AGE DEPENDENT VARIATION OF SODIUM CONCENTRATION IN HUMAN KNEE CARTILAGE MEASURED AT 7T

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Purpose: To determine the age dependent variation of molecular changes in human knee cartilage via sodium MRI at ultrahigh fields.

Introduction: Aggrecan along with Type-II collagen forms a major structural component of cartilage, particularly articular cartilage. Aggrecan (a large aggregating proteoglycan [PG]) has been shown to undergo predominant change in early OA. While OA is a disease of diverse etiologies cartilage tissue is primarily affected. The loss of PG is an initiating event in the OA. By monitoring sodium concentration in tissues via 23Na MRI, the integrity of the cartilage tissue can be analyzed in vivo, which provides OA diagnosis and disease evaluation. Before quantifying the OA changes with any noninvasive method, it is critical to estimate the natural course of molecular degeneration in cartilage matrix. In the present study, using sodium MRI at ultrahigh field, we investigated the natural, age dependent molecular changes in the healthy human knee cartilage in vivo.

Methods: All human studies were approved by the Institutional Review Board. The knees of three healthy young male (20-24 years) and three aged (43-59 years) male volunteers were imaged on 7T Siemens MRI scanner. We used a custom built birdcage tuned to sodium frequency with optimal SNR. Sodium MRI was performed using an Ultra short Echo (UTE) (Siemens medical solutions, Erlangen) 3D radial sequence with a mean SNR of 24 (TR=75ms, TE = 0.07ms, FOV=16cm, Matrix = 128×128×128 and 600 radial spokes for total scan time of 45 sec per image). 16 individual acquisitions (12 min total acquisition time) were co-added offline to further improve SNR for more accurate concentration measurements. The same parameters were maintained in all experiments for all subjects for direct comparison. For B1 field variation and concentration corrections, a phantom with 250 mM NaCl and 5% agarose was imaged and appropriate calibrations were computed. The images obtained using UTE sequence have higher SNR compared to 3D FLASH sequence (approximately by a factor of 20) for comparable scan time of 12 - 15min.

Results: The two MAP Kinases p38 and MEK/ERK are known to have opposite effects on chondrocyte differentiation: p38 is overall a positive regulator and MEK/ERK a negative regulator. We focused mostly on genes down-regulated by the SB and up-regulated by U treatments. 88 probe sets were both >1.45 down-regulated by SB and up-regulated by U. Among these genes we confirmed a subset by real-time PCR: Col10a1, Matn3, Lh, Paps2, Fgf3, Sp7 and Alpl. These common genes were also identified by IPA analysis in different functional categories such as: “development of osteochondrodysplasia”, “osteonecrosis” and “osteoblast differentiation”. One interesting hypertrophic chondrocyte/bone marker, Ibsp was highly down-regulated by SB (5 fold), but unaffected by U. In addition to the separate effects of the two inhibitors (SB and U) on gene expression, we also analyzed by real-time PCR the effects of a combined SB and U treatment (SU). For genes such as Ihh, SU treatment brought its expression level close to DMSO control, suggesting that the opposite effects of the two pathways in chondrocytes were canceled when they were simultaneously inactivated. This was not the case for all markers; e.g some terminal differentiated chondrocyte/bone markers such as Tnfrsf11a (RANK) and Tnfsf11 (RANKL) were down-regulated by both pathways.

In tibia organ culture, bone growth was significantly reduced by SB, increased in the presence of U and close to DMSO control in SU treatment. Upon safranin O staining of tibia sections, we noticed smaller cells in the proliferative area of SB treated bones that were organized in poorly defined columns. Both length and width of different growth plate zones were measured. Hypertrophic zone length was decreased in SB and U, but unaffected by U, while the proliferative zone was increased in U only. The whole growth plate area was decreased in SB and SU and increased in U, and the width of the hypertrophic zone was increased in U only.

Conclusion: In conclusion, the two MAPK pathways had opposite effects on specific growth steps of the endochondral ossification process, and these effects were canceled when the two pathways were inhibited simultaneously. However, other processes were mainly affected by one of the two pathways (e.g. Ibsp expression) or regulated by both p38 and MEK/ERK in the same direction (e.g. the expression of Tnfrsf11a and Tnfsf11).

Conclusion: These preliminary results demonstrate that it is feasible to obtain sodium maps of human knee in-vivo at 7T with reasonable acquisition times and quantify age dependent molecular changes in knee cartilage of healthy human subjects. Further work is in progress to quantitatively age dependent Aggrecan changes in larger groups of subjects to quantitatively establish the natural course of degenerative changes in cartilage.

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VALPROIC ACID SUPPRESSES INTERLEUKIN-1β-INDUCED MICROSOmal PROSTAGLANDIN E2 SYNTHASE-1 EXPRESSION IN CHONDROCYTES


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Purpose: Microsomal prostaglandin E2 Synthase (mPGES)-1 catalyzes the terminal step in the biosynthesis of PGE2. Early growth response factor-1 (Egr-1) is a key transcription factor in the regulation of mPGES-1. In the present study we examined the effects of valproic acid (VA), a histone deacetylase (HDAC) inhibitor, on interleukin (IL)-1β-induced mPGES-1 expression in human chondrocytes.

Methods: Chondrocytes were stimulated with IL-1 in the absence or presence of VA, and the level of mPGES-1 protein and mRNA expression were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction, respectively. The mPGES-1 promoter activity was analyzed in transient transfection experiments. Egr-1 recruitment to the mPGES-1 promoter was evaluated using chromatin immunoprecipitation (ChIP) assays.

Results: VA dose-dependently suppressed IL-1β-induced mPGES-1 protein and mRNA expression as well as its promoter activity. Treatment with VA did not alter IL-1-induced Egr-1 expression, nor its recruitment to the mPGES-1 promoter, but prevented its transcriptional activity.

Conclusions: Our study demonstrates that VA inhibits IL-1-induced mPGES-1 expression in chondrocytes. The suppressive effect of VA was not due to reduced expression or recruitment of Egr-1 to the mPGES-1 promoter.