Assessment of cellular, biochemical, and histologic effects of bipolar radiofrequency treatment of canine articular cartilage

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Objective—To assess the cellular, biochemical, and histologic effects of bipolar radiofrequency-generated heat on canine articular cartilage.

Sample Population—Articular cartilage explants (n = 72) from 6 canine cadavers and cultured articular chondrocytes from 5 canine cadavers.

Procedure—Cartilage explants were randomly assigned to receive no treatment or treatment with focal (3 seconds) or diffuse bipolar radiofrequency. Following treatment, methylene blue permeability assay was performed in the cartilage explants, and remaining samples (60) were cultured. Immediately and 5, 10, and 20 days after treatment, cultured explants were assessed for glycosaminoglycan (GAG) and collagen contents, type II collagen and matrix metalloproteinase (MMP)-13 immunoreactivity, and modified Mankin histologic scores. Liquid culture media were collected every 4 days and GAG content measured. Additionally, cultured chondrocytes were exposed for 3 seconds to media preheated to 37°C, 45°C, or 55°C. Cell viability was determined via 2 different assays immediately and 24 hours after treatment.

Results—Radiofrequency-treated cartilage had reduced permeability and considerable histologic damage, compared with control samples; most treated samples had reduced collagen II staining and increased MMP-13 immunostaining. Compared with other treatments, less GAGs were released from cartilage after diffuse radiofrequency treatment throughout the study period. Cell viability was significantly different between controls and cells treated at 55°C immediately and 24 hours after heat treatment.

Conclusions and Clinical Relevance—In this study, bipolar radiofrequency treatment had detrimental effects on normal articular cartilage cells and extracellular matrix with probable long-term clinical consequences. The usefulness of radiofrequency for treatment of osteoarthritic articular cartilage requires further investigation. (Am J Vet Res 2004;65:604–609)

Osteoarthritis is a complex disease involving gross, histologic, biochemical, and biomechanical changes in the articular cartilage and supporting tissues that elicit an irreversible cycle of inflammation and degradation leading to signs of pain, swelling, and dysfunction. There is no cure for osteoarthritis. Treatment strategies are aimed at palliation of clinical signs. Successful treatment of osteoarthritis is typically associated with decreased signs of pain, reduction in the progression of the disease, and improvement in the affected individual's function and quality of life. Palliative or symptomatic surgical procedures are directed at reducing inflammation in the joint, slowing degradation of the articular cartilage, and improving biomechanical function. Arthroscopic methods for palliative surgical treatment of osteoarthrosis have received great attention and include abrasion arthroplasty, subchondral bone penetration procedures, and thermal chondroplasty.

Abrasion arthroplasty and subchondral bone penetration techniques can induce ingrowth of reparative fibrocartilage. This repair tissue is biomechanically inferior to normal articular cartilage and has been reported to undergo substantial degradation within 2 years after surgery in humans. Data have suggested that these techniques may not significantly improve long-term functional outcome.

Because of the limitations of other currently available techniques, radiofrequency thermal chondroplasty has recently been applied arthroscopically as an innovative, palliative treatment for osteoarthrosis. Radiofrequency-generated heat can be delivered arthroscopically to osteoarthritic cartilage to ablate fibrillated cartilage and create a smoother appearance of the articular surface. Smoothing fibrillated cartilage may provide immediate improvement in the mechanical integrity and function of the treated cartilage. Biomechanical modification of the cartilage may subsequently restore joint function, thereby delaying disease progression and improving an individual's overall function and quality of life. Compared with abrasion arthroplasty, radiofrequency chondroplasty appears to have potential advantages. In a model of condromalacia in sheep, Turner et al compared bipolar radiofrequency arthroplasty to abrasion arthroplasty for treatment of experimentally roughened articular cartilage. In that study, subjective histologic scoring of harvested tissue was performed and treated limb scores were compared with the contralateral untreated limb scores in each animal. Overall, radiofrequency treatment resulted in 14% favorable responses and 36% equivocal responses, compared with untreated controls. In contrast, the abrasion technique yielded 0% favorable responses and 7% equivocal responses, compared with untreated controls. However, results of that study also included a high unfavorable response rate associated with radiofrequency treatment of articular cartilage. Staining of the extracellular matrix of the cartilage specimens with safranin-O fast green revealed 50% and 57% unfavorable response rates in the radiate and transitional zones of the radiofrequency-treated cartilage, respectively.

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Data collected regarding radiofrequency thermal chondroplasty has been primarily focused on its acute effects on chondrocyte viability in treated tissue and the ultrastructural appearance of the tissue immediately after treatment. In general, these studies have indicated that radiofrequency-generated heat can smooth the surface of fibrillated cartilage, but causes immediate loss of chondrocyte viability; the extent of the loss of viability depends on the generator type (bipolar vs monopolar), probe size, and generator setting (which influences the amount of heat and energy delivered) used to administer the treatment. Although results of many of these studies suggest that monopolar devices may have less detrimental effects on chondrocyte viability, other investigators have reported that bipolar devices produce less tissue heat and less damage. Debate continues regarding the relative merits of various generator types and settings; however, a consistent finding among studies is that the amount of heat typically used to smooth cartilage with any of these devices in a clinical setting (ie, > 65°C) also kills cells.

Although chondrocyte viability in response to radiofrequency treatment has been extensively studied, the nature of the cellular response to radiofrequency treatment has not been fully elucidated. Loss of chondrocyte viability may be a result of the heat generated by radiofrequency treatment or an effect of factors associated with radiofrequency energy or both. Determination of changes in chondrocyte viability in response to heat alone (at temperatures reached in radiofrequency-treated tissues) may help to identify the factors responsible for alterations in cell viability and function after radiofrequency treatment.

The extracellular matrix of articular cartilage is a complex structure comprised primarily of collagens, aggregated proteoglycans, and water. The extracellular matrix is synthesized and maintained by the chondrocytes. It is solely responsible for providing the compressive and tensile strength of articular cartilage; its integrity depends on a delicate balance between synthesis and degradation of the key matrix components, which is mediated by mechanical forces, soluble mediators, and degradative enzymes. Disruption of this balance can produce specific changes within the extracellular matrix that may induce the biomechanical alterations and cartilage destruction patterns observed in clinical cases of osteoarthritis. In terms of basic science, data regarding radiofrequency treatment of articular cartilage are lacking. To the authors' knowledge, the effects of radiofrequency-generated heat on articular cartilage with respect to changes in biochemical, histologic, and biomechanical variables have not been investigated. Such studies are needed to evaluate the immediate and long-term changes that develop in the cells and the extracellular matrix of radiofrequency-treated cartilage. The purpose of the study reported here was to assess the cellular, biochemical, and histologic effects of bipolar radiofrequency-generated heat on articular cartilage. We hypothesized that radiofrequency treatment immediately alters the metabolism of chondrocytes and the integrity of the matrix and that these effects are exacerbated over time. Furthermore, we proposed that the effects of radiofrequency treatment on these cells are heat-dependent and become evident via stress-activated, cell-signaling pathways.

Materials and Methods

Radiofrequency treatment—The forelimbs of 6 adult mixed-breed dogs were removed immediately after euthanasia (performed by reasons unrelated to this study) by use of an overdose of pentobarbital and phenytoin administered IV. The dogs were 1 to 3 years of age and mean weight was 28.9 kg. Limbs were disarticulated at the shoulder joint; the proximal portion of the humerus was further isolated by dissection and ostectomy at the junction of metaphysis and diaphysis. Six-millimeter-diameter templates (n = 72) were created on the caudal weight-bearing surface of the articular cartilage and randomly assigned to 1 of 3 treatment groups; cartilage specimens received no treatment (control group), local treatment for 3 seconds with bipolar radiofrequency, or diffuse treatment with bipolar radiofrequency (brushstroke treatment). Radiofrequency energy was delivered through a commercially available bipolar radiofrequency generator at a generator setting of 90 W (default setting) with an end-effect arthroscopic electrode in a noncontact mode, based on clinical recommendations. For each treatment group, the proximal portion of the humerus was placed in 100 mL of physiologic saline (0.9% NaCl) solution that was maintained at 25°C. For the focal bipolar radiofrequency treatment group, radiofrequency energy was applied through a handheld probe placed at the center of the template (treatment duration, 3 seconds). For the diffuse bipolar radiofrequency treatment group, radiofrequency energy was applied through a handheld probe that was moved at a rate of approximately 1 mm/s over the entire surface area of the template in a brushstroke fashion to mimic typical clinical application technique. To determine the effects of radiofrequency energy on cartilage permeability, 12 of the cartilage templates (4/treatment group) were left on the bones after treatment for methylene blue permeability assay. All other cartilage templates (n = 60) were removed from the subchondral bone as full-thickness cartilage explants and cultured in RPMI 1640 culture medium containing 10% fetal bovine serum and antimicrobials (penicillin [100 U/mL], streptomycin [100 µg/mL], and amphotericin [2.5 µg/mL]). Explants were cultured in 24-well tissue culture plates at 37°C with 5% carbon dioxide. The liquid medium was replenished every 4 days.

Sample collection—On days 0 (ie, immediately after treatment), 5, 10, and 20, cultured explants were collected and processed to determine glycosaminoglycan (GAG) content, collagen content, type II collagen and matrix metalloproteinase (MMP)-13 immunoreactivities, and modified Mankin histologic scores. The cartilage tissue in each well was divided into 2 equal portions. One portion was placed in neutral-buffered 10% formalin for processing for routine histologic evaluation; the other portion was lyophilized, weighed (dry weight recorded in grams), and then stored at −80°C for subsequent GAG and hydroxyproline assays. Liquid culture media were collected every 4 days and stored at −80°C for subsequent GAG assay.

Histologic evaluation—After routine processing, 5-µm sections were cut and stained with H&E and toluidine blue. All stained sections were examined via light microscopy by 2 investigators (J.L.C., K.Ku.) who were unaware of the treatment applied to the cartilage in each section; cartilage sections were subjectively scored for surface defects, cell number and morphologic features, and extracellular matrix staining by use of the modified Mankin scoring system.
Immunohistochemical evaluation—Unstained 5-µm sections were deparaffinized in xylene, rehydrated in graded ethanol solutions, and washed in 50 mM Tris buffered saline solution (pH 7.6) + 0.15 M NaCl. Sections were evaluated immunohistochemically by use of polyclonal anti-type II collagen (rabbit X bovine) and monoclonal anti-MMP-13 (Ab-1, mouse X human) antibodies, as described.28 All immunohistochemically stained sections were examined by light microscopy by 2 investigators (J.L.C., K.K.U) who were unaware of the treatment applied to the cartilage in each section; cartilage sections were subjectively assessed for the presence and intensity of MMP-13 and type II collagen immunoreactivities.

Glycosaminoglycan assay—Total sulfated GAG content was quantified by dimethylmethylen blue assay,15 as described.31 Total GAG concentration in samples of the liquid medium was reported as micrograms per milliliter. Results for cartilage explant samples were standardized to correct for differences in sample weights. Total GAG concentration for explant samples was reported as micrograms per milliliter per gram (dry weight).

Hydroxyproline assay—Total collagen content can be extrapolated from results of the hydroxyproline assay,21 as described.21 Total hydroxyproline concentration in samples of the liquid medium was reported in micrograms per milliliter. Results for cartilage explant samples were standardized to correct for differences in sample weights. Total hydroxyproline concentration for explant samples was reported as micrograms per milliliter per gram (dry weight).

Assessment of chondrocyte viability and response mechanisms—Chondrocytes were isolated from the humeral heads of 5 healthy dogs immediately after euthanasia (performed for reasons unrelated to this study) by use of an IV overdose of pentobarbital and phenytoin. Each chondrocyte line was cultured in monolayer with RPMI 1640 culture medium containing 10% fetal bovine serum and antimicrobials (penicillin [100 U/mL], streptomycin [100 µg/mL], and amphotericin [2.5 µg/mL]). At 50% confluency, each chondrocyte line was exposed for 3 seconds to medium that had been preheated to 45° or 55°C. Cells cultured in medium at 37°C were used as controls. The trypan blue exclusion assay was used to assess cell viability immediately and 24 hours after thermal treatment. An equal volume of trypan blue dye solution (0.4%) was added to each aliquot of cells. Cell solutions were loaded into a hemocytometer, and 100 cells were evaluated for dye uptake. The percentage of viable cells (those that did not take up the trypan blue dye) was calculated.

Colorimetric microassay was also performed immediately and 24 hours after heat treatment. Equal numbers (105) of cells in 100 µL of medium were placed in each well of a 96-well plate. Immediately after thermal treatment, 20 µL of a tetrazolium salt (MTS; dimethylthiazol, carbosxymethoxyphenyl, sulfophenyl, tetrazolium, inner salt) with an electron coupling agent (PMS; phenazine methosulfate) was added to each well according to the manufacturer’s instructions and incubated in 5% carbon dioxide and 95% humidity for 1 hour. Absorbance at 490 nm was determined for each well by use of a spectrophotometric micro plate reader.1 The absorbance value is directly correlated with the number of viable cells in each well; therefore, the absorbance values were used for comparisons among treatment groups with respect to viability for the colorimetric assay.

Statistical analyses—All statistical analyses were performed by use of a computer software program.6 Data from each group were pooled, and mean values ± SD were determined. One-way ANOVA with Tukey’s post hoc procedure was used to determine differences in GAG release into samples of medium, cell viability and GAG and collagen contents of cartilage specimens, and modified Mankin scores among and within treatment groups. Spearman correlation coefficients were used to measure correlations among assessments. Values of P < 0.05 were considered significant.

Results

Cartilage permeability—Bipolar radiofrequency energy delivered via the focal and diffuse methods caused significant (P < 0.05) reductions in depth of methylene blue penetration (a measure of cartilage permeability; data not shown).

Histologic effects—Significant (P < 0.001) tissue damage (as measured by modified Mankin histologic score; Fig 1) was observed immediately after treatment and throughout the study period in both radiofrequency-treated groups (Fig 2). Histologically, radiofrequency-induced damage was evident as surface changes...
ranging from small fibrillations to large defects extending into the transitional zone) and loss of cell viability (empty lacunae, pyknosis, and karyolysis).

**Glycosaminoglycan content and release**—Total GAG content of articular cartilage explants was largely unchanged during the study period. However, significantly ($P < 0.05$) higher GAG concentration was detected on day 20 in cartilage that had been treated focally with radiofrequency (Table 1). Assessment of GAG turnover revealed significantly ($P < 0.05$) less GAG release from cartilage into the culture medium after diffuse radiofrequency (brush-stroke) treatment throughout the study period (Fig 3), compared with GAG release from focally treated cartilage and controls.

**Table 1**—Mean glycosaminoglycan content ($\mu$g/mL/g) in cartilage explants (obtained from 6 dogs) immediately (day 0) and 5, 10, and 20 days after receiving no treatment (control group; $n = 20$ explants [5/collection day]), focal treatment for 3 seconds with bipolar radiofrequency (20 explants [5/collection day]), or diffuse treatment (brushstroke treatment) with bipolar radiofrequency (20 explants [5/collection day]).

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Treatment group</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 20</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td>210.5</td>
<td>193.3</td>
<td>180.4</td>
<td>169.9</td>
</tr>
<tr>
<td>Focal</td>
<td></td>
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<td>188.0</td>
<td>186.6</td>
<td>219.2*</td>
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<tr>
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<td>213.8</td>
<td>182.7</td>
<td>183.2</td>
<td>184.4</td>
</tr>
</tbody>
</table>

*Value significantly ($P < 0.05$) different from other groups at the same time point.

**Evaluation of collagen and MMP-13**—Total collagen content of the cartilage receiving diffuse radiofrequency treatment was significantly ($P < 0.001$) lower than that of untreated control samples on day 5 and remained lower (albeit not significantly) than that of untreated control samples on days 10 and 20 (Fig 4). When type II collagen was localized by use of immunohistochemical techniques and assessed subjectively, most of the radiofrequency-treated samples had diminished collagen staining, compared with that of untreated control samples. An increase in MMP-13 immunostaining was consistently detected in radiofrequency-treated cartilage, compared with that of untreated control samples (Fig 5).

**Chondrocyte viability**—Immediately after treatment, viability (as assessed by trypan blue exclusion) was not significantly ($P = 0.332$) different among groups. After 24 hours, the viability of chondrocytes treated with medium heated to $55^\circ$C was significantly ($P = 0.020$) lower than that of chondrocytes in control
cytes in control samples and that of cells treated at 55°C. However, during such treatment, radiofrequency-generated heat produces temperatures at which cells are killed and extracellular matrix is altered. Viability of cells can be lost at ≥ 45°C, the minimum tissue temperature reported in articular cartilage from 5 dogs immediately and 24 hours after exposure to culture media heated to 37°C (control; black bars), 45°C (gray bars), or 55°C (white bars). *Significant (P < 0.001) difference was present between control cells and cells treated with medium at 55°C both at immediately (time 0) and 24 hours after heat treatment. No significant differences were detected between 37°C-treated and 45°C-treated groups or between 45°C-treated and 55°C-treated groups.

**Discussion**

Radiofrequency treatment of articular cartilage in osteoarthritic joints is an attractive treatment option because of its minimally invasive method of delivery, its immediately evident effects on fibrillated cartilage, and some clinical data regarding its benefits for patients. However, during such treatment, radiofrequency-generated heat produces temperatures at which cells are killed and extracellular matrix is altered. Viability of cells can be lost at ≥ 45°C, the minimum tissue temperature reported in articular cartilage during radiofrequency treatment is approximately 55°C. In the study of this report, we attempted to differentiate between effects of heat and effects of radiofrequency-generated heat by assessing chondrocyte viability (via 2 methods) after exposure of articular cartilage to media heated to different temperatures.

Trypan blue exclusion is a cell viability assay that is based on the ability of the plasma membrane of live cells to exclude the vital dye trypan blue. Because the loss of membrane integrity usually occurs in the final stages of apoptosis, cells in the early stages of the process may retain their ability to exclude the vital dye and are scored as viable cells by this assessment technique. In the colorimetric assay of cell viability used in our study, the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) is reduced by viable cells to a formazan product that is soluble in DMSO. The absorbance of this formazan product is indicative of cell mitochondrial activity; results of immunohistochemical assessment of the presence of MMP-13 in radiofrequency-treated articular cartilage supported this. In addition, chondrocyte nutrition, cell signaling, and biomechanical function require the matrix to be permeable to be effective, and reductions in permeability may have detrimental effects on these biological processes leading to devastating changes in cell and tissue function over time. Interestingly, the biochemical changes detected in the study of this report that were attributable to alteration of tissue permeability may have both potentially positive (eg, decreased GAG release) and negative (eg, histologic damage, increased MMP-13 immunoreactivity, and collagen loss) effects. However, the pathophysiologic and clinical consequences of these findings have not been determined, and before comprehensive statements regarding
the biochemical effects of radiofrequency treatment of osteoarthritic cartilage can be made, further in vitro and in vivo investigations would be required.

As expected with a study of this design, more questions have been created than answered by its completion. The influence of the generator setting and delivery technique selected on outcome, indications and contraindications for radiofrequency treatment, and the potential beneficial and detrimental long-term clinical effects of its application still must be fully investigated. In our opinion, it is important to correlate the histologic, biochemical, and biomechanical data from in vitro and in vivo investigations of radiofrequency thermal chondroplasty to efficiently and effectively evaluate the usefulness of this technique for successful treatment of osteoarthritis. The data from the study of this report may be helpful in determining avenues of research in this area to better understand the scientific basis of this technique and assess its application.

References