Therapeutic Interventions to Inhibit Cartilage Degeneration, Promote Cartilage Repair, and Prevent the Development of Post-Traumatic OA.

ABSTRACT INTRODUCTION

Joints are subjected to a variety of insults including but not limited to injury, improper alignment, excessive weight, excessive activity, or repetitive mechanical demand. These injuries may go untreated resulting in cartilage wear and eventually lead to the development of post-traumatic osteoarthritis (PTOA). Additionally, osteoarthritis (OA) is the most common joint disease and among the leading causes of pain, disability and economic loss in all populations. The financial burden on society by PTOA constitutes approximately $3.06 billion annually, or about 0.15% of the total U.S. health care direct cost. The identification and development of therapeutic interventions aimed to prevent or delay the onset of the disease. Previous studies have provided valuable insight on what happens to cartilage after injury and the mechanisms that govern the progression to PTOA. Our objective was to determine when and how it could be prevented or cured by comparing varying classes of biologic interventions in an attempt to design a novel treatment algorithm based on the time and type of cellular responses in a human cartilage ex vivo acute injury model.

MATERIALS AND METHODS

Twelve normal human tali, age 16-58 years old, obtained from organ donors through the Gift of Hope Organ and Tissue Donor Network were impacted using a 4mm cylindrical indenter with 600N following a model previously described. Cartilage explants of 8mm diameter comprising the impacted core and the immediately adjacent non-impacted ring were removed from the joint and cultured for 14 days in media containing 5% fetal bovine serum (FBS) with or without pro-anabolic or anti-catabolic agents. Treatment groups consisted of 1) Impacted control (serum only), 2) Non-impacted control; 3) Impaction + BMP-7/OP-1, 100ng/ml, 4) Impaction + IL-1 receptor antagonist (IL-RA), 100ng/ml, 5) Impaction + IL-RA, 20ng/ml, 6) Impaction + tumor necrosis factor-α (TNF-α) antagonist, 100ng/ml, 7) Impaction + N-Acetyl-L-Cysteine (NAC), 2.5mM, 8) Impaction + Z-VAD-FMK pan caspase inhibitor, 100μM, 9) Impaction + Q-VD-OPh pan caspase inhibitor, 100μM. All treatments were administered on day 0 immediately after impaction and maintained for 48 hours. At day 2, all treatments were replaced with culture media containing 5% FBS only. Tissue and media samples were collected on days 0, 2, 7, and 14 to assess cell survival and metabolic activity. Samples were analyzed by live/dead assay, apoptosis (Tunel assay), matrix integrity (Safranin O staining, Mankin score), and proteoglycan synthesis and content.

RESULTS

A single impact to human articular cartilage resulted in cell death/apoptosis within the impacted core, which if untreated, expanded to the adjacent non-impacted ring. The anti-catabolic treatments were pan caspase inhibitors, anti-TNF-α, IL-RA, NAC. Both pan-caspase inhibitors had no significant effect on cell viability assessed by live/dead and Tunel assays. However, cells that survived impaction after the treatment with pan caspase inhibitors showed elevated PG synthesis and content, especially in the second half of culture between days 7 and 14. This resulted in preservation of matrix integrity (Fig.1) more so after the treatment with Z-VAD-FMK inhibitor. Anti-TNF-α treated samples showed an increase in cell death with a normal pattern of Safranin O staining, cell viability (live/dead assay), apoptosis (Tunel assay), matrix integrity (Safranin O staining, Mankin score), and proteoglycan synthesis and content.

NAC treated explants showed a two-fold decrease in percentage of apoptotic cells in the superficial layer at day 2 in comparison with the untreated impacted control. However, as NAC was removed from culture, the level of apoptosis reached the untreated impacted control levels. Similar observations were found for PG synthesis that peaked at day 2 and returned to the levels of the impacted control as the treatment was removed. Pro-anabolic growth factor BMP-7/OP-1 promoted cell survival, cartilage homeostasis and repair. At day 2, the percentage of viable cells in the superficial layer of the impacted region was 50% higher than in the corresponding control; at days 7-14 it was 2-fold higher. In the region adjacent to the impaction, cell survival was at the level of a normal intact non-impacted control. BMP-7 also induced PG synthesis and overall PG content. At day 2, PG synthesis was higher by about 25% in treated explants. This effect was sustainable for up to 7 days, but then returned to the levels of controls suggesting a direct correlation between treatment duration and outcome. PG content in the BMP-7 group was two-fold higher at day 2 than in the untreated control and remained higher during the entire culture period. An anti-catabolic agent QVD+P188 combined with BMP-7 resulted in preservation of matrix integrity and a normal pattern of Safranin O staining (Fig.1).

DISCUSSION

The ideal therapy to arrest and prevent the development and progression of PTOA must be multi-varied and include anabolic effects on chondrocyte metabolism and anti-catabolic treatments to prevent chondrocyte death and matrix loss. More specifically, the following are the key mechanisms that should constitute the basis for the design of intervention therapies: 1) Chondroprotection; 2) Anti-inflammatory; 3) Matrix protection; and 4) Pro-anabolic, stimuli of cartilage remodeling and regeneration. The most beneficial agents are those that target multiple pathways and mechanisms. Previous studies from our laboratory suggest the immediate use of P188 as a membrane sealant after acute cartilage trauma. Our current findings show that BMP-7/OP-1 possesses a wide variety of pro-anabolic and anti-catabolic activities, and support the idea of giving BMP-7 more than once to target various pathways and to ensure the sustainability of the effect. Our findings also support TNF-α antagonist as an agent with the potential to strengthen therapeutic effects of PTOA therapy. TNF-α antagonist seems to be the most appealing of tested anti-catabolic agents due to its ability to target more than one mechanism and its long-lasting effect even after the removal of the agent. In summary, future PTOA therapies will be most effective if the multi-mechanistic approach is utilized. Specifically, a triad of P188, BMP-7/OP-1, and TNF-α antagonist may show promise for rapid translation of our results in to clinic since all three factors have already been approved by the FDA for other use.

SIGNIFICANCE

Investigating the utility of pro-anabolic and anti-catabolic agents administered after acute articular cartilage trauma provides the basis for novel targeted therapeutic approaches to stimulate proper cartilage repair. Our results suggest an innovative combination of known biologies to treat post-traumatic cartilage degeneration.

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REFERENCES
