washed three times with HBSS containing 0.5 mg/mL heparin sulfate, detached with accutase, and resuspended in growth media for analysis. Cell-associated fluorescence was measured via flow cytometry. Live cells were discriminated via forward and side scatter; cell association was quantified using the mean fluorescence intensity (MFI). At each dosage, MFI values between control and TAT-peptides were compared using paired t-tests with an a priori significance level of 0.05.

Results: The TAT-PTD resulted in significantly more cell-associated fluorescence than the control peptide in a dose-dependent manner implying that intracellular uptake was facilitated. The MFI values for TAT-Treated cells (MFI: 313±61, 686±136, 2430±271 for peptide concentrations of 1.25, 2.5, and 5 μM, respectively) were significantly greater than those (110±15, 156±25, 280±36 for 1.25, 2.5, and 5 μM, respectively) for the chondrocytes treated with the control peptide (all p<0.02). Cell association of TAT peptides was observed in various types of chondrocytes from both male and female donors, from donors ranging in age from 24 to 64, and from normal and OA donors.

Conclusions: These data suggest that the TAT protein transduction domain facilitates peptide-uptake in primary human chondrocytes from diverse donor populations with varying age and OA grades. Future studies will determine whether the TAT PTD can be used to deliver therapeutic molecules to in vivo articular cartilage for modulating chondrocyte behavior toward treating osteoarthritis.

496 PHELLODENDRON AMURENSE REGULATE THE LEVELS OF MATRIX METALLOPROTEINASES, PROINFLAMMATORY CYTOKINES AND SIGNALING OF THE MITOGEN ACTIVATED PROTEIN KINASE (MAPK) PATHWAY IN HUMAN OSTEOARTICULAR CARTILAGE AND CHONDROCYTES


Purpose: Traditional medicine has widely been used Phellodendron amurense Rupr. (Rutaceae) to treat various inflammatory diseases including arthritis. In this study, we investigated the effects of Phellodendron amurense (P. amurense) in protecting cartilage, including regulating the levels of aggrecanases, matrix metalloproteinas (MMPs), tissue inhibitor of metalloproteinas (TIMPs), proinflammatory cytokines and signaling of the mitogen activated protein kinase (MAPK) pathway in human osteoarticular cartilage and chondrocytes.

Methods: Explants from human osteoarthitis cartilage were cultured alone or in IL-1α for 7 days with or without P. amurense ethanol extract or celecoxib (40, 100, 200 μg/mL). The effect of P. amurense on matrix degradation induced by IL-1α in human articular cartilage was assessed by staining, and the quantities of sulfated glycosaminoglycan (GAG) and type II collagen were calculated from the culture media. The levels of aggrecanases, MMPs, TIMPs, and PGE2 in the culture media were investigated using an enzyme-linked immunosorbent assay (ELISA). In addition, reverse transcription polymerase chain reaction (RT-PCR) evaluated the mRNA expression of aggrecanases, MMPs and TIMPs. Furthermore, Western blot analysis was performed to identify the roles of P. amurense played in the ERK, JNK and p38 signaling pathway.

Results: P. amurense showed no evident cytotoxicity on human articular cartilage. P. amurense significantly inhibited the IL-1α-induced degradation of GAG and type II collagen from human osteoarticular cartilage in a concentration-dependent manner. Celecoxib did not significantly inhibit IL-1α-induced release of GAG and only slightly reduced type II collagen. P. amurense also dose-dependently decreased the levels of aggrecanase-1 and -2, MMP-1, -3, and -13, whereas it increased TIMP-1 expression in human osteoarticular cartilage. Celecoxib only decreased MMP-1 and MMP-13 levels in human osteoarticular cartilage. In addition, P. amurense reduced the phosphorylation of extracellular signal regulated kinase (ERK)1/2, Jun NH2-terminal kinase (JNK) and activated phospho-p38 MAPK in a dose-dependent manner in human osteoarthritis chondrocytes.

Conclusions: These data suggest that P. amurense inhibited osteoarthritic cartilage and chondrocyte destruction by inhibiting proteoglycan release and type II collagen degradation, down-regulating aggrecanases, MMP activities and phospho-ERK1/2, JNK and p38 MAP kinase signalling, and up-regulating TIMP-1 activity. Therefore, our results suggest that P. amurense is a potential therapeutic agent to protection cartilage against OA progression.

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497 THERAPY SWITCHING, ADD-ON, AND DISCONTINUATION IN PATIENTS WITH OSTEOARTHRITIS IN THE UNITED KINGDOM


Purpose: To evaluate the patterns of therapy switching, add-on and discontinuation after initiation of treatment with select currently recommended treatments in patients with OA.

Methods: Using the United Kingdom THIN (The Health Improvement Network) database, patients at least 18 years of age, with at least two healthcare encounters with an associated diagnosis of OA starting January 1, 2005, with the two diagnoses being at least 30 days apart, newly prescribed (index event) non-selective non-steroidal anti-inflammatory drugs (NS-NSAIDs, N=6,639; average age ± SD, 67.2±11.4 years), Cyclooxygenase-2 inhibitors (Cox-2s, N=542; 60.1±11.2 years), acetylaminen (N=3,215; 74.1±10.1 years), tramadol (N=1,886; 70.1±11.2 years), weak opioids (N=5,886; 70.4±11.1 years) and strong opioids (N=178; 74.0±11.3 years) within 30 days prior, or anytime after the first diagnosis of OA were selected. Descriptive statistics, Kaplan Meier analyses, and COX proportional hazards model were used to evaluate patterns of therapy switching, add-on, and discontinuation during the 12-months post-index period. The study protocol was approved by the Cambriidgeshire 4 Research Ethics Committee.

Results: Substantial proportions of OA patients switched therapy within a year after treatment initiation and rates of therapy switching were significantly different (p<0.0001) across the evaluated drugs groups: NS-NSAIDs, 30.0%; Cox-2s, 44.6%; acetylaminen, 32.8%; tramadol, 57.8%; weak opioids, 33.7%; and strong opioids, 59.6%. Patterns of therapy add-on at 12-months were also significantly (p<0.0001) different, although rates of therapy add-on were much lower: NS-NSAIDs, 9.1%; Cox-2s, 13.5%; acetylaminen, 9.1%; tramadol 11.8%; weak opioids, 8.7%; and strong opioids, 15.2%. A majority of patients in each group discontinued therapy during the 12-months post-index period (91.9%, 86.9%, 91.4%, 89.7%, 93.2%, and 84.3% of patients in the NS-NSAIDs, Cox-2s, acetylaminen, tramadol, weak opioids and strong opioids groups, respectively). An evaluation of estimated probabilities suggested that over two thirds of patients who switched, augmented or discontinued therapy did so within the first 2-months, and a majority did so within 6-months of treatment initiation.

Conclusions: Results of this study suggest that therapy switching and discontinuation were very common among OA patients initiating treatment with the currently recommended medication classes for this painful condition. The observed high rates of therapy switching and discontinuation may be indicative of inadequate pain relief or potentially intolerable side-effects of therapies. Given the human and economic burden of OA, future research may benefit from a focus on comparative efficacy and safety parameters to further differentiate treatment options.

498 BIPHASIC POSITIVE EFFECT OF FORMONONETIN ON METABOLIC ACTIVITY OF HUMAN NORMAL AND OSTEOARTHRITIC SUBCHONDRAL OSTEOLASTS


Purpose: Osteoarthritis is a multifactorial disease characterized by loss of articular cartilage and subchondral plate thickening. Therefore,