The Effects of Ketorolac Tromethamine on Tendons - An In Vitro and In Vivo Study

Ting Yuan, Jianying Zhang, Guangyi Zhao, Binghua Zhou, James Wang.
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.


Introduction: Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug (NSAID), which is commonly used to treat tendon injuries because of its potent analgesic and anti-inflammatory effects. In clinical settings, the local application of KT has shown promising results with decreased incidences of NSAIDs-related side effects when compared to systemic application. However, the local injection of KT into peritendinous regions could directly affect tendon tissues, although whether these effects are beneficial or detrimental is not known. Therefore, in this study, we aimed to determine the effects of KT on tendons using both in vitro and in vivo models.

Methods: In vitro study - Human tendon cells were isolated from the patellar tendons of seven human donors (ages 26-51 years old). These cells, which are a mixture of tenocytes and tendon stem cells (TSCs), were grown in growth medium (DMEM + 10% FBS) with various concentrations of KT (1, 2, 5, 15, and 30 mg/ml) in 10% ethanol. The control cells were treated with 10% ethanol only. All groups of cells were cultured at 37°C with 5% CO2. After KT treatment for 48 hrs, cell proliferation was measured by CCK8 assay and cellular gene expression levels were determined using qRT-PCR.

In vivo study - Patellar tendons from six SD rats were assigned randomly to one of two groups: KT (15 mg/ml) injection or 10% ethanol injection. Under anesthesia, 20 μL of KT or 10% ethanol was injected into the patellar tendon percutaneously once a day for three consecutive days. On the fourth day all rats were euthanized and their patellar tendons and peritendinous tissues were harvested for histological analyses.

Statistical analysis - One-way ANOVA was used to assess the overall statistical differences among groups. Fisher’s predicted least-square difference (PLSD) for multiple comparisons or unpaired student’s t-test was performed wherever applicable. Differences between two groups were considered significant when P-value was less than 0.05.

Results: Control human tendon cells cultured in vitro adhered well to the surface of culture plates. In these cell cultures, two distinct cell shapes representing two different cell types could be identified: i) slender and spindle shaped tenocytes, and ii) ovoid or polygonal TSCs. Twenty-four hours after KT treatment, the number of adhering tendon cells decreased significantly when compared to the control. At high KT concentrations (15, 30 mg/ml) a significant number of cells detached from the monolayer. These cells became round and dead cells floated in the medium (Fig. 1).

KT treatment also significantly inhibited cell proliferation (Fig. 2A) (P < 0.001), indicating that KT is a potent inhibitor of cell proliferation particularly at higher concentrations. Further, KT treatment resulted in decreased gene expression of collagen type I (Coll I - tenocyte marker) in a concentration-dependent manner, but elevated expression of the following genes: lipoprotein lipase (LPL - adipocyte marker), aggrecan (chondrocyte marker), and Runt-related transcription factor 2 (Runx-2 - osteocyte marker) significantly at 5 mg/ml concentration. Aggrecan expression was higher at 1 mg/ml KT, but decreased at 5 mg/ml (Fig. 2B).
Finally, three days after KT injections (15 mg/ml) into the patellar tendons, peritendinous soft tissue edema, vessel formation, and a larger area of congestion were observed when compared to the controls (Fig. 3A). Histological staining with hematoxylin and eosin showed acute inflammation in rats treated with KT, as evidenced by abundant neutrophils around the patellar tendon (Fig. 3B).

**Discussion:** Our results show that addition of KT to human tendon cell cultures can result in detachment of cells from the culture plate and eventual death. Expression of the tenocyte marker, collagen I, was significantly reduced, but expression of non-tenocyte related genes, LPL and Runx-2, was higher. Aggrecan, the chondrocyte marker, expression levels was also increased at the concentration of 1 mg/ml KT. Finally, local injection of KT led to acute inflammation with accumulation of abundant neutrophils around the tendon, which may induce secondary damage to tendon tissue. Taken together, these findings suggest that short-term local injections of KT may be detrimental to tendon tissues. Further studies for long-term effects of KT on tendon characteristics such as collagen structure, matrix degradation, and mechanical strength are needed. Previous studies have shown that the NSAID, ibuprofen, increased neutrophil migration when the peak plasma concentration was less than 50 μg/ml, but above 50 μg/ml neutrophils migration decreased [1]. Whether similar threshold effects apply to KT needs further investigation.

**Significance:** Local application of KT in tendon tissues may be detrimental to tendons; therefore, caution should be exercised in the use of KT to treat tendon injuries in clinical settings.
Fig. 1 Treatment of human tendon cells in culture with KT causes cell detachment and cell death in a KT dose-dependent manner. At 2 and 5 mg/ml KT decreased cell proliferation; 15 and 30 mg/ml KT concentrations prevented cells from attaching to the culture plates.
Fig. 2A KT treatment decreases proliferation of human tendon cells in a dose-dependent manner. (P<0.001, * compared to the control (ctrl)). Fig. 2B The effects of KT on human tendon cell differentiation. After 3 days of KT treatment, Coll I gene expression decreased with increasing KT concentrations. LPL and Runx-2 reached maximum expression levels at 5 mg/ml KT. Aggrecan expression level was the highest at the lower KT concentration (1 mg/ml) (* P < 0.05 compared to the control group).
Fig. 3 KT treated patellar tendons (A, arrow) show marked peritendinous soft tissue edema, vessel formation, and a larger area of congestion compared to the control treated with 10% ethanol (B). Histological staining shows acute inflammation in the experimental group (C, KT), with abundant neutrophils around the patellar tendon. No inflammatory cells are found in the control (D). (* neutrophils; T: tendon; P: paratenon; magnification 40X).