Dietary administration of Curcumin modifies transcriptional profile of genes involved in inflammatory cascade in horse leukocytes

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ABSTRACT - Pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) play a key role in the pathogenesis of osteoarthritis (OA). Once released, these cytokines are potent stimulators for the *de novo* production of catabolic enzymes such as matrix metalloproteinases (MMPs) and cyclo-oxygenase-2 (COX-2). Anti-inflammatory agents capable of suppressing the production and catabolic actions of these cytokines may have therapeutic potential in the treatment of OA and a range of other osteoarticular disorders. The purpose of this study was to examine the therapeutic effect of Curcumin (diferuloylmethane), a pharmacologically safe phytochemical agent, on males and foals affected by degenerative joint diseases. Curcumin, in the form phytosome (CURCUVET®, Indena Spa, Milan, Italy) was administered to animals for fifteen days and gene expression was monitored before the treatment and after four, eight, and fifteen days. In mares, Curcumin inhibited the expression of COX-2, TNF-α, IL-1β, IL1RN, and IL6, even if only the downregulation of IL-1β and IL1RN were significant. In foals, Curcumin significantly inhibited the expression of COX-2, TNF-α, IL1RN and significantly increased that of IL6. These results indicate that Curcumin has nutritional potential as a natural anti-inflammatory agent for treating osteoarticular disorders through suppression of pro-inflammatory cytokines and catabolic enzymes.

Key words: Osteoarthritis, Horses, Curcumin, Cytokines.

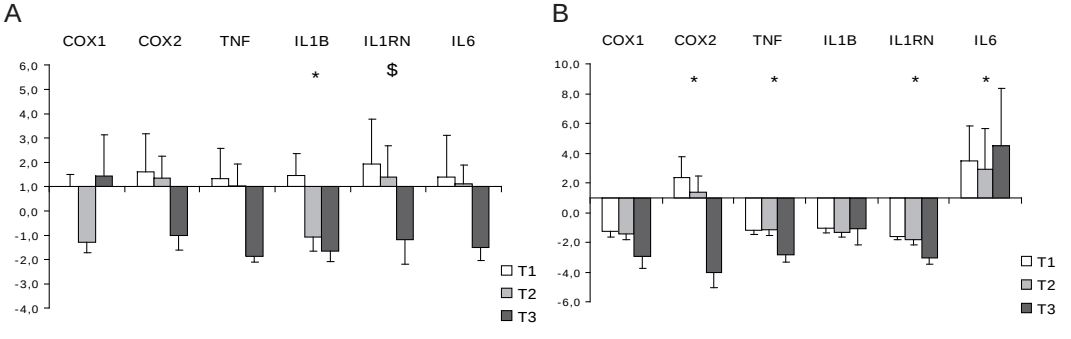
Introduction - Osteoarthritis (OA) is a well-known and common disease for the athletic horse and seriously affects the quality of life and career of affected animals, leading to economic losses. Among the causes of OA, joint disease is very frequently diagnosed (Todhunter and Lust, 1990). Also in growing horses, articular osteochondrosis is a major joint pathology with high prevalence and important economic impact (Gangl *et al.*, 2007). The onset of disease begins as an abnormal chondrocyte development and maturation, leading to altered endochondral ossification and subsequent damage to the cartilage, resulting in osteochondral lesions or fragmentation and incongruency of the articular cartilage (Jeffcott and Henson, 1998). It is known that articular cartilage is a metabolically active tissue and that chondrocytes are able to synthesize and digest matrix macromolecules, thus contributing to cartilage matrix homeostasis. In OA, the equilibrium between matrix deposition and degradation is disrupted, leading to the excessive digestion of matrix and progressive loss of important matrix components. Even in the absence of classic inflammation, elevated levels of inflammatory cytokines have been measured in OA synovial fluid, including interleukin-1β (IL-1β), interleukin 6 (IL6), transforming growth factor-β (TGF-β), and tumor necrosis factor-alpha (TNF-α) (Islam *et al.*, 2001). Inflamma-

tory cytokines, as TNF-α and IL-1β, have also been shown to induce expression of metalloproteases (MMPs) (Mengshol *et al.*, 2002) and cyclo-oxygenase-2 (COX-2). These synovium and chondrocyte-derived products represent potential targets for the development of therapeutic agents, which could be used to prevent or retard the progression of the OA articular lesion. Chronic arthritis and pain associated conditions are the most common conditions for which Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are used. However, NSAIDs have well-known adverse effects, including gastric ulceration and inhibition of cartilage resynthesis without reducing inflammation-associated cartilage breakdown. For these reasons, alternatives to allopathic medications, as nutraceuticals and complementary therapies, are often sought for arthritic conditions in horses and companion animals. In this study, the bioactivity of CURCUVET® (Indena Spa, Milan, Italy), a *Curcuma longa* stabilized extract associated to a phytosome complex, was investigated in 12 subjects, including 7 mares affected by osteoarthritis and 5 foals affected by osteocondhrosis. The effect was evaluated by measuring the expression levels of a set of genes associated to inflammatory cascade.

Material and methods - Seven adult mares (4-9 years) affected by chronic osteoarthritis and five foals affected by osteochondrosis were used for the study. The diagnosis was based on clinical signs and radiological examination, executed by the veterinary. The animals received 4 mg/kg LW of CURCUVET® every 24 hours before the morning meal for 15 consecutive days. Blood was collected from jugular vein in the morning before (T0) and after the CURCUVET® treatment, specifically on days 4, 8, and 15 (T1, T2, and T3 respectively) from the beginning of the trial. Blood was sampled after 2 hours from the CURCUVET® administration.

Whole blood (2.5 ml) was drawn directly into a PAX gene blood RNA tube (PreAnalytix, Qiagen, Cologne, Germany) and total RNA was purified with PAX gene blood RNA kits (PreAnalytix, Qiagen) according to the manufacturer's protocols. Reverse transcription was performed by using Improm-II Reverse Transcriptase (Promega, Milan, Italy) according to the manufacturer's instructions. Real-Time PCR reactions were performed in triplicate using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Milan, Italy). Oligonucleotide primers were designed with the help of Primer3 Input software (Rozen and Skaletsky, 2000). Target gene were COX-1, cyclo-oxygenase-1; COX-2, cyclo-oxygenase-2; TNF-α, tumor necrosis factor-alpha; IL1β, interleukin 1β; IL1RN, interleukin 1 receptor antagonist; IL6, interleukin 6. As reference housekeeping gene, 18S rRNA (18S ribosomal RNA) was used. The expression level of a given target gene at each sampling time, was analyzed by the $2^{-\Delta\Delta Ct}$ method (Bustin, 2000) where $2^{-\Delta\Delta Ct}$ represents the different regulation of a given target gene at T1, T2, or T3 *vs.* the T0. The *n*-fold expression was calculated as $2^{-\Delta\Delta Ct}$ for upregulated genes, and $-(1/2^{-\Delta\Delta Ct})$

Figure 1. Effect of CURCUVET® administration on the expression level (*n*-fold) of a set of genes of the inflammatory cascade in mares (A) and foals (B); *P<0.05, $P<0.1.



for downregulated genes. All the recorded variables were submitted to analysis of variance using the ANOVA model to assess significant differences between sampling times (SPSS Inc., 1997).

Results and conclusions - The treatment of mares with CURCUVET® induced a down regulation of COX-2, TNF-α, IL6, IL-1β and IL1RN, even if the effect was significant only for the last two genes (Figure 1). In particular, IL-1β gene expression was reduced at T2 andT3 ($P<0.05$), whereas IL1RN gene expression only at T3 ($P<0.1$). In foals treated with CURCUVET®, COX-1, COX-2, TNF-α, IL-1β, IL1RN resulted downregulated, even if the effect was significant only for COX-2, TNF-α, IL1RN. COX-2 transcription was reduced only at T3 ($P<0.05$), whereas TNF-α ($P<0.05$) and IL1RN ($P<0.05$) transcription diminished at T1, T2, and T3. Differently, IL6 gene expression resulted significantly upregulated at T1, T2, and T3 ($P<0.05$). The stimulation of IL6 expression could have a beneficial effect since IL6 has a protective role with respect to cartilage integrity (Goldring, 2000). Moreover, during the inflammatory response, IL6 has a regulatory effect inhibiting IL-1β and TNF-α expression (Feghali and Wright, 1997). These results are in agreement with previous studies showing that Curcumin inhibit IL-1β levels (Banerjee *et al.*, 2003), and IL-1β and TNF-α stimulated activity of NFkB (Nuclear Factor kB) and downstream targets, such as COX-2 and MMP9 (Matrix Metallopeptidase 9) (Shakibaei *et al.*, 2007). These results indicate that CURCUVET® has nutritional potential as an anti-inflammatory agent for treating osteoarticular disorders through suppression of pro-inflammatory cytokines and catabolic enzymes.

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