OA: Nutraceuticals/Dietary Supplements

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C′-CEO SLOWS POST-TRAUMATIC OA PROGRESSION AND RELIEVES OA-RELATED PAIN

D. Leong †, M. Choudhury †, R. Hanstein †, L. Xu †, D.M. Hirsh †, J.A. Hardin †, R.J. Majeska ‡, D.C. Spray †, M.B. Goldring §, N.J. Cobelli †, H.B. Sun †. † *Albert Einstein Coll. of Med., Bronx, NY, USA;* ‡ *The City Coll. of New York, New York, NY, USA;* § *Hosp. for Special Surgery, New York, NY, USA*

Purpose: There is currently no cure for osteoarthritis (OA) and no therapy that will slow or arrest progression of this disease. Our previous studies identified a transcriptional regulator, Cbp/p300 Interacting Transactivator 2 (CITED2) that mediates chondroprotection, at least in part by suppressing expression of proteases including the matrix metalloproteinase MMP-13 and the aggrecanase ADAMTS5. Using a targeted screening approach, we found four nutraceuticals (carvacrol [C′], curcumin [C], green tea polyphenol Epigallocatechin gallate [E], and oligomeric proanthocyanidins [O]), that individually increased the expression of CITED2 and reduced the expression of MMPs and ADAMTS in human chondrocytes in vitro. The objective of the present study was to determine the efficacy of the combination of these nutraceuticals (C′-CEO) in chondroprotection in vitro and in vivo using an OA disease model that includes symptom modification.

Methods: Human chondrocyte culture: Human chondrocytic cells (C28/I2) were treated with Carvacrol (C′, 1μM), Curcumin (C, 1μM), EGCG (E, 100μM), OPC (O, 50μg/ml), or C′-CEO (combination of all 4 compounds) for 24 hours in the presence of IL-1β (10ng/μl). Relative mRNA levels of MMPs 1, 3, 13, ADAMTS5, TNF-α, and CITED2 were analyzed by real-time PCR. n=6. * p<0.05 using one-way ANOVA and Tukey post-hoc test. Mice (C57BL/6, male, 6 months-old, n=8/group) were subjected to destabilization of the medial meniscus (DMM) and treated daily (7 days/week) with Carvacrol (C′: 50mg/kg), Curcumin (C: 50mg/kg), Epigallocatechin gallate (E: 5mg/kg), oligomeric proanthocyanidins (O: 50mg/kg), C′-CEO (combination of all 4 compounds), or vehicle for 8 weeks via oral gavage with naïve mice as an additional control. OA assessments: Arthritic pain was assessed at 8 weeks following DMM surgery using assays of von Frey (mechanical allodynia) and open field. OA severity was evaluated by OARSI scoring of Safranin O-stained tissue sections from decalcified, formalin-fixed hind limbs. Immunohistochemistry was used to detect cleaved aggrecan and type II collagen in the cartilage extracellular matrix and MMP-13, ADAMTS5, and CITED2 positive chondrocytes.

Results: In vitro, the combination treatment, C′-CEO, was more potent than any individual compound in reducing mRNA levels of MMP-3, MMP-13, ADAMTS-5 and TNF-α and increasing CITED2 mRNA (Fig 1), indicating a synergistic chondroprotective effect of the 4 compounds as a potential OA therapeutic. In vivo, while each individual compound reduced OA disease progression, C′-CEO-treated mice exhibited the lowest OA score and best preservation of the articular cartilage from thinning and surface damage compared to that observed in vehicle-treated DMM mice (Fig 2A). C′-CEO also protected against type II collagen and aggrecan cleavage, and reduced MMP-13 and ADAMTS5 immunostaining (Fig 2B). Furthermore, C′-CEO treatment restored the percentage of CITED2-positive chondrocytes from 28±3% (vehicle) to 55±6% (C′-CEO; C′-CEO vs. vehicle p<0.05), similar to that in naïve mice (51±3%, Fig 2C). With regards to pain, C′-CEO-treated DMM mice, like naïve controls, required a higher force to elicit paw withdrawal (p<0.05, Fig 3A), traveled a longer distance (p<0.05, Fig 3B), and increased rearing (p<0.05, Fig 3C), indicating that C′-CEO exerted a significant relief of pain symptoms related to OA.

Conclusions: A novel nutraceutical formulation, C′-CEO, increases levels of CITED2 and is effective in slowing OA progression and in relieving OA-related pain in an OA animal model. These findings provide a rational basis for advancing this product as an intervention for OA prevention and treatment.

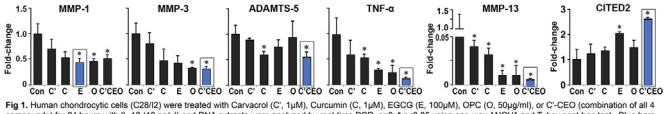

Fig 1. Human chondrocytic cells (C28/I2) were treated with Carvacrol (C′, 1μM), Curcumin (C, 1μM), EGCG (E, 100μM), OPC (O, 50μg/ml), or C′-CEO (combination of all 4 compounds) for 24 hours with IL-1β (10 ng/μl) and RNA extracts were analyzed by real-time PCR. n=6; * p<0.05 using one-way ANOVA and Tukey post-hoc test. Blue bars enclosed by rectangles indicate the highest efficacy among the treatments.

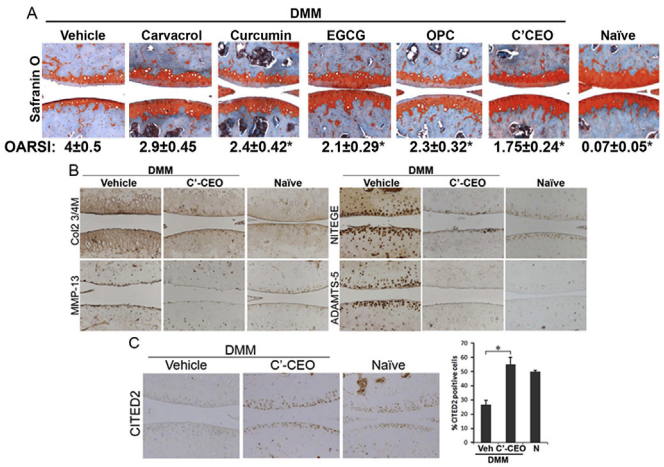

Fig 2. A. Carvacrol (C': 50mg/kg), Curcumin (C: 50mg/kg), EGCG (E: 5mg/kg), OPC (O: 50mg/kg), C'-CEO (combination of all 4 compounds), or vehicle was administered daily (7 days/week) for 8 weeks via oral gavage in DMM mice (C57BL/6, male, 6-months-old, n=8/group), with naïve as an additional control. OA severity assessed in Safranin O-stained sections by the OARSI guidelines. The OARSI score is a semi-quantitative histological scoring system with 0 indicating healthy cartilage and 6 representing severe cartilage erosion. Each result is presented as the mean OARSI scores of 8 animals/group ± standard deviation. *p<0.05, one-way ANOVA compared to vehicle-treated animals. B. Immunohistochemistry for cleavage epitopes of type II collagen (Col2 3/4M) or aggrecan (NITEGE), MMP-13, and ADAMTS-5 in the articular cartilage of vehicle or C'-CEO-treated DMM mice and naïve controls. C. Immunohistochemistry for CITED2 and % of CITED2 positive chondrocytes in the articular cartilage of vehicle- or C'-CEO-treated mice at 8 weeks following DMM and naïve (N) controls.

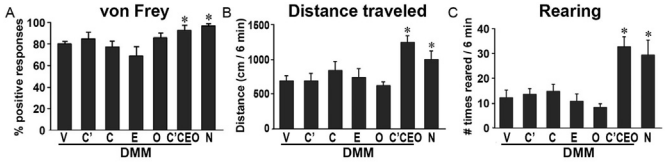

Fig 3. Arthritic pain was assessed in mice treated as described in Fig 2. At 8 weeks after DMM, OA-associated pain was assessed using assays of von Frey (mechanical allodynia) and open field. Briefly, the von Frey test consisted of exposing the hind paw to von Frey filaments in ascending order. Groups were evaluated using the number of withdrawal responses normalized to baseline. The open field behavioral test measured the distance (cm) traveled and frequency of rearing (standing on hind limbs) in 6 min. *p<0.05 vs. vehicle.

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THE EFFECTS OF TREATMENT ON DISEASE SYMPTOMS AND PROGRESSION OF STRUCTURAL CHANGES IN KNEE OSTEOARTHRITIS: PARTICIPANTS FROM THE OSTEOARTHRITIS INITIATIVE PROGRESSION COHORT

J. Martel-Pelletier †, C. Roubille †, J. Raynauld †, F. Abram ‡, M. Dorais §, J. Pelletier ‖. † *Univ. of Montreal Hosp. Res. Ctr., Montreal, QC, Canada;* ‡ *Imaging Res. & Dev., ArthroLab Inc, Montreal, QC, Canada;* § *StatSci. Inc., Notre-Dame de l'Île-Perrot, Quebec, QC, Canada;* ‖ *Osteoarthritis Res. Unit, Univ. of Montreal Hosp. Res. Ctr., Montreal, QC, Canada*

Purpose: The impact of meniscal extrusion on response to treatment could be a clinically relevant concern in the context of personalized management of osteoarthritis (OA).

The aim of this study was to evaluate the role of meniscal extrusion on the effects of conventional OA pharmacological treatment and those of glucosamine and chondroitin sulfate (Glu/CS) on knee structural changes, using data from the progression cohort of the OA Initiative (OAI).

The OAI allows the study of potential disease-modifying OA drug (DMOAD) effects in patients over time following the evolution of OA structural changes and the associated risk factors.

Methods: Participants (n=600) were selected from the OAI progression cohort (n=1,390) if they had 24 consecutive months follow-up with complete radiographic and magnetic resonance imaging (MRI) data for the most symptomatic knee based on the highest WOMAC pain score at the onset of the study (Time [T] 0).

Those participants were stratified into two main groups based on whether (+) or not (-) standard pharmacological treatment for OA (analgesics and non-steroidal anti-inflammatory drugs [NSAIDs]) was taken for disease symptoms over a continuous period of 24 months