Increased oxidative DNA damage in porcine osteoarthritic articular cartilage explants

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Introduction: The pathogenesis of osteoarthritis (OA) may involve oxidative damage due to the over production of nitric oxide (NO) and other reactive species, and decreased antioxidants. Interleukin-1 or nitric oxide (NO) donors can cause oxidative DNA damage in porcine articular chondrocytes cultured in alginate beads. The aims of this study were to determine (i) if greater oxidative DNA damage occurs with increasing severity of OA in articular cartilage compared with non-OA cartilage explants, (ii) the percentage of OA chondrocytes that contain DNA damage at different grades, (iii) an estimation of the number of DNA breaks per OA chondrocyte based on x-ray calibration.

Materials and Methods: Spontaneous OA articular cartilage from pigs were macroscopically graded using the Collins scale. Articular chondrocytes were enzymatically isolated from porcine distal femoral cartilage explants. A sample was fixed in 4% paraformaldehyde for histological grading of OA using a modified Mankins scale. DNA damage was determined immediately on the isolated chondrocytes using a single cell gel electrophoresis assay, the "comet" assay (Trevigen). DNA was stained with SYBR® green and DNA damage identified by migration of nuclear material to give a comet-like appearance. Images were captured using LSM scanning confocal microscopy and analyzed using CASP™ and ImageJ (NIH) software. DNA damage was quantified by multiplying tail length and the distribution of DNA in the tail to give an "Olive tail moment" (OTM). DNA repair enzymes were used in the modified comet assay to determine the type of DNA damage. Formamidopyrimidin (Fpg, 1 μg/ml) recognizes modifiedpurines, Endonuclease III (EndoIII, 1 μg/ml) recognizes modified pyrimidines, and 8-oxoguanine glycosylase (OGG, 1 μg/ml) recognizes 8-oxoguanine. DNA repair enzymes excise damaged bases that result in increased OTM. A calibration curve was generated for non-OA pig chondrocytes using x-ray irradiation. Non-OA chondrocytes were enzymatically isolated, embedded in agarose, placed on ice, and exposed to 0-12 Gray of x-rays, using a tungsten anode tube (SRO 0950 ROT 350, Philips Medical Systems) and a mobile MOSFET® wireless dosimetry system (Model TN-RD-70-W, Best Medical Canada, Ottawa, Canada). The comet assay was performed on each sample. NO production was measured as NO2- and NO3- (NOx) using the Griess assay. Cell viability was determined with the live/dead assay (Molecular Probes). Statistical analysis was performed by ANOVA with Duncan’s post-hoc comparison.

Results: A significant increase (p < 0.05) in NOx levels were found in the synovial fluid of Collins Grades I and II compared with Collins Grade 0 joints.

Discussion: Our data suggest significant levels of oxidative DNA damage occur in OA chondrocytes compared with non-OA chondrocytes, and 8-oxoguanine formation maybe associated with OA. It appears that a sub-population of OA chondrocytes do not contain DNA damage. The population of chondrocytes that do contain DNA damage when exposed to x-rays, interleukin-1 or NO donors is much higher than observed in OA explants. Since the chondrocytes exposed to these agents were isolated from the extracellular matrix, it suggests the extracellular matrix may protect the chondrocytes from DNA damage, possibly via extracellular superoxide dismutase. Alternatively, chondrocytes in the deeper zone may be protected, by the pericellular matrix of the chondron.


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Fig.1: (a) Levels of DNA damage associated with different histological Grades of OA pig articular cartilage explants. Mean ± SEM, N = 3, n = 150. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. (b) Levels of DNA damage after exposure to 0-12 Gy of X-ray irradiation. OTM = 0.7043-2.818Gy. Mean ± SEM, N = 3, n = 150/group.

A significant increase in DNA damage was associated with OA explants in joints with Grades 3 or greater compared with non-OA explants from joints with Grade 0 histological OA (Fig.1a). The percentage of chondrocytes without DNA damage compared with the mean OTM of chondrocytes from Grade 0 joints decreased with increased severity of OA (56.1±9.1, 57.7±6.8, 16.4±9.4 for Grades 1-2, 3-5; or 6-7 respectively). The approximate number of bases with damage were calculated (Fig.1b), using the conversion factor 1 Gy = 0.31 breaks/1012 Da of DNA. Porcine chondrocytes contain 5.44 pg DNA/chondrocyte, equivalent to 3.28 X 1012 Da of DNA/chondrocyte. Therefore, 1 Gy causes 1017 breaks/chondrocyte. Chondrocytes contained approximately 1247, 1641, 2204 or 5092 breaks/chondrocyte for Grades 0, 1&2, 3-5, or 6&7 respectively. 2 Gy x-ray irradiation did not cause damage to approximately 15% of the chondrocytes.

When DNA repair enzymes were used in the modified comet assay (Fig.2), EndoIII, Fpg, or OGG1 caused a significant increase in excised bases in OA chondrocytes, compared with OA chondrocytes without DNA repair enzyme.

Fig.2: Addition of DNA repair enzymes in the modified comet assay on OA chondrocytes, causes excision of damaged bases leading to increased DNA damage. Mean ± SEM, N=3, n = 150/group, *** = p < 0.001.