C>B>A. The 95 % confidence limit was 0.2065-0.7847, 0.2041-0.7276, and 0.1823-0.7213 g/L, respectively. 4) The inhibition by P-A, P-B, and P-C at the concentration of 0.34 mg/L on superoxide free radicals detected by ESR was 5.99 %, 9.15 %, and 9.15 %, respectively, also confirming the results of xanthine oxidase assay. **CONCLUSION:** Prousion has the properties scavenging hydroxide and superoxide free radicals.

S-06 Steroids modulate placental CRH gene transcriptional activity through cAMP regulatory element

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AIM: To explore the regulation of corticotropin-releasing hormone (CRH) gene expression by steroid hormones in placenta and its molecular mechanism. **METHODS:** Human primary cultured placental trophoblasts were transfected with CRH-luciferase reporter genes and treated with estrogen, progesterone, and their antagonists. **RESULTS:** Estradiol (E2) inhibited basal and cAMP-stimulated CRH promoter activity. A pure estrogen antagonist ICI182, 780 not only blocked repression of CRH gene expression by E2, but up-regulated CRH promoter activity. This effect appeared to occur specifically through ERα-mediated mechanism, as similar effects were found in the ERα over-expression trophoblasts. Through deletion and mutagenesis analyses we found that estradiol inhibition of the CRH gene required cAMP regulatory element (CRE). Progesterone (P4) treatment resulted in a decrease in CRH promoter activity. Addition of Ru486 or inhibition of endogenous P4 production by placental cells, led to an increase of promoter activity. It was also found that the repression of CRH gene expression by P4 also required CRE. This CRE sequence conferred E2 and P4 inhibitions upon a heterologous promoter (rabbit β-globin). **CONCLUSION:** Estrogen and progesterone play inhibitory roles in the regulation of CRH gene transcription in human placental trophoblasts. These effects occurred through CRE of CRH gene.

S-07 Soluble protein of a novel costimulatory molecule, Dectin-2, breaks ultraviolet B-induced immune tolerance in contact hypersensitivity in mice

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AIM: One of hazardous adverse effects of ultraviolet B light (UV) exposure to skin may be best exemplified with its immunosuppressive property on contact hypersensitivity (CHS) and the subsequent induction of tolerance. Bearing in mind thoughts that artificial break of impaired cutaneous immune response may lead to regain host's immune competence, we focused on a novel costimulatory molecule, Dectin (Dec)-2, shown to be selectively expressed on antigen presenting cells, such as epidermal Langerhans cells (LC). **METHODS & RESULTS:** Mice exposed to low doses of UV and sensitized thereafter with hapten, dinitrofluorobenzene (DNFB) through the exposed skin area showed markedly ameliorated CHS upon resensitization and rechallenge to the same animals. When soluble Dec-2 (sol-Dec2), an inhibitor of cognate Dec-2, was injected iv before resensitization, following rechallenge led to full occurrence of CHS, indicating that sol-Dec2 breaks UV-induced tolerance. This is the first demonstration that functional neutralization of costimulatory molecule restores impaired cutaneous immune response. To fur-

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