Osteoarthritis and Cartilage 21 (2013) 918–922

Osteoarthritis and Cartilage

Brief report

An explorative study comparing levels of soluble mediators in control and osteoarthritic synovial fluid

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ARTICLE INFO

Article history:
Received 11 November 2012
Accepted 9 April 2013

Keywords:
Synovial fluid
Cytokines
Chemokines
Growth factors

SUMMARY

Objective: Soluble mediators in synovial fluid (SF) are acknowledged as key players in the pathophysiology of osteoarthritis (OA). However, a wide-spectrum screening of such mediators in SF is currently lacking. In this study, the levels of 47 mediators in the SF of control donors and osteoarthritic (OA) patients were compared.
Materials & Methods: SF was collected from control donors ($n = 16$) and end-stage knee OA patients ($n = 18$) and analysed for 47 cytokines, chemokines and growth factors using several multiplex enzyme-linked immunosorbent assays (ELISAs). A Mann–Whitney *U* test was used to determine differences between OA and control controls. A principal component analysis (PCA) was performed to cluster the 47 mediators.
Results: The majority of the mediators could be detected in both control and OA SF. Interleukin (IL)-6, interferon inducible protein (IP)-10, macrophage derived chemokine (MDC), platelet derived growth factor (PDGF)-AA and regulated on activation normal T cell expressed and secreted (RANTES) levels were found to be higher in OA compared to control SF ($P < 0.001$). Leptin, IL-13, macrophage inflammatory protein (MIP)-1β, soluble CD40 (sCD40L) levels were higher and eotaxin and granulocyte colony-stimulating factor (G-CSF) levels were lower in OA SF than in control SF, albeit borderline significant ($P < 0.05$). The PCA enabled identification of six clusters of mediators, which explained 76% of the variance.
Conclusions: The current study provides the first extensive profile of cytokines, chemokines and growth factors present in control and OA SF. Increased levels of mediators such as MDC and IL-6 imply involvement of inflammatory processes and might be associated with the influx of inflammatory cells in OA synovial tissue. Moreover, the performed cluster analysis indicated multiple clusters, which could indicate different pathophysiological pathways in the joint.

Summary

Soluble mediators, e.g., cytokines, chemokines and growth factors, are acknowledged as key players in the pathophysiology of osteoarthritis (OA)[1,2]. However, a wide-spectrum screening of such mediators in the joint environment is currently lacking. In this study, synovial fluid (SF) was collected from control donors and end-stage knee OA patients and analysed for 47 cytokines, chemokines and growth factors using several multiplex ELISAs. In addition, a principal component analysis (PCA) was performed to cluster the measured mediators. Interleukin (IL)-6, interferon inducible protein (IP)-10, macrophage derived chemokine (MDC), platelet derived growth factor (PDGF)-AA and regulated on activation normal T cell expressed and secreted (RANTES) levels were found to be higher in OA compared to control SF ($P < 0.001$). Leptin, IL-13, macrophage inflammatory protein (MIP)-1β, soluble CD40 (sCD40L) levels were higher and eotaxin and granulocyte colony-stimulating factor (G-CSF) levels were lower in OA SF than in control SF, albeit at borderline significance ($P < 0.05$). Increased levels of inflammatory mediators and chemokines, such as MDC

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1063-4584/$ – see front matter
http://dx.doi.org/10.1016/j.joca.2013.04.002

and IL-6, imply involvement of inflammatory processes in OA and might be associated with the influx of inflammatory cells in OA synovial tissue. Additionally, the PCA enabled identification of six different clusters, which explained 76% of the variance, and in this way could indicate different pathophysiological pathways in the joint. This dataset is valuable as a reference for future experiments to study pathophysiological pathways, and useful in more extensive profiling studies for OA.

Brief report

A control group of knee SF samples ($n = 16$) were collected from *post mortem* donors within 24 h after death. Control donors had no history of OA, other joint pathology and possessed macroscopic healthy cartilage. OA SF samples ($n = 18$) were collected during total knee arthroplasty. All OA patients were diagnosed according to the American College of Rheumatology (ACR) criteria for OA[3]. Exclusion criteria were rheumatoid arthritis (RA) or infection. SF samples were centrifuged at 3,000 rpm for 3 min to spin down any cells or debris. The supernatant was stored at −80°C until further analysis. The control SF samples were stored for 1–10 years and OA SF samples were stored for 1–3 years. None of the samples had ever been thawed before. Collection of the SF was done according to the Medical Ethical regulations of the University Medical Centre Utrecht and according to the guideline 'good use of redundant tissue for clinical research' constructed by the Dutch Federation of Medical Research Societies on collection of redundant tissue for research. As according to these guidelines, no information about the patients' characteristics could be obtained. Gender and age information was available for limited donors. Control donors had an average age of 39.6 ± 9.3 and consisted of 55% female. OA donors had an average age of 69.9 ± 7.9 and consisted of 64 % female. Due to the limited availability these data could not be linked to any of the outcomes.

Two hundred microlitre of each of the OA SF samples was pre-treated with 20 μl of hyaluronidase (Sigma, St. Louis, MO, USA; 10 mg/ml) for 15 min at 37°C. Samples were spun down in a X-column (Corning, Amsterdam, Netherlands; Costar 8169). Finally, 150 μl of the SF sample was dissolved in 300 μl high performance ELISA (HPE)-0.1375% Tween (Sanquin, Amsterdam, Netherlands). The pre-treated SF samples were used for all Multiplex ELISA assays mentioned below.

To determine a wide panel of soluble mediators the commercially available human inflammation 42-multiplex and the human adipokine 13-multiplex (Millipore, Bellirica, MA, USA) were used according to the manufacturer's protocol. Additionally, 12 different soluble mediators were measured with the Bio-Plex suspension system (Bio-Rad laboratories, Hercules CA, USA) as previously described[4]. The levels of cytokines in the SF samples were expressed as pg/ml. All samples were measured in the same plate and in duplo. Levels below the lower limit of quantification (LLOQ) were indicated as the value of the lowest point on the calibration curve divided by 2. The measured mediators are listed in Table I. Data are expressed as median ± interquartile range (IqR) as the

Table I
Overview of the measured mediator concentration (pg/ml) in control and osteoarthritic (OA) SF. Data are indicated as median ± IqR. The coefficient of variation (CV) in percentage and lower limit of quantification (LLOQ) with the number of samples are given. Data are subjected to non-parametric statistical analysis Mann–Whitney *U*; $^{*}P < 0.05$ and $\#P < 0.001$. GM-CSF, IL-12(p70), IL-13, IL-17, IL-2, IL-4, IL-5, IL-7, IL-9, TNFα, TNFβ, VEGF and NGF could not be detected

	Healthy SF (median ± IqR)	CV (%)	<LLOQ or 0 (*n*)	OA SF (median ± IqR)	CV (%)	<LLOQ or 0 (*n*)	Detection limit	Mann–Whitney *U* (*P*-value)
EGF	4.8 ± 13.1	102.3	11	4.8 ± 0	189.3	14	5.3	0.65
Eotaxin	14.6 ± 39.6	96.0	6	0 ± 0	236.7	15	12.1	*0.02
Fibroblast growth factor (FGF)-2	37.2 ± 70.4	137.5	2	21.6 ± 231.4	134.1	4	16.0	0.67
Flt-3 ligand	135.9 ± 119.7	59.0	0	103.5 ± 76.4	53.1	0	6.1	0.23
Fractalkine	0 ± 0	387.7	14	0 ± 19	168.5	12	7.6	0.14
G-CSF	34.8 ± 151	105.9	1	16.9 ± 15	66.9	0	3.9	*0.03
Growth regulated oncogene (GRO)	59.2 ± 79.5	74.4	0	84.2 ± 96	81.6	0	11.4	0.38
IFNα2	7.2 ± 10.5	67.4	7	16.4 ± 46.8	89.3	7	27.2	0.09
IFNγ	40.7 ± 12.9	23.30	0	28.0 ± 21.0	136.74	2	2.14	0.1
IL-10	3.3 ± 6.3	127.6	6	2.1 ± 2.7	103.7	3	0.3	*0.04
IL-12 (p40)	4.8 ± 1.7	60.5	9	4.8 ± 11.9	137.7	10	12.4	0.30
IL-15	9.9 ± 6.0	37.1	0	12.8 ± 5.7	31.2	0	0.6	0.32
IL-1α	0 ± 1.8	217.8	9	0 ± 4.9	153.4	12	1.5	0.74
IL-1ra	0 ± 6.9	125.6	8	0 ± 0	288.4	14	2.3	0.14
IL-1β	0 ± 1.5	205.4	11	4.8 ± 11.9	298.6	14	0.7	0.61
IL-3	0 ± 0	0.0	16	4.8 ± 0	179.3	16	9.8	0.18
IL-6	4.8 ± 0	196.9	13	135.8 ± 224.6	120.3	3	0.4	#0.001
IL-8	16.2 ± 43.5	92.2	0	30 ± 23.5	170.9	0	0.3	0.24
IP-10	302.1 ± 280.8	107.6	0	710.4 ± 597.1	93.1	0	1.3	#0.001
MCP-1	542.4 ± 839.2	102.1	0	824.8 ± 645.5	47.8	0	1.2	0.58
MCP-3	4.8 ± 36	123.5	10	4.8 ± 5	215.5	14	5.2	0.83
MDC	52.2 ± 38.4	43.9	0	189.5 ± 119.8	41.8	0	2.4	#0.001
MIP-1α	4.8 ± 0	26.7	16	4.8 ± 0	172.7	15	6.6	0.06
MIP-1β	9.6 ± 24.0	83.7	5	21.8 ± 23.5	127.6	3	3.2	*0.04
PDGF-AA	0 ± 2.1	201.3	11	72.6 ± 116.9	72.8	0	0.3	#0.001
PDGF-AB/BB	43.2 ± 42.9	93.6	1	34.2 ± 69.7	137.8	0	12.2	0.44
RANTES	15.6 ± 28.1	152.7	0	408.2 ± 910.9	114.3	0	1.6	#0.001
sCD40L	0 ± 0	230.9	13	5.9 ± 45.1	144.6	7	5.2	*0.005
sIL-2ra	37.5 ± 76.8	90.8	1	63.0 ± 72.5	65.9	0	7.5	0.11
TGFα	0 ± 0.6	201.2	9	0 ± 0	401.5	17	1.4	0.4
HGF	2554.4 ± 3505.7	80.2	0	2303 ± 1695.2	38.12	0	1.6	0.32
Leptin	82.2 ± 1565.3	133.21	4	1637.5 ± 2414.1	125.33	0	27.4	*0.01
Resistin	2713.4 ± 1854.6	87.25	0	3824.8 ± 3550.7	72.71	0	4.5	0.12
Adiponectin	>250,000	na	na	>250,000	na	na	80.3	na
OSM	0 ± 0	447.83	16	0 ± 0	307.79	12	1.59	0.09