Assessment of collagen integrity in articular cartilage by using magnetization transfer imaging: In vivo application to the goat knee

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Introduction

Among leading theories, structural damage to the collagen network of articular cartilage may play a major role in the etiology of osteoarthritis (OA). Assuming that both the collagen content and its degree of cross-linking varies with location, age and pathology, the resulting change in its swelling property is likely to influence functional integrity of cartilage. Magnetization transfer (MT) imaging shows a lot of promise as a method that can non-invasively examine the severity of a cartilage abnormality with respect to its high content of collagen (1, 2). By selectively saturating the broad signal of macromolecule bound water, the MRI observable resonance of the bulk water is attenuated, the degree of signal reduction depending on the exchange rate. In this study, we applied MT imaging in order to investigate 1) the role of collagen concentration on the saturation transfer, 2) the influence of collagen type and the exact contribution of proteoglycans (PG) to the MT effect, 3) the effect of enzyme treatments on collagen integrity and 4) its *in vivo* applicability to the goat knee.

Methods

Preliminary experiments were performed *in vitro* on phantoms made of collagen (type I or II) or chondroitin sulfate (CS) as well as *ex vivo* on cartilage plugs. For consistency reasons, MT measurements were done using the same imaging parameters as for *in vivo* experiments. Of importance to note, type I collagen gels were made as an acid soluble solution at concentrations varying from 0% to 20% w/v, while type II collagen solutions were prepared by proteolytic hydrolysis. Bovine articular and nasal plugs (8-mm diameter) were made using a dermal punch and differentially treated for 24-h with either trypsin or bacterial collagenase (Bac CLN). As an *in vivo* model of cartilage degradation, an intra-articular injection of papain (30 to 390 units/knee) was performed in the goat knee the day prior to MRI. On the day of MRI, the goat was intubated and kept under 2% isoflurane in O_2 anesthesia. MRI experiments were conducted at room temperature in a 3T/60cm Bruker magnet equipped with a 33-cm i.d. actively shielded gradient insert. The RF antenna was a home-built semi-circular Alderman-Grant resonator with an i.d. of 14 cm. It was designed open to the side to accommodate the knee of large animals. Single 2-mm thick slices were obtained using a 2D gradient-recalled standard sequence with a TE of 7.6 ms, a TR of 200 ms, a sinc pulse of 3 ms, 4 averages and spatial resolution in plane of 312 μm^2. For measurements on the live goat, the position of the coronal section was adjusted from a previous series of sagittal and transverse scout images. MT contrast was obtained using a pre-saturation 50-ms pulse operated with a 210 Hz bandwidth and directed 1 kHz off-resonance from the bulk water signal. This off-resonance frequency was chosen based on preliminary measurements demonstrating the largest difference in terms of the MT ratio (MTR) between collagen and water samples while other macromolecules (i.e., CS) had a minimum contribution to the MT effect. The amount of MT was quantified by calculating $MTR=((M_0-Ms)/M_0)$ with M_0 and Ms referring to signal intensity (SI) before and after saturation, respectively. Since it is generally accepted that signal attenuation follows a first-order rate process, we also measured the rate constant k for MT in cartilage plugs and the goat knee, as described previously (3). With a similar imaging setup but varying pre-saturation times (24, 50 and 100 ms), k was derived from the initial slope of the exponential decay $Ms/M_0=(1+kT_1)$. Biochemical determinations of collagen and PG contents in cartilage plugs were performed using the OH-proline and safranin O methods, respectively. Data are presented as means±SEM.

Results

MTR was found to increase as a power function of type I collagen concentration ($y=0.88x^{0.43}$, $r^2=0.90$). Up to a concentration of 10% w/v, the contribution of CS to the MT effect can be considered as negligible. Type II collagen exhibited a lower MT effect than measured in type I collagen samples at similar concentrations. This may be due to structural differences between the two types of collagen or bias in the phantom preparation that affected levels of hydration. The magnetization rate as well varied with the type I collagen concentration ($y=0.58x^{0.43}$, $r^2=0.98$). For a 50% decrease in type I collagen content (ie. from 10 to 5% w/v), a ~35% drop (1.59 to 1.05 s^{-1}) in the apparent k could be detected. When applied to bovine cartilage plugs, trypsin treatment did not result in obvious changes of MTR (trypsin: 0.33±0.04 vs. control: 0.36±0.03, NS). However, a ~50% drop in the MT effect was measured in articular cartilage when treated with Bac CLN (MTR=0.14±0.04, vs. control $p<0.05$). The MTR for bovine nasal cartilage was consistently lower (20 to 30%) as compared with articular cartilage. None of the enzymatic treatments changed MTR in the thicker nasal cartilage disks during 24h incubation. These data are in good agreement with biochemical assessments of collagen in articular cartilage. Of interest to note, both trypsin and Bac CLN treatments resulted in significant PG depletion. Fig. 1 shows a typical MTR image series obtained with various MT pulse lengths and used to calculate the *in vivo* rate constant k. In average, ~40% signal attenuation (MTR=0.35±0.01, n=3) was measured in articular cartilage of the normal knee. The corresponding magnetization rate was k=3.18±0.25 s^{-1}. These values remained unchanged when the knee was injected with 30 papain units (MTR=0.34±0.02, k=3.09±0.08 s^{-1}, n=3). However a slight decrease was observed in goats that received 100 units (MTR=0.31±0.02, n=2; k=2.60 s^{-1}, n=1), an effect that became even more pronounced in goats pre-injected with 390 units (MTR=0.22±0.01, n=2). Interestingly, additional measurements based on $Gd(DTPA)^{2-}$-enhanced MRI (4) have shown significant papain-induced PG depletion in the articular cartilage of these goats even when only 30 papain units were injected.

Discussion

We have demonstrated that MTR is dependent on collagen concentration (see data obtained on phantoms and cartilage plugs). This result is in line with data from Wachsmuth et al. (5). Considering that PG only marginally contributes to MT contrast, MT imaging specifically probes on collagen status. However, this technique may be relatively insensitive to the small change of collagen content normally observed in OA tissue (~5%). In fact, any large variation of MTR should be attributed to factors like structural changes of collagen, as suggested from data obtained on type I and type II collagen phantoms. In the goat experiment, while PG degradation certainly followed papain treatment, our study also reported change in collagen integrity when using high amounts of papain (>30 units/goat). Promising results on the apparent magnetization exchange rate have also shown good sensitivity to the extent of the damage. We conclude that *in vivo* MT measurements may be applied for the routine evaluation of collagen integrity in normal and abnormal articular cartilage.

References

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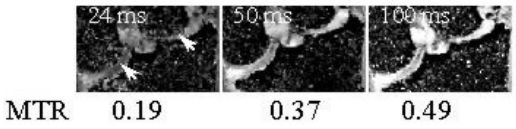

Fig.1 Effect of varying MT pulse length on articular cartilage of a normal goat knee