A Promising Natural Therapy for Equine Osteoarthritis

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Equine osteoarthritis is a debilitating, degenerative condition affecting horses of various age groups and with a multitude of causes, ranging from conformational flaws, trauma to infectious origins. Osteoarthritis is also one of the main causes of lameness in the equine athlete, resulting in lost time, performance and high expenditures on various therapeutics. Curcumin, the active constituent of turmeric, has been shown in human research trials to actively downregulate inflammatory mediators through two main mechanisms: NF-kB inhibition and through activities as a secondary antioxidant. Equine osteoarthritis is primarily mediated by various cytokine release from damaged cells within the joint, resulting in high levels of PGE-2 and MMP-9, which further result in cartilage degredation and pain. Through NF-kB inhibition, curcumin has been shown to downregulate many inflammatory mediators, including PGE-2 and MMP-9, along with COX-2 enzymes. Curcumin has not only been shown to be fairly effective in human trials, but has also been shown to be safe even at high dosages with no noted side effects.

Introduction

Curcumin is a polyphenol derived from the rhizome of the herb *Curcuma longa* and has been shown to have a wide range of biological and pharmacological activities. Curcumin has been shown to be the active principle of tumeric and has been demonstrated to exhibit anti-inflammatory, antioxidant, antimicrobial and anti-carcinogenic effects. Various animal and human studies has proven that curcumin is extremely safe, even at very high dosages.[1] The yellow pigmented fraction of turmeric contains curcuminoids, which are chemically related to the main ingredient, curcumin. The major curcuminoids present in turmeric are demethoxycurcumin (curcumin II), bisdemethoxycrucmin (cucumin III) and cyclocurcumin.[2] The major components of commercially available curcumin include curcumin I, curcumin II and curcmin III, with curcumin I being the major component.[3]

   Traditional therapies aimed at relieving clinical signs associated with equine osteoarthritis include non-steroidal therapy, polysulfated glucosamines, intra-articular steroids and hyaluronic acid derivatives. Newer therapies include shock wave and IRAP therapy. The main goal of therapies include reduction of pain, reduction of localized inflammation and slowing of the degenerative process. This is a complicated task as there are many different cytokines and enzymes involved in this process. The primary mediators that we are interested in include PGE-2, MMP-9, IL-1, TNF-α, COX-2 and 5-LOX. Almost all of the inflammatory mediators linked to arthritis have been shown to be regulated by transcription factor nuclear factor-κB (NF-κB).[4] Given all of the evident factors involved, it is difficult for one type of therapy to actively target all entities. Current therapeutics for equine osteoarthritis range in expense for the owner and have a wide range of effects, some potentially being harmful. The effects, if seen, are generally short lived and thus, necessitate repetitive treatments, which can increase the possible negative side effects and overall cost to the owner. Curcumin, being a natural option, can potentially be more effective and cost effective for long term therapy.

Materials and Methods

Two separate trials were conducted evaluating the effects of a proprietary formula consisting of curcumin, vitamin E and vitamin C on horses with varying degrees of clinical osteoarthritis. The formula was created by Tom Schell, D.V.M and formulated with the assistance of a human nutraceutical pharmacy.

In the first trial, two horses were evaluated before and after therapy, which consisted of top dressing the curcumin formula on the feed once daily for 30 days. Lameness scores were assessed at the beginning and end of the trial. Bloodwork was assessed at the beginning and end of the trials, consisting of a routine blood chemistry profile as well as a complete blood count. Synovial fluid was also retrieved from the affected joint prior to therapy and at the end of the trial. The synovial fluid was then submitted to the MD Anderson Cancer Research Center for evaluation of eicosanoid levels. The average age of the horses in this trial was 15 years and all horses were geldings of varying breeds and disciplines.

In the second trial, 6 total horses were evaluated with varying degrees of osteoarthritis. The horses were again assessed pre and post for lameness scores, as well as complete blood counts, general chemistry panels, lymphocyte phenotypes, C-reactive protein and RBC EFA levels. Synovial fluid was retrieved from an affected joint before and at the end of the trial. Synovial fluid was submitted to the Ohio State University for matrix-metalloproteinase 9 evaluation and to the MD Anderson Cancer Research Center for evaluation of eicosanoids. The average age of the horse in the second trial was 16 years and all horses were geldings of varying breeds and disciplines.

Horse	Breed	Gender	OA Location	Pre-grade Lame	Post-grade Lame	Prior-meds
1	QH	Gelding	Hock	2/5	1/5	Bute prn
2	Morgan	Gelding	Hock	2/5	1/5	IA steroids

Table 1.0: Trial One Patient Assessment.

Horse	Breed	Gender	OA location	Pre-grade Lame	Post-grade Lame	Prior-meds
1	QH	Gelding	Carpus/hock	4/5	3/5	Bute prn
2	QH	Gelding	Hock	4/5	2/5	Cosequin
3	QH	Gelding	Hock	3/5	1/5	Cosequin
4	THB	Gelding	Diffuse	3/5	1/5	MSM
5	Arabian	Gelding	Fetlock	4/5	2/5	Bute prn
6	Paso-fino	Gelding	Pastern	4/5	3/5	Bute prn

Table 1.1: Trial Two Patient Assessment.

Results

Effects of Curcumin Formula on Overall Lameness. Lameness scores in all horses improved by at least one grade, with the majority of horses being improved by 2 grades. Both horses in the initial clinical trial improved by one grade of lameness. One of the horses in the first trial had recurrent and persistent synovial effusion of the tarso-crural joint that had previously only responded intermittently to intra-articular steroids and HA. During this trial, the synovial effusion resolved by 70% as well as the associated stiffness in that particular limb. In the second trial, all horses improved by at least one grade of lameness. The second trial had a variety of lameness scores and severity of osteoarthritis, primarily localized to the tarsus. One horse in the second trial had diffuse carpal OA, collapsed suspensory apparatus in the rear limbs and moderate OA of the tarsal-crural joint. This particular horse was generally very stiff and categorized as a grade 4/5 lameness. Prior therapy on this particular horse included intermittent phenylbutazone administration by the owner. This particular horse improved by one grade of lameness as perceived by myself. He was generally perceived as moving more freely, but still overall stiff. Another horse in the trial had a prior history of intra-articular ringbone in the left forelimb which had been treated with localized intra-articular injections of methylprednisolone. This particular horse experienced a one grade improvement in lameness as well. The remaining horses in the study did very well by our perception with an average of a two grade improvement in the lameness. Some of the remaining horses were able to be removed from daily administration of other joint supplements and phenylbutazone administration. Overall, there was an 80% perceived response rate in the patients and owner satisfaction. The biggest notation by the owners was that the horses seemed to feel better overall and moved about much looser, with some of the horses exhibiting youth like activity.

Effect of Curcumin Formula on C-reactive Protein. The horses in the second trial were evaluated for serum C-reactive protein levels, utilizing the laboratory at the Bio-Center located in Wichita, KS. We were unable to appreciate any change in the C-rap levels and all horses exhibited a level of less than 0.10 mg/L on the pre and post samples.

Effects of Curcumin Formula on General Chemistry Values and Complete Blood Counts. Serum and whole blood was submitted non-fasting on all patients at the initiation and conclusion to the trial. There were no appreciable changes in the blood chemistry panels or the complete blood counts over the 30 day trial. Hepatic, renal values and protein levels were unchanged at the cessation of the trial.

Effects of Curcumin Formula on Lymphocyte Phenotypes.

Horse	CD21 Pre/post	CD8 Pre/post	CD4 Pre/post	CD5 Pre/post	CD4/8 Ratio Pre/post	T/B ratio Pre/post	% lymph Pre/post	% neut Pre/post	% monocyte Pre/post
1	9.0/ 2.5	23.0/5.41	42/ 9.4	67/ 49	1.83/1.74	7.4/19.6	20/14	56/65	6/3
2	7/ NA	24/ NA	63/NA	87/NA	2.15/ NA	10/ NA	24 / NA	57/NA	6/NA
3	10/ 4	15/ 17	68 /43	87/ 68	4.5 / 2.5	8.7 / 17	27/ 23	58/ 48	4 /6
4	5/ 25	23/ 16	40/ 34	82/ 50	1.7/ 2.1	16/ 2	20/ NA	57/ NA	9/ NA
5	8/ 2	17/ 9	13/ 60	36/ 77	0.7/ 6.6	4.5/ 38	17/ 23	65/ 53	3/ 4
6	8/ NA	20/NA	43/ NA	80/NA	2.1/ NA	10/ NA	24/ NA	57/NA	6/ NA

Table 2.1: Lymphocyte phenotype analysis: pre and post 30 day treatment. Noted that horse 2 and 6 experienced sampling errors due to handling resulting in no post value determination.

Effects of Curcumin Formula on Synovial Eicosanoids:

Horse	PGE-2 pre/post	PGE-2 Post
1	1.08 ng/ml	0.07 ng/ml
2	0.08 ng/ml	0.03 ng/ml

Table 2.2: Trial one synovial fluid eicosanoid levels

Horse	PGE-2 Pre	PGE-2 Post
1	0.54 ng/ml	0.36 ng/ml
2	1.31 ng/ml	0.74 ng/ml
3	0.27 ng/ml	0.08 ng/ml
4	0.22 ng/ml	0.15 ng/ml
5	0.08 ng/ml	0.06 ng/ml
6	0.87 ng/ml	0.21 ng/ml
7	0.19 ng/ml	0.15 ng/ml

Table 2.3: Trial two synovial fluid eicosanoid levels.

Effects of Curcumin Formula on Synovial MMP-9 Levels:

Horse	Pro-dimer pre/post	Act-dimer Pre/post	Pro-mono pre/post	Act-mono pre/post
1	825/ 664	464/ 0.0	564/ 603	1425/ 531
2	283/ 340	392/ 0.0	349/ 184	1065/ 482
3	0.0/ 0.0	564/ 0.0	274/ 0.0	1461/ 461
4	0.0/ 0.0	508/ 423	237/ 166	2542/ 277
5	450/ 592	0.0/ 0.0	204/ 222	241/ 276
6	635/ 710	0.0/ 0.0	242/ 302	241/ 276
Average	393/ 404	275/ 60	300/ 248	1075/ 375

Table 2.4: Trial two synovial MMP-9 determination: pro-dimer and activated dimer, pro-monomer and activated monomer.

Discussion

Equine osteoarthritis is an inflammatory process secondary to various factors including increased stress on a particular joint from conformational flaws to repetitive trauma including stress from infectious processes. The underlying pathophysiology to condition is a release of inflammatory mediators, cytokines and reactive oxygen species from the synovium and chondrocytes, as well as neutrophils and other immune related cells. The cytokines and reactive oxygen species (ROS) then trigger further release of other inflammatory mediators from surrounding cells, which leads to further degradation, inflammation, pain and recruitment of immune cells.

Typical non-steroidal antiinflammatories, flunixen meglumine and phenylbutazone, result in a reduction in cyclooxygenase enzymes and are non-selective for the most part. This results in a reduction in COX-1 and COX-2 enzymes. This inhibition of COX-2 results in a reduction in the production of PGE-2 in the arachidonic acid cascade. The inhibition of COX-1 can impart potential degradation to the prostaglandin lining of the intestinal tract as well as influencing renal circulation. The potential side effects of a non-selective COX inhibitor are gastrointestinal ulceration and renal compromise.

Steroidal anti-inflammatory medications, oral or intra-articular, impact the NF-κB transcription factor, resulting in varied levels of inhibition. This leads to decreased pro-inflammatory mediator release, decrease circulating COX 1-2 enzymes as well as potential immunocompromise due to decreased circulating interleukin levels. Side effects of long term or repetitive steroid usage are well known and include immunosuppression, gastrointestinal ulceration, hepatic compromise, renal compromise, water retention and potentially laminitis in horses.

A new form of intra-articular therapy in the horse is IRAP or interleukin antagonistic protein. This form of therapy is targeted at reducing the circulating levels of interleukin-1 in the synovial fluid. This form of therapy is new and highly researched. Side effects are minimal at this time but do include risk of joint sepsis due to invading the joint for injection purposes.

Curcumin is a unique, natural alternative to traditional therapeutics. Curcumin interacts with a number of apparent targets. It binds to and inhibits the activity of enzymes, growth factor receptors, metals, albumin and other molecules. Curcumin has been shown in human research to be a potent, dose dependent, inhibitor of NF-κB. This is a transcription factor present within the cytoplasm of a multitude of cells. Activation of NF-κB results in the production of many proteins, of which a high number of them can be considered pro-inflammatory as well as contributing to tumorigenesis, cell survival, cell proliferation, invasion and angiogenesis.[3] For purposes of this discussion, we will focus on the inhibition of inflammatory mediators. The role of inflammatory cytokines such as TNF-α, IL-1, IL-6 and chemokines; inflammatory enzymes such as COX-2, 5-lipoxygenase and matrix metalloproteinase and adhesion molecules in the pathogenesis of arthritis have been well documented in humans. Almost all inflammatory mediators associated with arthritis have been shown to be regulated by the transcription factor nuclear factor kappa-B (NF-κB).[4] Curcumin has been shown to inhibit NF-κB by suppressing the degradation of the inhibitory unit I-κB alpha and hindering the nuclear translocation of the functionally active subunit of NF-κB, therefore blocking improper NF-κ B activation.[5]

The other main advantage of curcumin therapy above and beyond traditional therapies are minimal side effects. Traditional therapies such as NSAIDs and corticosteroids can exhibit non-selective inhibition of the COX enzymes, resulting in COX-1 inhibition. This inhibition can lead to gastrointestinal side effects with long term usage. Curcumin inhibits COX-2 but not COX-1, based on colon cancer cell studies, thus the effects attributed to nonselective COX inhibitors can be alleviated.[5] In terms of toxicity, there have been multiple phase I human clinical trials that indicated that curcumin is well tolerated when take at doses as high as 12 g/day.[3,6] These results were also recently verified in a dose-escalation trial to determine curcumin's maximum tolerated dose and safety.[3,7] Throughout the trial, only minimal toxicity, non-dose related toxicity was seen in 30% of the patients.[3] In one equine in-vitro study, researchers analyzed the effect of varying concentrations of curcumin on cultured equine chondrocytes and synoviocytes. They discovered that there was apparent apoptosis of both chondrocytes and synoviocytes at concentrations above 50 µM.[8]

Despite curcumin's apparent efficacy and safety, it has not been readily approved as a therapeutic agent. The reasoning behind this appears to be related to concerns over bioavailability.[1] In human clinical trials, patients were dosed with 500 mg/day and gradually increased to 1000, 2000, 4000, 8000 and finally 12,000 mg/day. Curcumin was not evidently toxic at these dosages, reaching peak serum concentrations of 0.51 µM at 4 grams, 0.63 µM at 6 grams and 1.77 µM at 8 grams.[3] It has been theorized that with typical equine dosing of curcumin, it would be theoretically impossible to achieve serum concentrations higher than 5 µM, considering bioavailability factors.[9] Given these speculations, concerns over chondrocyte or synoviocyte damage can potentially be dismissed. In our research trial, we were able to achieve serum curcuminoid levels of 1.5µM at 2 hours post ingestion. Recent human research has shown problems or concerns with curcumin bioavailability such as low serum levels, limited tissue distribution, apparent rapid metabolism and short half life. Several research trials utilizing rats, analyzed various tissue sample for curcumin levels. After variable dosing, the tissue levels of curcumin were highest in intestinal and liver tissue, while only trace amounts

were noted in other tissues throughout the body. [1] It has been determined that curcumin undergoes extensive reduction via alcohol dehydrogenase, followed by conjugation, which mainly took place in the liver. In rats, the major billiary metabolites of curcumin are glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC). The ultimate question is whether the metabolites of curcumin are as active as curcumin itself.[1]

Aside from being a potent inhibitor of NF-κB, curcumin has proven to be a potent antioxidant as well, helping to inhibit reactive oxygen species (ROS) or free radicals. Curcumin, and its metabolites, have been shown to provide protection of hemoglobin from oxidation at levels as low as 0.08 μM. Lipid peroxidation (LPO) is generally implicated in inflammation, cancer and cardiovascular disease. In rat models, curcumin has been shown to actively inhibit LPO. The antioxidant activities of curcumin are thought to be potentially mediated through superoxide dismutase, catalase and glutathione peroxidase, resulting in lower intracellular levels of GSH. It has also been postulated that curcumin exhibits far superior antioxidant activity than vitamin E.[10]

In terms of actual clinical data on the use of curcumin on inflammatory conditions, there is very little veterinary data. In a human study, 18 patients with a history of corticosteroid responsive rheumatoid arthritis, received either 1200 mg/day of curcumin or 300 mg daily of phenylbutazone. At the end of the trial, the dose of curcumin utilized was evidently well tolerated, no evident side effects and exhibited anti-inflammatory capabilities similar to that of phenylbutazone.[3,11] In human research, IL-1 is the main cytokine instigator of cartilage degeneration in cases of arthritis. IL-1 induces MMP-3 and MMP-13 RNA and protein in chondrocytes through the activation of mitogen-activated protein kinase (MAPK), AP-1 and NF-κB transcription factors. Based on human studies, curcumin resulted in 48-99% suppression of MMP-3, 45-97% of MMP-13 in human and 8-100% MMP3 and 32-100% MMP-13 in bovine chondrocytes. Evidently, inhibition of IL-1 signal transduction could be useful for reducing cartilage resorption by MMP's in cases of arthritis.[10,12]

The equine osteoarthritis trials that we performed were small in nature and varied as to the degree of clinical osteoarthritis in each patient. The trial or study was intentionally created in this manner in order to initially gain an overview of the clinical improvement in a wide range of clinical settings. Overall, we did note an improvement in each patient with a high degree of owner satisfaction in the majority of patients. There were two candidates that did not exhibit marked improvement and both of those patients had marked changes on radiograph, in one case in multiple joints, characterized by moderate osteophyte formation and joint collapse. Despite the severity of their clinical conditions, all horses in the trial exhibited reduced levels of the activated monomer form of MMP-9 and PGE-2 within the synovial fluid. As mentioned earlier, all involved horses also exhibited a change for the better in their demeanor, with an improved attitude.

Overall, the administration of curcumin orally to horses with osteoarthritis seemed to improve their overall function and attitude. It does appear that there is a more beneficial response in cases of lower grade osteoarthritis clinically than those higher grade patients with marked degeneration of the joint on radiograph. Despite a lowering of inflammatory cytokines within the synovial fluid, it is felt that there still exists a mechanical lameness attributed to the formation of osteophytes, eroded cartilage surface as well as soft tissue contracture associated with chronicity. In the short term of our trial, we appreciated some improvement clinically to the higher grade cases of osteoarthritis,

but the changes clinically were minimal. It is possible that with a more prolonged study or administration of curcumin, that some of the mechanical issues attributed to higher grades of joint degeneration could improve.

Curcumin appears to be a promising natural alternative to traditional NSAID's, intra-articular injections, hyaluronic acid and PGAG administration. The results are encouraging after oral administration and there appears to be minimal to no side effects. The powdered preparation was deemed palatable by the majority of our patients and can be improved by the addition of flavoring enhancers. Future areas of further exploration would include inflammation associated with other soft tissue injuries, possible usage in cases of enteritis/colitis, septic shock, navicular syndrome and laminitis.

References:

1. Anand, P.; Kunnumakkara, A.; Newman, R. and Aggarwal, B. Bioavailability of Curcumin: Problems and Promises. *Molecular Pharmaceutics* 2007, Vol xxx ,No. xxx
2. Kiuchi, F, Goto Y, Sugimoto N, Akao N, Kondo K, Tsuda Y. Nematocidal Activity of Turmeric: Synergistic Action of Curcuminoids. *Chem Pharm Bull* (Tokyo) 1993; 41:1640-3.
3. Goel A, et al., Curcumin as "Curecumin": From Kitchen to Clinic. *Biochem Pharmacol* 2007, doi:10.1016/j.bcp.2007.08.016.
4. Khanna, D, et al., Natural Products as a Gold Mine for Arthritis Treatment, *Current Opinion in Pharmacology* 2007, 7:344-351.
5. Thangapazham, RL, et al., Multiple Targets of Cancer Chemoprevention by Curcumin. *AAPS Journal*. 2006; 8(3): E443-E449.
6. Cheng, AL. et al., Phase I clinical Trial of Curcumin, A Chemopreventative Agent in Patients with High-Risk or Pre-Malignant Lesions. *Anticancer Res*. 2001;21:2895-900.
7. Lao, CD; Demierre, MF; Sondak, VK. Targeting Events in Melanoma Carcinogenesis for the Prevention of Melanoma. *Expert Rev Anticancer* Ther. 2006;6:1559-68.
8. Mobasheri, A., abstract on equine chondrocytes.
9. Personal communication; Ali Mobasheri, University of Knottinham, UK. 2008.
10. Aggarwal et. al., Curcumin- Biological and Medicinal Properties. *Turmeric: The Genus Curcuma*. 2006: 297-368.
11. Dodhar SD, Sethi R, Srimal RC. Preliminary Study on Antirheumatic Activity of Curcumin. *Indian J Med Res*. 1980;71:632-4.
12. Liacini, A., Sylvester, J., Li, WQ., and Zafarullah, M. Inhibition of Interleukin-1 Stimulated MAP Kinases, Activating Protein-1 (AP-1) and Nuclear Factor Kappa B (NF-κB) Transcription Factors Down Regulates Matrix Metalloproteinase Gene Expression in Articular Chondrocytes. Matrix Biol. 2002; 21(3): 251-262.