Eur Radiol (2009) 19: 1512–1518
DOI 10.1007/s00330-008-1286-1

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Glucosamine sulfate effect on the degenerated patellar cartilage: preliminary findings by pharmacokinetic magnetic resonance modeling

Received: 16 July 2008
Accepted: 25 November 2008
Published online: 13 February 2009
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Abstract Normal and degenerated cartilages have different magnetic resonance (MR) capillary permeability (K^{trans}) and interstitial interchangeable volume (v_e). Our hypothesis was that glucosamine sulfate treatment modifies these neovascularity abnormalities in osteoarthritis. Sixteen patients with patella degeneration, randomly distributed into glucosamine or control groups, underwent two 1.5-Tesla dynamic contrast-enhanced MR imaging studies (treatment initiation and after 6 months). The pain visual analog scale (VAS) and American Knee Society (AKS) score were used. A two-compartment pharmacokinetic model was used. Percentages of variations (postreatment-pretreatment/pretreatment) were compared (*t*-test for independent data). In the glucosamine group, pain and functional outcomes statistically improved (VAS: 7.3±1.1 to 3.6±1.3, $p<0.001$; AKS: 18.6±6.9 to 42.9±2.7, $p<0.01$). Glucosamine significantly increased K^{trans} at 6 months (−54.4±21.2% vs 126.7±56.9%, $p<0.001$, control vs glucosamine). In conclusion, glucosamine sulfate decreases pain while improving functional outcome in patients with cartilage degeneration. Glucosamine sulfate increases K^{trans}, allowing its proposal as a surrogate imaging biomarker after 6 months of treatment.

Keywords Pharmacokinetics · Cartilage · Glucosamine · Osteoarthritis · MR

Introduction

Patellar cartilage degeneration is one of the leading causes of knee pain and physical disability, impairing the quality of life in industrialized countries. Degeneration results in chronic pain and diminished functionality, leading finally to osteoarthritis with social limitations [1].

Different pathophysiologic processes serve as targets of behavioral and pharmacologic interventions. Nowadays, cartilage degeneration can be tracked with several imaging biomarkers, such as measurements of cartilage volume, thickness, T1 and T2 relaxation times and delayed enhancement after intravenous contrast media administration. Actual pharmacokinetic biomarkers of cartilage abnormalities can be efficient even in the early stages of the disease [2]. These imaging biomarkers of cartilage degeneration may be useful not only to detect osteoarthritis before it is severe enough but also to follow-up the lesions and evaluate treatments.

Both chondromalacia and degenerative osteoarthritis are common diseases of the articular cartilage [3]. In both conditions, there are several inflammatory mediators [1] and abnormal vascular changes at the deeper cartilage layers and the subchondral bone involved in the disease progression [2, 4–9]. It has been shown that loss of resistance to vascular invasion distinguishes osteoarthritis cartilage from normal articular cartilage [9]. Nowadays, magnetic resonance (MR) imaging can be used to trace the

pharmacokinetic properties of the patellar cartilage in the different degenerative stages, offering good reproducibility in the evaluation of the capillary tissue and extravascular extracellular space (EES) properties [10–13]. Parametric mappings can be used as disease biomarkers in the evaluation of permeability between vessels and EES (known as K^{trans} and expressed in $ml \cdot min^{-1} \cdot 100\ ml^{-1}$); the extraction rate between EES and vessels (known as k_{ep} and expressed in $ml \cdot min^{-1} \cdot 100\ ml^{-1}$); the EES volume fraction (known as v_e and expressed as a percentage) and the intravascular volume fraction (v_p also expressed as a percentage).

After the intravenous administration of contrast medium and adequate MR acquisition strategies and computer modeling, it has been shown that there are statistical differences in the capillary permeability (K^{trans}) and the interstitial component volume (v_e) between both normal and degenerated cartilage. All these parameters show an increment with the progression of disease, being higher in cartilages with osteoarthritis compared with chondromalacia [2].

The evaluation of disease-modifying osteoarthritis drugs is quite important in the tracking of cartilage degenerative disorders. Glucosamine sulfate preparations are widely used as prescription drugs against osteoarthritis. Two long-term trials [14, 15] have shown a potential for joint-structure modification based only on plain radiography findings. Although glucosamine reduces joint space narrowing in patients with osteoarthritis [15], in-vivo monitoring the effect of glucosamine on the cartilage properties has not been evaluated. More importantly, the rationale for glucosamine sulfate having a role as a cartilage protector is still not well established.

Our hypothesis is that the neoangiogenesis related to cartilage degeneration, as seen in chondromalacia and osteoarthritis by MR pharmacokinetic analysis, may show significant differences between patients treated with glucosamine sulfate and a control group.

Materials and methods

Patients

Twenty patients with non-advanced degenerative patellar conditions, where surgery was not indicated, were included. All the patients were autonomous for daily life activities and did not need help to stroll. None were under treatment (neither corticosteroids nor nutraceuticals) before enrolling in the study. None had rheumatic diseases, severe articular inflammation, neither previous history of significant traumatic knee injury. Four patients were withdrawn after the first MR examination because they failed in therapeutic adherence.

Finally, there were 16 patients fulfilling all the study enrolling conditions. All patients signed a written informed consent for the contrast-enhanced MR examination with the use of contrast media and the use of the data for further analysis. No Ethics Committee approval was required, as the study was defined as a clinical observation and contrast-enhanced MR is used in cartilage examination in our hospital in daily clinical practice.

They were classified as having chondromalacia (seven cases) or osteoarthritis (nine cases) by means of MR imaging (all cases had signal intensity variations and patients with osteoarthritis also had cartilage surface ulcerations), arthroscopy (one case) and surgery (one case). Patella surface analysis was performed with the transversal T2-weighted (T2W) high-resolution images. For all patients, mean age was 39±18 (mean ± standard deviation). As expected, there were differences between clinical groups in age (29±12 for chondromalacia vs 59±9 for osteoarthritis) and sex (1/6 vs 5/4, men/women respectively).

Patients were distributed in the glucosamine sulfate (three with chondromalacia and seven with osteoarthritis) or control (two with chondromalacia and four with osteoarthritis) groups using a randomized generated list (four patients of the control group dropped out of the study after the first MR examination). The groups were not statistically different regarding disease condition (chi-square test, $p=0.38$). Also, there were no differences in the treatment groups for age (Student's *t*-test after normal data distribution confirmation, $p=0.30$) or sex (chi-square test, $p=0.13$).

Both groups started treatment immediately after the first MR study. All patients took the medication uninterruptedly until the second MR examination. The glucosamine group had 1,500 mg (Xicil, Rottapharm, Valencia, Spain) orally once a day, while the control group had oral 650 mg of acetaminophen (Dolostop, Química Farmaceútica Bayer, Barcelona, Spain) once a day.

Neither the patients nor those responsible for image acquisition and analysis (LMB, RSR and AAB) knew to which group an individual patient belonged to.

Clinical evaluation

All patients were clinically evaluated twice; the first examination was performed before starting the treatment and the second one 6 months later. Two scores were used in both clinical evaluations, again before and 6 months later: the pain visual analog scale (VAS) (range, 1–10, best to worse) and the widely used functional outcome American Knee Society (AKS) score (range 1–100, worst to best). The AKS is divided into two parts: the Knee Score, which considers pain, stability and range of motion, with deductions for flexion contractures, extension lag and malalignment; and the Function Score, which utilizes walking distance and stair climbing, with deduction for the use of a walking aid [16].

Values are expressed as mean±standard deviation and percentage of variation calculated as [(second-first)/first×100].

Data acquisition

Two MR examinations were obtained from each patient, with a 6-month interval (± 2 weeks). MR imaging was acquired on a 1.5-Tesla magnet (Philips Gyroscan Intera, Philips Medical Systems, Best, The Netherlands). A dynamic fat suppression T1-weighted (T1W) contrast-enhanced spoiled gradient echo (TR=3.47 ms, TE=1.93 ms, flip angle=10°, reconstruction matrix=256×256, pixel size of 0.78×0.78 mm, slice thickness=10 mm, 10 slices) was obtained in the transverse orientation centered on the patellar cartilage. Fifty dynamic series were continuously obtained with a temporal resolution of 2.9 s each and a total acquisition time of 2 min 25 s (Fig. 1). Other acquired sequences included T1W TSE-SPIR, T2W SSFP and T2*W GRE with magnetization transfer.

The intravenous contrast agent (Gd-DTPA-BMA, Omniscan, GE Healthcare, UK) was injected after the completion of the acquisition of the third dynamic (serving as a non-contrast baseline) as a bolus (0.2 ml/kg at 4 ml/s), followed by 40 ml of saline flush at the same rate.

Pharmacokinetic modeling

A one-input two-compartment model was used, with the popliteal artery as the arterial input function (AIF) and the two compartments being the blood plasma and the EES. From this model, three pharmacokinetic parameters were calculated: permeability (K^{trans}), extraction ratio (k_{ep}) and extravascular extracellular space volume fraction (v_e). It has been shown that in normal or weakly irrigated cartilages, the vascular volume fraction (modeled in terms of v_p) can be neglected in order to increase the accuracy of fit, the reproducibility and the stability of the model [2]. To calculate these pharmacokinetic parameters (K^{trans}, k_{ep} and v_e), a non-linear least-square fit using the Levenberg-Marquardt method was performed [17]. The pharmacokinetic model can be expressed as a convolution equation:

$$C_t(t) = K^{trans} \int_0^t C_p(u) e^{-k_{ep}(t-u)} du$$

Where $C_t(t)$ is the cartilage contrast concentration vs the time curve and $C_p(u)$ is the arterial input contrast concentration vs the time curve.

Image analysis

Several regions of interest (ROIs) encircling the patellar cartilage were manually defined by an experienced radiologist in all the slices of the baseline dynamic acquisition. The popliteal artery ROI was manually drawn in the central single slice during the early enhanced phase. To obtain the arterial input function, the intensity values of all the

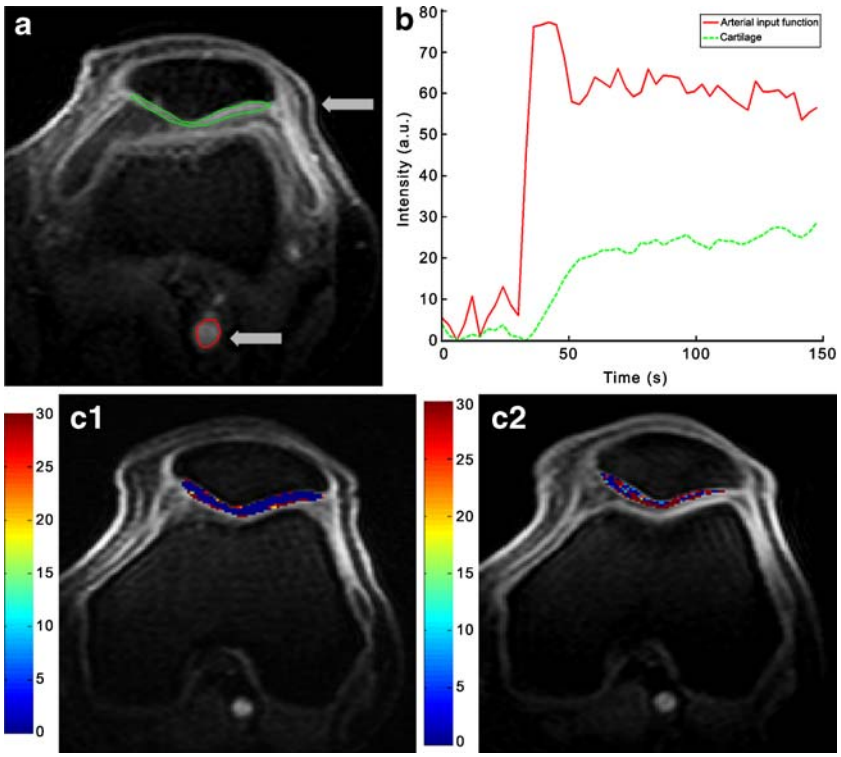

Fig. 1 **a** Dynamic contrast-enhanced MR imaging slice showing the regions of interest for the cartilage (*upper arrow*) and the popliteal artery (*lower arrow*). **b** Enhancement curves for the popliteal artery and the cartilage region. **c** Cartilage parametric maps for K^{trans} in a patient with osteoarthritis at first (**c1**) and second (**c2**) MR examination. It can be seen there is an increase in the values of permeability. Colours *blue* and *green* represent lower values, while *yellow* and *red* represent higher values

popliteal pixels were averaged at every dynamic to construct the intensity vs time curve.

The pharmacokinetic analysis was performed only on those pixels defined as cartilage by the manual segmentation. Pixel averaging was used to smooth the contrast enhanced curves. The pharmacokinetic parameters of enhanced pixels were calculated and color-coded as parametric images. These colored maps were overlapped over the anatomical slices (Fig. 1).

After a pixel-by-pixel pharmacokinetic analysis, mean cartilage values were obtained by averaging [2]. K^{trans}, k_{ep} and v_e were extracted for each patient and MR examination. The difference between the values measured in the second and first MR examinations was calculated and expressed as a percentage of change [(second-first)/first×100].

Statistical analysis

The normal distribution of the data was assessed with the Kolmogorov-Smirnov test. All the variables had a normal distribution. A *t*-test for independent data was used to evaluate differences (glucosamine vs controls, expressed in percentage of change) after evaluating the equality of the variance with Levene's test. A *p*-value ≤ 0.05 was considered statistically significant. Statistical results were obtained with SPSS 13.0 (SPSS, Chicago, Ill., USA) and are expressed as mean ± standard deviation. Calculations of power statistics were also performed for variables showing statistical differences and the curves for power vs difference in means were obtained.

Results

Glucosamine sulphate treatment was considered clinically effective. Statistically significant differences were found in both the pain and functional outcome scores for patients under glucosamine compared with the control group. Treatment with glucosamine sulfate reduced patient's pain and improved their knee function compared with the controls ($p<0.01$ for both clinical indexes). Table 1 summarizes the results for the clinical evaluations before the first MR study and 6 months afterwards.

In the pharmacokinetic analysis, there was a statistically significant difference in the controls vs glucosamine for K^{trans}, with higher values in the glucosamine group (−54.4% vs 126.7%, controls vs glucosamine respectively). The results were statistically significant ($p<0.001$) (Fig. 2). The k_{ep} differences were not statistically different. Regarding the EES volume fraction, there was a tendency for higher v_e differences in percentage in the glucosamine group ($p=0.11$). Table 2 summarizes the values of the parameters and the differences in the MR-calculated parameters between the two MR examinations.

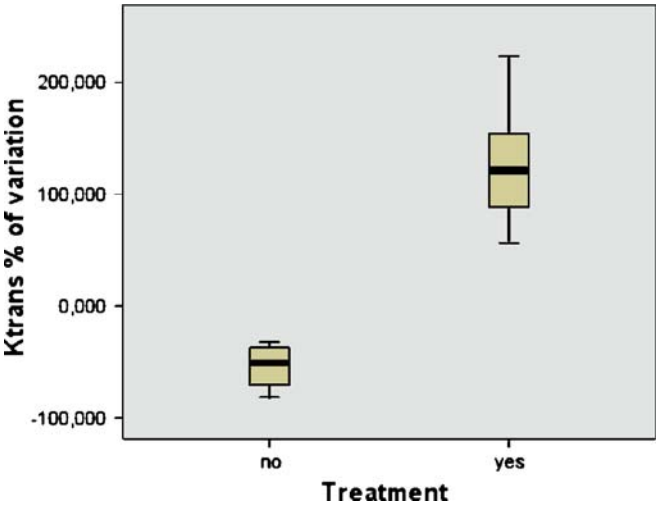

Fig. 2 Box-plots for the difference (%) in K^{trans} for all patients and for both chondromalacia and osteoarthritis. The chart shows the five statistics (minimum, first quartile, median, third quartile and maximum)

The power calculation curve for K^{trans}, obtained for the worst case (highest standard deviation, 56.9% for the glucosamine group, alpha=0.05, *n1*=10 and *n2*=6), showed a value of 0.64 for a difference of 72.3% (mean percentage difference between glucosamine and controls). For VAS, the power calculation (highest standard deviation, 12.6%, alpha=0.05, *n1*=10 and *n2*=6) showed a value of near 1 for a difference of 55.1%. For AKS no power calculation was obtained since the control group had null standard deviation and the difference was 165.4%.

Discussion

In an effort to increase our in-vivo knowledge of the cartilage status, MR imaging can be used to quantitatively assess different biomarkers. The most evaluated parameters

Table 1 Summary of results for the clinical evaluation of the patients

	Controls		Glucosamine		*p*
	Baseline	At 6 months	Baseline	At 6 months	
VAS	6.8±0.4	7.0±0.0 (3.3±7.4%)	7.3±1.1	3.6±1.3 (−51.8±12.3%)	<0.001
AKS	26.0±5.5	26.0±5.5 (0±0%)	18.6±6.9	42.9±2.7 (165.5±114.6%)	<0.01

Values express the mean ± standard deviation and the 6 months percentage of change *in parenthesis*

Table 2 Summary of results for the difference in the main pharmacokinetic parameters in both proposed models

Parameter	Group	MR1	MR2	Difference (%)	
K^{trans}	Controls	17.1±7.1	12.5±3.2	−54.4±21.2 (9.5)	$p<0.001$
	Glucosamine	9.1±6.5	14.4±8.3	126.7±56.9 (21.5)	
k_{ep}	Controls	367.0±130.5	314.5±237.3	4.6±96.2 (43.0)	$p=0.76$
	Glucosamine	287.9±342.4	373.4±130.4	−11.7±85.1 (32.2)	
v_e	Controls	14.2±11.4	9.3±2.7	−36.1±90.8 (40.6)	$p=0.11$
	Glucosamine	9.3±6.2	20.4±16.1	118.4±204.7 (77.4)	

The values shown are the mean ± standard deviation (standard error of the mean) (K^{trans} values are ml·min^{-1}·100 ml^{-1}, k_{ep} values are ml·min^{-1}·100 ml^{-1}, v_e values are percentages)

are cartilage thickness and volume, delayed contrast enhancement and the physical properties of the T2 and T1 relaxation times.

Mature articular chondrocytes in physiological condition are essentially devoid of vascular structure. However, blood vessel growth into the articular cartilage may contribute to cartilage damage. Different studies have pointed to a correlation between articular cartilage degeneration and the presence of an abnormality in its blood supply and nutrition [2, 4–9, 18, 19]. Although diffusion from the synovial fluid into the cartilage matrix is a known mechanism for cartilage nutrition, a direct blood supply from the subchondral bone is observed in osteoarthritis patients [18, 19].

In this sense, dynamic contrast-enhanced MR imaging pharmacokinetically derived parameters can be used as a biosignature for cartilage degeneration [2]. Pharmacokinetic models focus directly on the tissue microvascular function, providing a reliable and reproducible measurement of angiogenesis and capillary membrane permeability properties. This technique, although lacking global standardization, is considered an accurate and validated neoangiogenesis biomarker even in complex models [20]. Reproducibility of the pharmacokinetic cartilage calculations for 1.5-Tesla acquisitions is high, with a very low test–retest root mean square coefficient of variation (9–19% for K^{trans}, 8–15% for k_{ep} and 14–23% for v_e) [2, 21]. In our series, influence of time and treatment was much larger than methodological variability (control group, root mean square coefficient of variation of 30% for K^{trans}, 110% for k_{ep} and 71% for v_e; glucosamine group, root mean square coefficient of variation of 76% for K^{trans}, 98% for k_{ep} and 98% for v_e). Even more, when compared with previous studies using the same methodology in different populations [2, 21], all the results had a similar range of values.

It has been shown that as cartilage degeneration progresses, its vascular status is modified. Different cartilage pharmacokinetic parameters increase, being higher in cartilages with osteoarthritis than with chondromalacia, both groups having larger values than normal non-degenerated cartilages [2]. These changes can be directly related to cartilage degeneration, inflammation and increased metabolic activity. Modeling of these pharmacokinetic properties of cartilage may be useful in stratifying the disease and follow-up the different treatment options.

We have found in this study a clear influence of glucosamine sulfate treatment in the pharmacokinetic results. There is a significant difference in K^{trans} after glucosamine administration compared with the control group. Patients treated with glucosamine sulfate had an increment in their cartilage capillary permeability as evaluated after 6 months of medication. There was also an increment in the volume of the interstitial space v_e, but the difference was not significant, most probably due to the small number of cases and the large standard deviation. The k_{ep} did not show any significant change between both examinations, in neither group of patients, due to the fact that the cartilage enhancement curves had very slow washout (the curve decay modeled with the k_{ep} parameter). This means that k_{ep} cannot be used as a biomarker to assess differences in cartilage status.

Glucosamine sulfate stimulates the synthesis of proteoglycans and, together with other biochemical properties, improves the quality of the cartilage matrix. We, therefore, anticipated an effect of treatment on the pharmacokinetic parameters. As found in this study, glucosamine sulfate administration has an effect on the capillary properties of cartilage, at least if evaluated 6 months after treatment onset.

Although we initially expected a decrease in the angiogenesis parameter values with treatment, the observed effect was opposite: an increase in the cartilage vascular permeability after treatment. This result could be associated with an increment in the angiogenesis associated with the promotion of reparative cartilage processes and with the metabolic cascades linked to the drug, as this repairing process may be energy consuming. As the 6-months pharmacokinetic evaluation seems to relate to the increased metabolic effect of glucosamine sulfate on cartilage, it seems reasonable that the curative effect of glucosamine sulfate should be evaluated over a longer period, most probably at least 12 months after starting the administration of the drug. In order to test if pharmacoki-

netic MR evaluates an increase of cartilage metabolism and perfusion in patients treated with glucosamine sulfate, another study will be required with different methodology and examination times to be confirmed.

As pharmacokinetic analysis from dynamic contrast-enhanced MR imaging may model the vascular and interstitial behavior of different pathological conditions in tissues, the obtained parameters in our study must be considered reproducible, as similar MR sequences, contrast injection protocols and measurement procedures were used. The fact that the molecular weights of glucosamine sulfate (456 Daltons) and gadolinium (gadodiamide DTPA-BMA, Omniscan) (573 Daltons) are quite similar makes this pharmacokinetic modeling even more promising to evaluate the effect of glucosamine sulfate on the capillary properties of the diseased cartilage.

If our finding of a significant increase in K^{trans} in osteoarthritic patients receiving glucosamine sulfate is replicated in a larger series, this parameter could be considered a surrogate endpoint of treatment effect, reflecting most probably an increase of the microvascularity and membrane permeability in the glucosamine sulfate group.

It has to be noted that we have used the prescription formulation of glucosamine sulfate (1,500 mg once a day), which has an established pharmacokinetic profile and clinical trial evidence. It is not known whether our results would be applicable to other glucosamine or glucosamine sulfate dietary supplement or generic preparations.

One bias of this study is the use of mean values to characterize cartilage. This whole analysis may hide the presence of degenerated regions whose values are minimized when averaged with the rest of the cartilage. Parametric maps, histograms or regional analysis and visualization tools must be used to depict abnormal regions and interpret local results. These representations will offer additional information on degeneration location, size and grading.

Also, the choice of the arterial input function for the pharmacokinetic study may affect the reproducibility among different sites, as other studies related to pharmacokinetic analysis use different input curves, such as population averaged or reference curves [22, 23]. In our study we have used individual input curves, chosen directly from the popliteal artery in each case, as both the spatial and temporal resolutions of our sequence were high enough.

As cartilage degeneration is also associated with changes in synovial and subchondral vascularization, this may be a source of bias. We carefully segmented cartilage tissue, excluding synovial and subchondral bone, making sure that only cartilage was included in the image analysis. Therefore, the contribution of other "non-cartilage" structures to the cartilage evaluation can be neglected. Also, as we evaluated the differences between the two groups of patients, variability associated with the methodology should be present in both groups and cannot explain the different group results. The values obtained from the power calculation allow an acceptable degree of confidence in our results.

In conclusion, glucosamine sulfate decreases pain and improves the functional outcome in patients with cartilage degeneration. Glucosamine sulfate treatment has also an effect on the vascular properties and cartilage metabolism. The strong relationship between treatment and increment in the cartilage capillary permeability (K^{trans}) allows us to propose the measurement of capillary permeability as a surrogate marker for the 6-months glucosamine sulfate effect on the degenerated cartilage metabolism.

Acknowledgments *Grant support* Part of this work was supported by a grant from Rottapharm S.L., Valencia, Spain.

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