structures, which were barely observable in 1-weekold rats, were networked in specific areas of the brain, for example, the lateral lobes and the cerebella, in 4-week-old rats. In 7-week-old rats, those structures were found to have become larger and better networked. With phase contrast microscopy, we found that in 1-week-old rats, chromium-hematoxylin-stained granules were scattered in the same areas of the brain in which the network structures would later be observed in the 4- and 7-week-old rats. Such age-dependent network structures were examined by using optical and transmission electron microscopy, and the following results were obtained. The scattered granules fused into networks with increasing age. Cross-sections of the age-dependent network structures demonstrated heavily-stained basophilic substructures. Transmission electron microscopy revealed the basophilic substructures to be clusters with high electron densities consisting of nanosized particles. We report these data as evidence for the existence of age-dependent network structures in the dura mater, we discuss their putative functions of age-dependent network structures beyond the general concept of the dura mater as a supporting matrix.

Keywords: brain, chromium-hematoxylin staining, dura mater, Fascia, hormone, nerve regeneration, neural regeneration, primo vascular system

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Prolonged Repeated Acupuncture Stimulation Induces Habituation Effects in Pain-Related Brain Areas: An fMRI Study

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Abstract

Most previous studies of brain responses to acupuncture were designed to investigate the acupuncture instant effect while the cumulative effect that should be more important in clinical practice has seldom been discussed. In this study, the neural basis of the acupuncture cumulative effect was analyzed. For this experiment, forty healthy volunteers were recruited, in which more than 40 minutes of repeated acupuncture stimulation was implemented at acupoint *Zhusanli* (ST36). Three runs of acupuncture fMRI datasets were acquired, with each run consisting of two blocks of acupuncture stimulation. Besides general linear model (GLM) analysis, the cumulative effects of acupuncture were analyzed with analysis of covariance (ANCOVA) to find the association between the brain response and the cumulative duration of acupuncture stimulation in each stimulation block. The experimental results showed that the brain response in the initial stage was the strongest although the brain response to acupuncture was time-variant. In particular, the brain areas that were activated in the first block and the brain areas that demonstrated cumulative effects in the course of repeated acupuncture stimulation overlapped in the pain-related areas, including the bilateral middle cingulate cortex, the bilateral paracentral lobule, the SII, and the right thalamus. Furthermore, the cumulative effects demonstrated bimodal characteristics, i.e. the brain response was positive at the beginning, and became negative at the end. It was suggested that the cumulative effect of repeated acupuncture stimulation was consistent with the characteristic of habituation effects. This finding may explain the neurophysiologic mechanism underlying acupuncture analgesia.

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Single Intramuscular-dose Toxicity of Water soluble Carthmi-Flos Herbal Acupuncture (WCF) in Sprague-Dawley Rats

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Abstract

Objectives: This experiment was conducted to examine the toxicity of Water soluble Carthmi-Flos herbal acupuncture (WCF) by administering a single intramuscular dose of WCF in 6-week-old, male and female Sprague-Dawley rats and to find the lethality dose for WCF.

Methods: The experiment was conducted at Biotoxtech according to Good Laboratory Practices under a request by the Korean Pharmacopuncture Institute. This experiment was performed based on the testing standards of "Toxicity Test Standards for Drugs" by the Ministry of Food and Drug Safety. Subjects were divided into 4 groups: 1 control group in which normal saline was administered and 3 test groups in which 0.1, 0.5 or 1.0 mL of WCF was administered; a single intramuscular dose was injected into 5 males and 5 females in each group. General symptoms and body weights were observed/measured for 14 days after injection. At the end of the observation period, hematological and clinical chemistry tests were performed, followed by necropsy and histopathological examinations of the injected sections.
Results: No mortalities were observed in any group. Also, symptoms, body weight, hematology, clinical chemistry and necropsy were not affected. However, histopathological examination of the injected part in one female in the 1.0-mL group showed infiltration of mononuclear cells and a multi-nucleated giant cell around eosinophilic material.
Conclusion: Administration of single intramuscular doses of WCF in 3 groups of rats showed that the approximate lethal dose of WCF for all rats was in excess of 1.0 mL, as no mortalities were observed for injections up to and including 1.0 mL.
Keywords: *Carthamus tinctorious L.*, Cathami Semen, Carthmi-Flos herbal acupuncture, water soluble Carthmi-Flos herbal acupuncture, pharmacopuncture, intramuscular toxicity test

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Single Dose Toxicity of Chukyu (spine-healing) Pharmacopuncture Injection in the Muscle of Rats

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Abstract
Objectives: This study was performed to analyze the single dose toxicity of Chukyu (spine-healing) pharmacopuncture.
Methods: All experiments were conducted at the Biotoxtech, an institution authorized to perform non-clinical studies under the regulations of Good Laboratory Practice (GLP) regulations. Sprague-Dawley rats were chosen for the pilot study. Doses of Chukyu (spine-healing) pharmacopuncture, 0.1, 0.5 and 1.0 mL, were administered to the experimental groups, and a dose of normal saline solution, 1.0 mL, was administered to the control group. This study was conducted under the approval of the Institutional Animal Ethic Committee.
Results: No deaths or abnormalities occurred in any of the four groups. No significant changes in weight, hematological parameters or clinical chemistry between the control group and the experimental groups were observed. To check for abnormalities in organs and tissues, we used microscopy to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs or tissues except in one case, where interstitial infiltrating macrophages were found in one female rat in the 0.5-mL/animal experimental group.
Conclusion: The above findings suggest that treatment with Chukyu (spine-healing) pharmacopuncture is relatively safe. Further studies on this subject are needed to yield more concrete evidence.
Keywords: Chukyu (spine-healing) pharmacopuncture, toxicity test

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Isolation and Characterization of a 32-kDa Fibrinolytic Enzyme (FE-32kDa) from *Gloydius blomhoffii siniticus* Venom

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Abstract
Objectives: This study was undertaken to isolate a fibrinolytic enzyme from the snake venom of *Gloydius blomhoffii siniticus* and to investigate its enzymatic characteristics and hemorrhagic activity as a potential pharmacopuncture agent.
Methods: The fibrinolytic enzyme was isolated by using chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and fibrin plate assay. The characteristics of the enzyme were investigated using fibrin plate assay, protein hydrolysis analysis, and hemorrhage assay. Its amino acid composition was determined.
Results: The fibrinolytic enzyme with the molecular weight of 32kDa (FE-32kDa) from *Gloydius blomhoffii siniticus* showed a fibrin hydrolysis zone at the concentration of 0.2 mg/mL in the fibrin plate assay. The fibrin hydrolysis activity of the enzyme was inhibited completely by ethylenediaminetetraacetic acid (EDTA), ethyleneglycoltetraacetic acid (EGTA), and 1, 10-

phenanthroline, thiothreitol and cysteine, and partially by phenylmethanesulfonylfluoride (PMSF). Metal ions such as Fe^{2+} and Hg^{2+} inhibited the fibrin hydrolysis completely, but Zn^{2+} enhanced it. FE-32kDa hydrolyzed α-chain but did not hydrolyze β-chain and γ-chain of fibrinogen. High-molecular-weight polypeptides of gelatin were hydrolyzed partially into low-molecular-weight polypeptides, but the extent of hydrolysis was limited. FE-32kDa induced hemorrhage beneath back skin of mice at the dose of 2 μg.

Conclusions: FE-32kDa is a α-fibrin(ogen)olytic metalloprotease that requires Zn^{2+} for fibrinolytic activity and causes hemorrhage, suggesting that the enzyme is not appropriate for use as a clinical pharmacopuncture.

Keywords: fibrinolytic enzyme, metalloprotease, *Gloydius blomhoffii siniticus*, snake venom

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