2019-09-25 Class

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1. Addis Kassahun (DVM, MSc)

Title: Psoralea corylifolia extract induces vasodilation in rat arteries through both endothelium-dependent and -independent mechanisms involving inhibition of TRPC3 channel activity and elaboration of prostaglandin.

Journal: *Pharm Biol.* (2017)

Abstract

CONTEXT: Fructus Psoralea, Psoralea corylifolia L. (Leguminosae), has been widely used in traditional medicines for the treatment of dermatitis, leukoderma, asthma and osteoporosis.

OBJECTIVES: In this study, we sought to study mechanisms underlying the vasoactive properties of Psoralea corylifolia extract (PCE) and its active ingredients.

MATERIALS AND METHODS: To study mechanisms underlying the vasoactive properties of PCE prepared by extracting dried seeds of Psoralea corylifolia with 70% ethanol, isometric tension recordings of rat aortic rings and the ionic currents through TRPC3 (transient receptor potential canonical 3) channels were measured with the cumulative concentration (10-600 μg/mL) of PCE or its constituents.

RESULTS: Cumulative treatment with PCE caused the relaxation of pre-contracted aortic rings in the presence and absence of endothelium with EC_{50} values of 61.27 ± 3.11 and 211.13 ± 18.74 μg/mL, respectively. Pretreatment with inhibitors of nitric oxide (NO) synthase, guanylate cyclase, or cyclooxygenase and pyrazole 3, a selective TRPC3 channel blocker, significantly decreased PCE-induced vasorelaxation ($p < 0.01$). The PCE constituents, bakuchiol, isobavachalcone, isopsoralen and psoralen, inhibited hTRPC3 currents (inhibited by 40.6 ± 2.7, 27.1 ± 7.9, 35.1 ± 4.8 and 47.4 ± 3.9%, respectively). Furthermore, these constituents significantly relaxed pre-contracted aortic rings (EC_{50} 128.9, 4.5, 32.1 and 114.9 μg/mL, respectively).

DISCUSSION AND CONCLUSIONS: Taken together, our data indicate that the vasodilatory actions of PCE are dependent on endothelial NO/cGMP and also involved in prostaglandin production. PCE and its active constituents, bakuchiol, isobavachalcone, isopsoralen and psoralen, caused dose-dependent inhibition of TRPC3 channels, indicating that those ingredients attenuate Phe-induced vasoconstriction.

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2. HAN XUEHAO (한설호)

Title: P2Y6 Receptor-Mediated Spinal Microglial Activation in Neuropathic Pain

Journal: *Pain Research and Managemen*t, 2019

Abstract

Objective: To explore the role of purine family member P2Y6 receptors in regulating neuropathic pain (NP) via neuroinflammation in the spinal cord.

Methods: Chronic constriction injury of the sciatic nerve (CCI) of NP was classic in setting up models on Sprague-Dawley (SD) rats. Experiments were performed on rats with sham surgery, CCI, CCI + MRS2578 (a P2Y6 receptor antagonist), and UDP (a P2Y6 receptor agonist). The hyperalgesia intensity was mirrored by paw withdrawal threshold (PWT) and thermal withdrawal latency (TWL). Immunofluorescence staining and western blot were used to evaluate activated microglial marker Iba-1. Enzyme-linked immunosorbent assay (ELISA) was used to access levels of IL-6. Conventional reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis were used to detect the expression of P2Y6 mRNA and activation of JAK/STAT signaling.

Results: Among all groups, CCI caused decreased PWT and TWL compared to sham surgery, meaning a successful establishment of the NP model. These decreased values of PWT and TWL tests could be prevented by intraperitoneally injected MRS2578 and enhanced by UDP administration. Similarly, CCI induced increase of Iba-1 protein, P2Y6 mRNA expression, and circulating IL-6 secretion, as well as increased JAK2/STAT3 mRNA expression and phosphorylating modification in spinal cord tissues could also be diminished by MRS2578 treatment and exacerbated by UDP.

Conclusions: These findings indicated the crucial role of the P2Y6 receptor in modulating the microglial and inflammatory responses in the process of NP in vivo. Results from this study would provide insights into targeting the P2Y6 receptor to treat NP in the near future.

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3. LI LAN

Title: Anti-Inflammatory and Antioxidative Effects of *Camellia japonica* on Human Corneal Epithelial Cells and Experimental Dry Eye: In Vivo and In Vitro Study
Journal: *Investigative Ophthalmology & Visual Science* (2017)

Abstract

Purpose: To analyze the anti-inflammatory and antioxidative effects of Camellia japonica (CJ) on human corneal epithelial (HCE) cells and its therapeutic effects in a mouse model of experimental dry eye (EDE).

Methods: Camellia japonica extracts of varying concentrations (0.001%, 0.01%, and 0.1%) were used to treat HCE cells. Dichlorofluorescein diacetate (DCF-DA) and dihydroethidium

(DHE) assays were performed. The production of peroxiredoxin (PRX) 1-6 and manganese-dependent superoxide dismutase (MnSOD) in HCE cells was assessed using Western blot analysis. Furthermore, eye drops containing 0.001%, 0.01%, or 0.1% CJ extract or a balanced salt solution (BSS) were applied to the EDE. Clinical parameters were measured 7 days after treatment. The levels of inflammatory markers and intracellular reactive oxygen species (ROS) were measured.

Results: Treatment with 0.01% and 0.1% CJ extracts decreased apoptosis in HCE cells. In addition, band intensities of PRX 1, 4, and 5, as well as MnSOD, after hydrogen peroxide (H2O2) treatment showed a significant improvement after pretreatment with 0.01% and 0.1% CJ extracts. Mice treated with 0.1% CJ extract showed significantly improved clinical parameters when compared to those of the EDE control and BSS groups. A significant decrease in the levels of inflammatory markers and intracellular ROS was observed in the 0.01% and 0.1% CJ extract groups.

Conclusions: Camellia japonica extracts promoted antioxidative protein expression and suppressed apoptosis in HCE cells. Furthermore, CJ extracts improved clinical signs of dry eye and reduced oxidative stress and the expression of inflammatory markers, suggesting that eye drops containing CJ extract could be used as an adjunctive treatment for dry eye.

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4. Adityanarayan Mohapatra

Title: A Lipophilic IR-780 Dye-Encapsulated Zwitterionic Polymer-Lipid Micellar Nanoparticle for Enhanced Photothermal Therapy and NIR-Based Fluorescence Imaging in a Cervical Tumor Mouse Model.
Journal: *International Journal of Molecular Sciences* (2018)

Abstract: To prolong blood circulation and avoid the triggering of immune responses, nanoparticles in the bloodstream require conjugation with polyethylene glycol (PEG). However, PEGylation hinders the interaction between the nanoparticles and the tumor cells and therefore limits the applications of PEGylated nanoparticles for therapeutic drug delivery. To overcome this limitation, zwitterionic materials can be used to enhance the systemic blood circulation and tumor-specific delivery of hydrophobic agents such as IR-780 iodide dye for photothermal therapy. Herein, we developed micellar nanoparticles using the amphiphilic homopolymer poly(12-(methacryloyloxy)dodecyl phosphorylcholine) (PCB-lipid) synthesized via reversible addition–fragmentation chain transfer (RAFT) polymerization. The PCB-lipid can self-assemble into micelles and encapsulate IR-780 dye (PCB-lipid–IR-780). Our results demonstrated that PCB-lipid–IR-780 nanoparticle (NP) exhibited low cytotoxicity and remarkable photothermal cytotoxicity to cervical cancer cells (TC-1) upon near-infrared (NIR) laser irradiation. The biodistribution of PCB-lipid–IR-780 showed higher accumulation of PCB-lipid–IR-780 than that of free IR-780 in the TC-1 tumor. Furthermore, following NIR laser irradiation of the tumor region, the PCB-lipid–IR-780 accumulated in the tumor facilitated enhanced tumor ablation and subsequent tumor regression in the TC-1 xenograft model. Hence,

these zwitterionic polymer-lipid hybrid micellar nanoparticles show great potential for cancer theragnostic.

Keywords: micelle; hydrophobic; IR-780; near-infrared; imaging; cervical cancer; photothermal
therapy; zwitterion

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5. Nguyen Phuoc Quang Huy.

Title: Targeting and Therapy of Glioblastoma in a Mouse Model Using Exosomes Derived From Natural Killer Cells
Journal: Frontiers in Immunology (2018)

Abstract:

Objective: Glioblastoma is a highly aggressive primary brain tumor that is resistant to radiotherapy and chemotherapy. Natural killer (NK) cells have been used to treat incurable cancers. Recent studies have investigated the effectiveness of NK-cell-derived exosomes (NK-Exo) for treating incurable cancers such as melanoma, leukemia, and neuroblastoma; however, NK-Exo have not been used to treat glioblastoma. In the present study, we investigated the antitumor effects of NK-Exo against aggressive glioblastoma both in vitro and in vivo and determined the tumor-targeting ability of NK-Exo by performing fluorescence imaging.

Methods: U87/MG cells were transfected with the enhanced firefly luciferase (effluc) and thy1.1 genes; thy1.1-positive cells were selected using microbeads. U87/MG/F cells were assessed by reverse transcription polymerase chain reaction (RT-PCR), western blotting, and luciferase-activity assays. NK-Exo were isolated by ultracentrifugation, purified by density gradient centrifugation, and characterized by transmission electron microscopy, dynamic light scattering (DLS), nanoparticle-tracking analysis (NTA), and western blotting. Cytokine levels in NK-Exo were compared to those in NK cells and NK-cell medium by performing an enzyme-linked immunosorbent assay (ELISA). NK-Exo-induced apoptosis of cancer cells was confirmed by flow cytometry and western blotting. In vivo therapeutic effects and specificity of NK-Exo against glioblastoma were assessed in a xenograft mouse model by fluorescence imaging. Xenograft mice were treated with NK-Exo, which was administered seven times through the tail vein. Tumor growth was monitored by bioluminescence imaging (BLI), and tumor volume was measured by ultrasound imaging. The mice were intraperitoneally injected with dextran sulfate 2 h before NK-Exo injection to decrease the liver uptake and increase the tumor specificity of NK-Exo.

Results: RT-PCR and western blotting confirmed the gene and protein expression of effluc in U87/MG/F cells, with the bioluminescence activity of U87/MG/F cells increasing with an increase in cell number. NTA and DLS results indicated that the size of NK-Exo was ~100

nm, and the western blot results confirmed that NK-Exo expressed exosome markers CD63 and Alix. We confirmed the in vitro cytotoxic effects of NK-Exo on U87/MG/F cells by performing BLI, and the killing effect on U87/MG and U87MG/F cells was measured by CCK-8 and MTT assays ($p < 0.001$). ELISA results indicated that NK-Exo contained tumor necrosis factor-α and granzyme B. In vivo NK-Exo treatment inhibited tumor growth compared to in control mice ($p < 0.001$), and pretreatment of xenograft mice with dextran sulfate 2 h before NK-Exo treatment increased the antitumor effect of NK-Exo ($p < 0.01$) compared to in control and NK-Exo-alone-treated mice.

Conclusion: NK-Exo targeted and exerted antitumor effects on glioblastoma cells both in vitro and in vivo, suggesting their utility in treating incurable glioblastoma.
