Efficient Incorporation and Slow Release of Manganese Indicating its Potential Use as a Contrast Agent for Articular Cartilage

G. Saar[1], H. Shinar[1], G. Navon[1]

[1]School of Chemistry, Tel Aviv University, Tel Aviv, Israel

Introduction: Imaging of cartilage is of paramount importance in the diagnosis of osteoarthritis. However its small thickness (about 2 mm) requires high degree of resolution, which is usually limited by the scan time. One way to decrease the scan time is shortening T_1, by introducing a contrast agent. Having high concentrations of the negatively charged proteoglycans it is expected that cartilage will take up positively charged ions such as Mn^{2+} more effectively than the negatively charged $Gd(DTPA)^{2-}$. Indeed, this has been previously demonstrated (1,2). However Mn^{2+} is toxic at high concentrations. Here we present data showing that Mn^{2+} is taken up by articular cartilage from solutions containing very low Mn^{2+} concentrations and that its release is slow relative to that of $Gd(DTPA)^{2-}$.

Materials and Methods: Cartilage bone plugs were excised from bovine femoral condoyles, equilibrated in saline and T_1 weighted GE images (FOV=1x1 cm, 64x64, TR/TE=300/1.07 ms, 8.45T) as well as T_1 maps (inversion recovery spin echo) were recorded. The saline solution was changed to saline containing either 0.1 mM $MnCl_2$ or 1.0 mM $Gd(DTPA)^{2-}$. T_1 weighted images were recorded as a function of time, for approximately 4 hours. At this stage T_1 maps were recorded again, after which the solution was changed to agent-free saline. T_1 weighted images were recorded for approximately 8 hours followed by T_1 mapping. For PG depletion intact plugs were equilibrated in 1 mg/ml trypsin in PBS for 12 h, at 25 ^{0}C. For decalcification intact plugs were immersed in continuously stirred solution of 5% formic acid for 3 days. Plugs were then equilibrated in TRIS or PBS, pH=7.4, for 12 hours. In both cases plugs were then re-equilibrated in saline and treated like the intact plugs. All measurements were done at 37 ^{0}C.

Results The time course of the signal intensity upon addition of 0.1 mM $MnCl_2$ in saline to an intact cartilage bone plug as well as the washout is given in Fig. 1, for three regions of interest: near the surface, at the center and close to the bone. The rate of penetration and washout of manganese is given in Fig 2 for intact, PG depleted and decalcified plugs at the three regions of interest. In all cases, the penetration rate is much larger than the washout (e. g. 0.06 min^{-1} vs. 0.008 min^{-1} at the center for intact plug). The penetration as well as the washout is fastest at the surface and slowest near the bone (penetration rate at the surface: 0.18 min^{-1}, and near the bone: 0.02 min^{-1} for the intact plug). It should be noted that the washout was significantly quicker for the PG depleted plugs (0.011min^{-1} near the bone) as compared to the intact (0.005min^{-1}) and the decalcified (0.005min^{-1}).

T_1 values before (A) and after (B) the addition of Mn^{2+} and after the washout (C), in the same three regions, for intact, PG depleted and decalcified cartilage-bone plugs are summarized in Fig.3. Throughout the plugs, there is a significant decrease of T_1 after the equilibration with manganese (e.g. 1.5 s to 0.3 s in the center of the intact plug). In the PG depleted cartilage after the addition of Mn^{2+}, T_1 is longer (0.4 s) than in the intact and decalcified plugs indicating that the amount of Mn^{2+} that has penetrated to the depleted cartilage is smaller than the amount in the intact and decalcified plugs. Moreover, a full recovery of T_1 was detected after washout in the depleted plug and only partial recovery for the intact and decalcified plugs.

The exact same series of experiments was done with 1mM $Gd(DTPA)^{2-}$ and the results are in line with results previously obtained by A. Bashir et al. (3). In this case the depletion results in a threefold decrease of the washout rate while for Mn^{2+} the effect is opposite – a twofold increase. This result is expected since in the depleted plug the amount of the negatively charged PG is small.

Discussion: The effect of manganese on T_1 of the intact plug is larger than that of $Gd(DTPA)^{2-}$ in spite of the 10 fold lower concentration of Mn^{2+}. This finding can point to the possibility of using Mn^{2+} as a contrast agent for connective tissues in spite of its toxicity at high concentrations. Two possible ways by which Mn^{+2} can be introduced to the body in a slow release manner are by *i.v.* injection of mangafodipir (Teslascan) which is metabolized by dephosphorylation and transmetallated by Zn^{2+}, gradually releasing Mn^{2+} ions to the blood (4) or *by os*. Mn^{2+} in the form of manganese ascorbate is widely used as a constituent in health supplements for people with osteoarthritis Although the concentration of Mn^{2+} in the blood would be small in these two cases, on the basis of the present results it is expected to be effectively taken up by articular cartilage reducing significantly its T_1. Moreover, the washout rate is smaller than the washout rate of $Gd(DTPA)^{2-}$by a factor of 4, allowing a greater time window for the measurements.

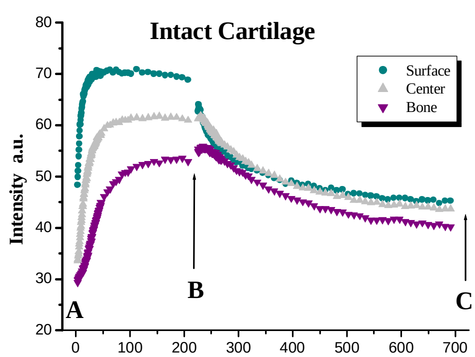

Fig 1.

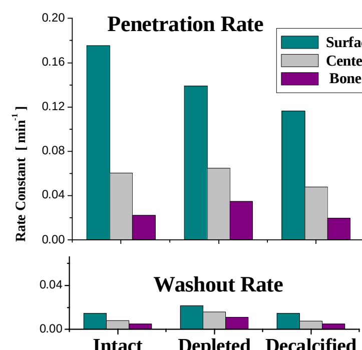

Fig 2.

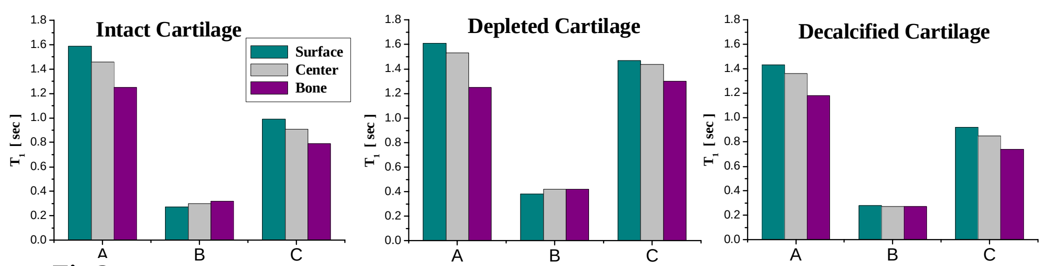

Fig 3.

Conclusion: Manganese should be considered as a contrast agent for connective tissues.

References: 1) Y.Kusaka et.al, MRM,24,137,1992. 2) G. Navon et.al, Proc. ISMRM 12, 825,2004 3) A. Bashir et.al, MRM 36, 665, 1996. 4) K. G. Toft et.al, Acta Radiol. 38, 677,1997