TOXICOLOGIC/EXPERIMENTAL PATHOLOGY

A Novel and Effective Long-Acting Follicle-Stimulating Hormone Analog, LAPS-FSH

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Background: One of the disadvantages of follicle-stimulating hormone (FSH) is its extremely short half-life, which requires women to receive 1-2 injections per day for 8-12 days under most protocols. Therefore, we developed a novel long-acting FSH analog by conjugating recombinant human follicle-stimulating hormone (rhFSH) and the constant region of the human immunoglobulin G4 fragment via nonpeptidyl linkers. Methods: The efficacy of the FSH analog was evaluated in vitro by cAMP level assessments, and in vivo by pharmacokinetic studies, ovarian weight measurements and comparing these findings with other FSH analogs. After 7 days of treatment, the total number and the size of the antral and graafian follicles were compared in the control group, 6 µg/kg follitropin beta, 6, 12, and 42 µg/kg corifollitropin alpha, and 3, 6, and 12 µg/kg LAPS-FSH groups. Results: Elevated cAMP levels indicated that LAPS-FSH was biologically active and the PK data of LAPS-FSH showed slow elimination of LAPS-FSH. In addition, the rats treated with LAPS-FSH demonstrated higher ovarian weights and produced larger and more abundant follicles. These data demonstrate that LAPS-FSH promotes growth and inhibits atresia of the ovarian follicle compared to other FSH drugs, indicating that LAPS-FSH enhances the efficacy and duration of treatment. Conclusions: Our novel long-acting FSH analog, LAPS-FSH, will reduce the number of injections, and also provide a higher chance of pregnancy in patients who are unresponsive to other drugs.

Key Words: Follicle stimulating hormone; LAPS-FSH; Ovulation; Infertility

Induction of Rat Liver Tumor Using the Sleeping Beauty Transposon and Electroporation

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Background: The sleeping beauty (SB) transposon system has been receiving much attention as a gene transfer method, as it is able to show permanent gene expression after insertion into the host chromosomes. However, it has been known to show low transposition frequency in higher eukaryotes, which limits its use in commonly-used mammalian species. Researchers have therefore focused on the modification of gene delivery and expression to overcome this property. In mouse liver, tumor induction using SB introduced by the hydrodynamic method has been successfully accomplished. Liver tumor in rat models using SB could also be of great use; however, dose of DNA, injection volume, rate of injection and achieving back pressure limit the use of the hydrodynamics-based gene delivery. Methods: We combined the electroporation, a relatively simple and easy gene delivery method, with the SB transposon system. After introduction of the oncogenes, c-Myc and HRAS, and shp53, tumors were developed in the liver and then phenotyped. Phenotyping methods included animal positron emission tomography, histopathology, immunohistochemistry, and quantitative real-time reverse transcription polymerase chain reaction. Results: The electroporation-enhanced SB transposon method can produce tumors at desired anatomical locations and in the desired species. The tumor phenotype was determined as a sarcomatoid carcinoma. Conclusions: This is the first demonstration of induction of tumor in the rat liver using the electroporation-enhanced SB transposon system. In addition, utilizing this method, it is possible to cause tumorigenesis in other large-size laboratory animals, which can be of great advantage. The present results indicate that the electroporation-enhanced SB transposon system can be used to produce tumors in genes and sites of desired animals.

Key Words: Sleeping beauty transposon; Electroporation; Liver neoplasms; Rat
HRAS oncogenes and shp53 was demonstrated by immunohistochemistry. **Conclusions:** Based on morphological characteristics and immunohistochemistry, we diagnosed this tumor as a sarcomatoid carcinoma. To the best of our knowledge, this study is the first report of the histological and immunohistochemical identification of subcutaneous tumors induced by sleeping beauty transposon system.

**Key Words:** Phenotyping; Sleeping beauty; Neoplasms; Oncogenes; Immunohistochemistry

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2-Year Carcinogenicity Study of CKD-501 by Oral Administration in SD Rats

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**Background:** The carcinogenicity of CKD-501, a peroxisome proliferator-activated receptor agonist, peroxisome proliferator-activated receptor agonist has been controversial due to its carcinogenic potential in rodents even though it is effective for diabetes. In order to investigate, 2-year carcinogenicity study in SD rat was conducted. **Methods:** Four week old male and female SD rats from the same colony were used in this study, and four groups using different doses of CKD-501 were assigned. 60 male and 60 female rats were distributed to each group. The male rats received 0, 0.03, 0.12, and 1.0 mg/kg/day while the female rats received 0, 0.03, 0.06, and 0.12 mg/kg/day for 94-101 weeks. CKD-501 was administered by the oral route. Observations such as clinical examinations, hematology and serum biochemistry, necropsy and histopathology were conducted. **Results:** Total mortality rate was 61% in males and 63% in females. The survival rate of the male 1.0 mg/kg/day group was 67%, which was tested statistically significant. Microscopic examinations demonstrated the following treatment related lesions: neoplastic lesions such as lipoma and liposarcoma in the female 0.12 mg/kg/day group, and non-neoplastic lesions such as bladder transitional cell hyperplasia in females and fat proliferation and bone marrow hypoplasia in both gender groups. No bladder tumors were observed. **Conclusions:** This two year carcinogenicity study demonstrates that CKD-501 caused lipoma and liposarcoma but did not produce bladder tumors.

**Key Words:** Carcinogenicity; Peroxisome proliferator-activated receptors; Rat; Lobeglitazone

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The Therapeutic Effects of Melittin on Propionibacterium acnes-Induced Inflammatory Responses In Vitro and In Vivo

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**Background:** Melittin is the main component in the venom of the honey bee (Apis mellifera). It has multiple effects that include anti-bacterial, anti-viral, and anti-inflammatory, in various cell types. However, the anti-inflammatory mechanisms of melittin have not been elucidated in the Propionibacterium acnes induced keratinocyte or inflammatory skin disease animal models. **Methods:** In this study we examine the effects of melittin on the production of inflammatory cytokines in heat-killed P. acnes-induced HaCaT cells. Subsequently, we examine the living P. acnes (1 × 10⁵ CFU) were intradermally injected into the ear of mice. **Results:** Heat-killed P. acnes treated keratinocytes were increased expression of pro-inflammatory cytokines and toll like receptor 2. However, melittin treatment significantly suppressed the expression of these cytokines through the regulation of the nuclear factor-kB and mitogen-activated protein kinase signaling pathways. Living P. acnes injected ears showed cutaneous erythema, swelling, and granulomatous response at 24 hours after injection. However, melittin-treated ears showed markedly reduced swelling and granulomatous responses compared with the ears injected with only living P. acnes. **Conclusions:** These results demonstrate the feasibility of applying melittin for the prevention of the progression of inflammatory skin diseases induced by P. acnes in vitro and in vivo inflammatory models.

**Key Words:** Melittin; Propionibacterium acnes; Inflammation; Skin diseases

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Inhibitory Effect of Bee Venom on Compound 48/80-Induced Allergic Symptoms

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**Background:** The itch-scratch cycle aggravates chronic inflammatory skin diseases. Honeybee (Apis mellifera) venom (BV) has been traditionally used for the treatment of pain and inflammation in various chronic diseases. However, the precise mechanism of BV in scratching behavior is not still cleared. **Methods:** In order to evaluate the effect of BV, we used a mouse skin scratching model induced by compound 48/80. Intraperitoneal administration of BV (0.01, 0.1 mg/kg) was inhibited compound 48/80-induced scratching behaviors. **Results:** The anti-scratching behavior effect of BV is in proportional to their vascular permeability effects. Treatment of bee venom also inhibited the degranulation of mast cells and the production of pro-inflammatory cytokines vascular permeability in compound 48/80-treated skin tissues. **Conclusions:** Based on these results, BV may improve pruritus by inhibiting the mast cell degranulation and proinflammatory cytokine expression.

**Key Words:** Chronic inflammatory skin diseases; Bee venom; Anti-scratching behavior; Mast cell degranulation
The Papaya Fruit Effect in Infertile Wistar Rat’s Seminal Vesicles Induced by Paracetamol

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Background: Oxidative stress is a part of the overall etiology of infertility in men. Paracetamol is one of the drugs that has the oxidative stress effect. One organ that could undergo changes because of the oxidative stress is the seminal vesicle. Red papaya fruit (Carica papaya L.) with lycopene can maintain the health of seminal vesicle. This study aim to determine the effect of red papaya on microscopic feature of the seminal vesicles with paracetamol exposure. Methods: Research subject were 28 male Wistar rats divided equally into 4 groups. The control group was given water, the papaya group was given 5 g of papaya juice, the paracetamol group was given 72 mg of paracetamol, and the paracetamol-papaya group was given 5 g of papaya juices along with 72 mg of paracetamol. Treatment was done for 28 days. Results: On the microscopic examination, the percentage of normal epithelial cell in paracetamol-papaya group had decreased significantly compared to the control group (p = 0.000) but without any separation of the epithelium as in paracetamol group which epithelial percentage also decreased significantly. For the percentage of secretions and the number of lumens, only paracetamol group had significantly decreased compared to control (p = 0.000). In measuring the thickness of the tunica muscular, only papaya group has significant improvement compared to control (p = 0.001). Conclusions: There are protective effects of papaya fruit against paracetamol’s oxidative stress on microscopic feature of the seminal vesicles.

Key Words: Oxidative stress; Papaya fruit; Seminal vesicles

Klotho and Peroxisome Proliferator-Activated Receptor Agonists Show Synergistic Anti-fibrotic Effects on the Mouse Glomerular Mesangial Cells Stimulated by Transforming Growth Factor β

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Background: Klotho mainly expressed in the renal distal tubule is a trans-membrane protein and acts as co-receptor for fibroblast-growth factor 23. It is originally reported as an anti-aging protein and also correlates with an anti-inflammatory and anti-fibrotic function. Klotho is a target molecule of peroxisome proliferator-activated receptor γ (PPAR γ) which also has an anti-inflammatory and anti-fibrotic effects. In this study, we investigated whether Klotho and PPAR agonist had anti-fibrotic effects and interactions of these two molecules on the transforming growth factor β (TGFβ) stimulated glomerular mesangial cells. Methods: Mouse glomerular mesangial cells stimulated by TGFβ were treated with AZ242 (PPARα/γ dual agonist), pioglitazone (PPAR γ agonist), Klotho, AZ242 + Klotho, pioglitazone + Klotho for 4 hours. Western blots for TGFβ1, PAI-1, phosphorylated Smad2 (pSmad2), PPAR γ, and Klotho were performed. Results: Klotho and PPAR γ protein expression were increased in all treated groups compared to TGFβ stimulated group (control group). PAI-1 expression was decreased in the AZ242 stimulated group and pioglitazone + Klotho treated group compared to control group. However, other treated groups showed no difference compared to control group. pSmad2 expression was markedly decreased in the AZ242 + Klotho treated group. Other treated groups revealed no difference of pSmad 2 expression compared to control group. TGFβ expression was decreased in the AZ242 + Klotho group and pioglitazone + Klotho group. Conclusions: Our results suggest that Klotho and PPAR agonists have interaction each other and shows synergistic anti-fibrotic effects on the TGFβ stimulated mouse glomerular mesangial cells.

Key Words: Klotho protein; PPAR agonist; Synergistic effect; Anti-fibrotic effect; Mesangial cell

Ethylmercury Induces Estrogen Receptor Stress and Mitochondrial Dysfunction Mediated Autophagy in Renal Proximal Tubular Cells

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Background: Mercury (MC) is one of the most important environmental and industrial pollutants throughout the world. The renal proximal tubular cell is one of representative target sites for MC toxicity. However, the molecular mechanism of MC induced tubular cell toxicity remains poorly understood. We investigated the role of mitochondrial dysfunction and endoplasmic reticulum (ER) stress on the ethylmercury chloride (EMC) induced cytotoxicity in proximal tubular cells. Methods: EMC was treated with 2, 5 and 10 μM to HK-2 cells, respectively. And in mice, 1, 2, 5, and 10 mg/kg of body weight was administrated intraperitoneally. Results: EMC induced reactive oxygen species production, changed the mRNA expression of MT-I and Hic-5 and caused the significant reduction of mitochondrial membrane potential. The gene expression and protein of UPR target genes, GRP78, CHOP and the levels of spliced XBP-1 mRNA, phospho-eIF2 protein and cytosolic Ca2+ increased. After the exposure of 2 μM EMC to cells, LC3II formation was time-dependently increased, however cleavage of caspase-9, caspase-12, and poly(ADP-ribose) polymerase were weakly activated. Co-treatment of N-acetyl cysteine (antioxidant) or phenylbutyric acid (an ER chemical chaperone) attenuated EMC induced ER stress or oxidative stress and protected against autophagy in cells. In mice with 5 or 10 mg/kg, proximal tubular cell necrosis esp. in the outer medulla was increased dose-dependently. EMC induced ER stress related proteins and autophagy treated 1 or 2 mg/kg doses, which did not show any histopathologic changes. Conclusions: Crosstalk between ER stress and mitochondrial dysfunction pathway mediates EMC induced autophagy in renal proximal tubular cells.

Key Words: Ethylmercury chloride; Toxicity; Mitochondrial dysfunction; Autophagy; Kidney tubules, proximal