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A proteomic investigation of synovial fluid in patients with knee osteoarthritis treated with intra-articular metallic gold micro particles

Sten Rasmussen[1, 2, 3], Martin Kaae Kristiansen[4], Jacob Skallerup Andersen[4], Joachim Bavnhøj Hansen[4], Kristian Kjær Petersen[1], Lars Arendt-Nielsen[1], Allan Stensballe[4]

[1)]Center for Sensory-Motor Interaction (SMI), CNAP, School of Medicine, Aalborg University, Aalborg Denmark
[2)]Department of Clinical Medicine, School of Medicine, Aalborg University, Aalborg Denmark
[3)]Department of Orthopedic Surgery, Aalborg University Hospital, Aalborg, Denmark
[4)]Deapartment of Health Science and Technology, School of Medicine, Aalborg University, Denmark

Background and aims

Intraarticular gold may decrease osteoarthritis (OA) inflammation. Gold implant treatment correlate with clinically significant improvements in joint movement and pain management. Gold may decrease inflammation because of various mechanisms such as regulation of the NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway (Figure 1 - 4) (1-6).

The aim of this study was to investigate if treatment with intra-articular solid gold micro particles induces any significant proteomic changes in the synovial fluid (SF) in knee OA.

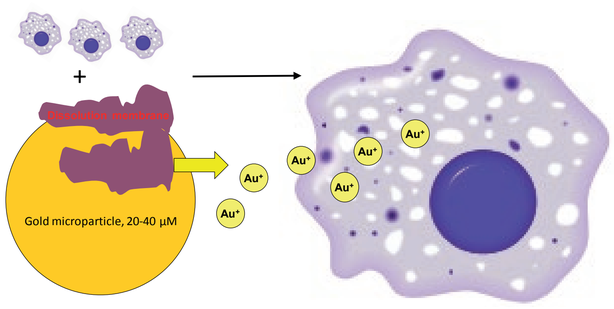

Figure 1. Macrophages controls the dissolution membrane which liberate the gold ions by oxidation of the surface. Once the ions are liberated, most likely as Au(CN) , they are free to diffuse through the immediate microenvironment. The gold-loaded molecules are taken up into the cells, primarily macrophages, mastcells and histocytes.

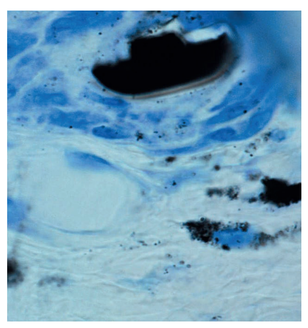

Figure 2. Close to the gold implant gold-loaded molecular clusters are located outside cells. The two loaded cells are believed to be macrophages loaded with gold ions. The gold ions accumulate primarily in the lysosymes (3).

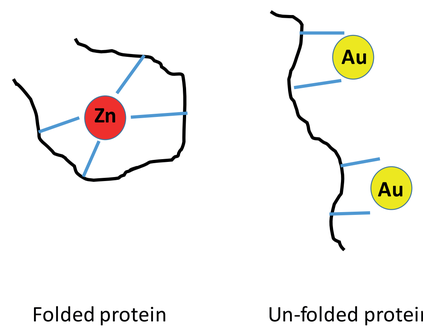

Figure 3. Once in the intercellular fluid and the intracellular compartments, the gold ions act in the same ways that have been demonstrated for systemically administered gold ions. The effect is related to the ability of the gold ions to unfold the protein structures.

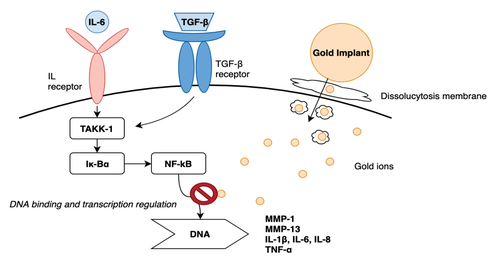

Figure 4. Gold ions suppress inflammation locally by affecting certain signalling molecules and binding enzymes essential for the inflammatory process. The DNA binding activity and transcription regulation of NF-kB is abolished when AU- ions replace Zn2+ ions. (4)

Methods

A cohort of 30 patients, aged ≥18years, pain ≥ 3 months, synovial effusion on MRI, and Kellgren-Lawrence OA grade 3-4 were included. Metallic gold 20 mg, 72.000 pieces, 20-40 µ-meter (Berlock-Micro-Implants, Human-GoldInject) (7-9) were injected into the knee joint using the patient's own synovial fluid as the carrier.

SF samples was investigated, before and 8 weeks after treatment in 17 patients. To determine protein concentration, a bicinchoninic acid (BCA) assay was used. Gel electrophoresis by SDS-PAGE was used to visualize the synovial proteome and relative qualitative changes between the treated and untreated samples. Mass spectrometry sample preparation was performed with filter aided sample preparation (FASP). The global proteome was investigated through LC-MS/MS in Orbitrap Q Exactive

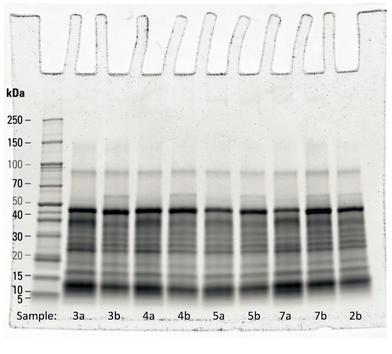

Figure 5. SDS-PAGE showing paired patients where the number identifies the patient and the 'a' or 'b' indicates if it is prior to or after treatment respectively. The column furthest to the left is the BroadRange Unstained Protein Ladder. Each gel contains four paired patients, before and after treatment, and one unpaired patient after treatment in the last column.

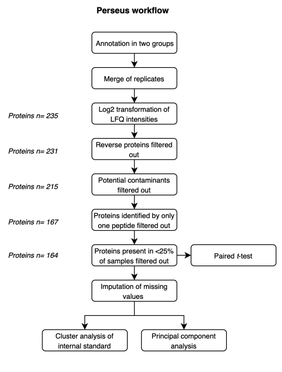

Figure 6. A scheme showing the chronological workflow of filtrations and MS analyses performed in Perseus Version 1.6.5.0 to investigate the proteomic changes of the samples.

Protein names	Gene Names	Regulation	Fold change	P-value
Aggrecan core protein	2 ACAN	Up	0.49	0.0081
Alpha-1-acid glycoprotein	2 ORM2	Down	-0.39	0.0001
Apolipoprotein	C-III APOC3	Down	-0.25	.0424
Cartilage acidic protein 1	CRTAC1	Up	0.21	0.0446
Cartilage oligomeric matrix protein	COMP	Up	0.33	0.0038
Clusterin	CLU	Up	0.28	0.0022
Complement component C6	C6	Down	-0.34	0.0142
Complement component C8 g-chain	C8G	Up	0.30	0.0195
Complement factor D	CFD	Up	0.47	0.0134
Complement factor H-related 1	CFHR1	Up	0.86	0.0049
Gelsolin	GSN	Up	0.26	0.0075
Hemoglobin subunit delta	HBD	Up	1.06	0.0398
Hemopexin	HPX	Down	-0.11	0.0369
Ig gamma-1 chain C region	IGHG1	Up	0.32	0.0035
Ig gamma-2 chain C region	IGHG2	Up	0.35	0.0043
Ig kappa chain C region	IGKC	Up	0.29	0.0337
Ig mu chain C region	IGHM	Up	0.25	0.0261
Monocyte differentiation antigen	CD14	Up	0.67	0.0269
Tetranectin	CLEC3B	Up	0.16	0.0455
Thyroxine-binding globulin	SERPINA7	Up	0.19	0.0005
Transforming growth factor	TGFBI	Down	-0.41	0.0332
Versican core protein	VCAN	Up	0.41	0.0355
Vitamin D-binding protein	GC	Up	0.15	0.0257

Table 1. Results from paired t-test showing proteins with significantly altered expression between samples before treatment and samples post treatment. The figure also shows the gene names of the proteins, the fold-change in expression and p-values.

Results

A distinctive protein band was visible in the treated patient columns at approximately 60 kDa in all of the patients (Figure 5). The MS analysis (Figure 6) revealed 23 of 164 proteins was significantly changed after treatment (Table 1). The expression of five proteins were down-regulated and 18 were upregulated. In the band between 50 and 70 kDA we found a significant elevation of clusterin (P = 0.0022), vitamin D binding protein (DPB) (P = 0.026) and cartilage acidic protein1 (CAP1) (P = 0.045).

Conclusions

The elevated clusterin may be a sign of increased protection of cartilage and cells, which correlates with a regulation of the NF-kB pathway. DBP correlate with increased vitamin D level, but the overall effect is uncertain. CAP1 has been found to marker of mesenchymal stem cells undergoing chondrogenic differentiation. It is shown that gold microparticles induce differentiation of mesenchymal stem cells. This indicate gold particles induce chondrogenic differentiation of resident mesenchymal stem cells.

We hypothesize gold particles inhibit macrophage mediated inflammation, induce chondrogenic differentiation of resident mesenchymal stem cells and stimulate the release of cartilage protective protein. Not all regulated proteins were correlated with positive effects on OA and some inconclusive. Further studies need to investigate the mechanism and proteins involved in gold treatment of OA.

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