Mitochondrial dysfunction promotes and aggravates the inflammatory response in normal human synoviocytes

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Abstract

Objectives. In RA, synoviocytes cause increased oxidative stress, leading to mitochondrial alterations that may participate in the pathogenesis of RA. Here we investigated whether mitochondrial dysfunction induces inflammatory responses in cultured normal human synoviocytes, a hallmark of RA.

Methods. Mitochondrial dysfunction was induced with the inhibitor oligomycin. The effects of mitochondrial dysfunction on cyclooxygenase-2 (COX-2), prostaglandin E₂ (PGE₂) and IL-8 expression; cellular and mitochondrial reactive oxygen species (ROS) production; nuclear factor-κB (NF-κB) activation and p65 translocation were studied. ROS scavengers (N-acetylcysteine and mitoTEMPO) and an NF-κB inhibitor (BAY-117085) were used to investigate the pathways involved. The natural anti-inflammatory antioxidant resveratrol was also tested.

Results. Mitochondrial dysfunction per se significantly stimulated mitochondrial ROS production as well as low-grade expressions of COX-2, PGE₂ and IL-8. Interestingly, mitochondrial dysfunction induced by pretreatment of synoviocytes with oligomycin synergized with IL-1β to increase the expression of these inflammatory mediators. The inflammatory effects of mitochondrial damage appeared to be dependent on ROS production and NF-κB activation since the inflammatory response was counteracted by both N-acetylcysteine and mitoTEMPO and it was also reduced by BAY-117085. Antimycin A and paraquat (inhibitors of mitochondrial function) also induced inflammatory responses. Furthermore, resveratrol significantly reduced the inflammatory response by decreasing ROS production and NF-κB activation.

Conclusion. These data suggest that mitochondrial dysfunction could induce an inflammatory response in normal human synoviocytes and sensitize these cells, causing a significant amplification of the inflammatory response induced by IL-1β. Resveratrol may represent a promising strategy in controlling the synovial inflammatory response.

Key words: mitochondria, oxidative stress, inflammation, synoviocytes, rheumatoid arthritis, resveratrol.

Introduction

Inflammatory arthritis (e.g. RA) is characterized by chronic cytokine-mediated inflammation of the synovial joints and by abnormal synovial hyperplasia associated with local infiltration of various types of immune and inflammatory cells [1]. RA synoviocytes drive inflammation and degradation of the joint by producing inflammatory cytokines and matrix-degrading molecules that lead to cartilage and bone destruction [2].

A large body of evidence supports the important role of oxidative stress in chronic arthropathies such as RA. In fact, a correlation has been found between the disease activity of RA and the presence of oxidative stress [3], especially oxidative DNA damage [4]. Previous studies have demonstrated increased levels of oxidative stress markers and decreased antioxidant levels in RA sera.
Mitochondrial damage aggravates inflammation

and synovial tissue and fluid, as well as oxidative damage in cartilage [3, 5–7]. Finally, the oxidative process of RA is modulated by drugs used in chronic arthropathies [8].

Mitochondria play a key role in oxidative stress. Because of their central role in adenosine triphosphate (ATP) formation via the mitochondrial respiratory chain (MRC), mitochondria are an important site of reactive oxygen species (ROS) production, but are also targeted by these molecules. Oxidative damage to mitochondria includes oxidation of membrane lipids, proteins and mitochondrial DNA (mtDNA) [9]. Oxidative mtDNA damage caused by ROS could potentially be a main source of mitochondrial genomic instability that leads to respiratory chain dysfunction [10]. MRC dysfunction increases ROS production, leading to a vicious cycle of oxidative stress. In fact, the clinical importance of mitochondria in inflammatory and age-related diseases such as rheumatoid disorders has become apparent [11–14].

With regard to inflammatory arthritis, a deficiency of a subunit of complex IV of the MRC has been described in the synovium of RA and JIA, suggesting a role for mitochondrial dysfunction in these types of arthritis [10, 15]. Furthermore, we recently demonstrated that nitric oxide, which is highly produced in RA synovium, alters the mitochondrial function of human synovial cells [16]. However, despite the potential involvement of mitochondrial dysfunction in arthritis, the role of mitochondria in the proinflammatory response of synoviocytes has not yet been defined.

Thus the purpose of the present study was to investigate the role of mitochondrial dysfunction in the inflammatory response of normal human cultured synoviocytes and to determine whether mitochondrial dysfunction increases the vulnerability to cytokine-induced synoviocyte inflammation. Because of their key role in RA, we decided to study prostaglandin E$_2$ (PGE$_2$) and IL-8. Furthermore, we investigated the effect of the natural polyphenol resveratrol on the above processes.

Materials and methods

Ethics statement

The study was conducted according to the Spanish Law for Biomedical Research (Law 14/2007-3 of July) and complied with the Declaration of Galicia. Written informed consent was obtained when samples were obtained from patients who underwent limb amputation or joint replacement surgery. When samples were obtained from autopsies, authorization was obtained from the family and approved by the ethics committee. All samples were collected anonymously.

Culture and cell stimulation of fibroblast-like synoviocytes

Normal human synovial tissue was obtained as previously described from the knee joint of 31 adult donors and patients [mean age 64 years (s.o. 14)] with no history of joint disease [17]. The tissue was minced and digested with dispase (Gibco-BRL, Paisley, UK) at 37°C for 60 min. Cells were grown in Roswell Park Memorial Institute (RPMI) medium (Lonza, Basel, Switzerland) supplemented with 20% fetal calf serum (FCS), 100 U/ml penicillin or 100 µg/ml streptomycin (Gibco) and 0.12 U/ml human insulin (Novo Nordisk, Bagsvaerd, Denmark). After three passages, isolated synoviocytes were subjected to FACS analysis to verify the culture purity. Before performing the experiments (24 h) and during the experiments cells were maintained in RPMI with 0.5% FCS. Oligomycin (OLI) (Sigma-Aldrich, St Louis, MO, USA) was used as inhibitor of mitochondrial ATP synthase [18, 19]. IL-1β (Sigma-Aldrich) was used to induce an inflammatory response [20]. The ROS scavenger N-acetylcysteine (NAC; Sigma-Aldrich), the mitochondrial antioxidant mitoTEMPO (Santa Cruz Biotechnology, Dallas, TX, USA) and the natural antioxidant resveratrol (Sigma-Aldrich) were also tested. BAY-117085 (BAY; Calbiochem, Darmstadt, Germany) was used to prevent nuclear factor-κB (NF-κB) activation. The NSAID drug diclofenac (Sigma-Aldrich) was used as an inhibitor of cyclooxygenase-2 (COX-2). Cell viability after stimulation was confirmed by trypan blue staining.

ROS detection

Cellular ROS production was determined in synoviocytes using dihydroethidium (DHE; Molecular Probes, Eugene, OR, USA), which is oxidized by superoxide exhibiting red fluorescence. Synoviocytes were loaded with DHE (10 µM) for 30 min in Hanks’ balanced salt solution. Then cells were fixed with 4% paraformaldehyde, washed and counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 10 µg/ml). Fluorescent images were taken using a Nikon Eclipse Ti microscope and further analysed with the ImageJ software. Fluorescence intensity was calculated from five separate fields for each treatment condition.

Measurement of COX-1/COX-2 protein expression

COX-1/COX-2 protein expression was analysed by flow cytometry using the FACSCalibur [Becton Dickinson (BD), Mountain View, CA, USA]. Stimulated cells were collected, washed and permeabilized with 0.2% saponin (Sigma-Aldrich) in FACS lysing solution (BD). Non-specific binding was blocked with PBS containing 1% BSA. Next, cells were incubated with FITC-labelled anti-COX-1/phycoerythrin (PE)-labelled anti-COX-2 (BD) or with an isotype control (FITC-IgG plus PE-IgG). Data are expressed as median fluorescence intensity (MFI) relative to the basal condition, which was set at one.

Analysis of COX-2 and IL-8 mRNA expression

To isolate mRNA, Trizol reagent (Invitrogen) was used. Isolated mRNA was treated with DNase I (Invitrogen) and then amplified with a Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Abingdon, UK). COX-2 and IL-8 expressions were normalized against that of the housekeeping gene porphobilinogen deaminase. PCR analyses were conducted with the LightCycler 480 SYBR Green I Master kit using a Real Time LightCycler

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PGE2 and IL-8 assays

The levels of PGE2 and IL-8 released were determined with commercially available ELISA kits (Amersham, Buckinghamshire, UK and BD, respectively) according to the manufacturer’s instructions. Data are expressed as picograms released per 50,000 cells.

Immunofluorescence detection of p65

Stimulated cells were fixed for 15 min with 4% paraformaldehyde, washed in PBS and permeabilized with a solution containing 0.3% Triton X-100 and 5% goat serum for 1 h. Then synoviocytes were incubated with a mouse anti-p65 antibody (Santa Cruz Biotechnology) for 2 h at room temperature, washed with PBS and incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG (Life Technologies) for 1 h at room temperature. The cells were then washed and counterstained with DAPI. Images were taken using a Nikon Eclipse Ti microscope and further analysed with ImageJ software.

NF-κB binding activity assay

Nuclear proteins were extracted with the Nuclear Extraction Kit from Panomics (Fremont, CA, USA). NF-κB activation was determined with a commercially available electrophoretic mobility shift assay (EMSA) kit (Panomics) according to the manufacturer’s instructions. The probe was detected by chemiluminescence and images of the membrane were taken with the LAS-3000 imaging system (Fujifilm, Stanford, CT, USA).

Statistical analysis

All statistical calculations were performed using GraphPad PRISM version 5 statistical software (GraphPad Software, La Jolla, CA, USA). Data are expressed as mean (S.E.M.) or as representative results, as indicated. Data were analysed using the Wilcoxon test. Where multiple comparisons were performed, the Friedman test was used. Differences were considered significant when P < 0.05.

Results

OLI-induced mitochondrial dysfunction per se stimulates COX-2 expression and PGE2 production in cultured normal human synoviocytes

Mitochondrial dysfunction was induced by the commonly used inhibitor OLI [18, 19]. As expected, inhibition of ATP synthase with OLI induced dose-dependent mitochondrial depolarization as determined by tetramethylrhodamine methyl ester (TMRM) staining and a significant dose-dependent increase in both cytosolic and mitochondrial ROS production as determined by DHE and MitoSOX red staining, respectively (see supplementary Fig. S1 and methods, available at Rheumatology Online).

The effect of mitochondrial dysfunction on the inflammatory response was first quantified on COX-2 protein expression. As shown in Fig. 1A and B, incubation of cells with OLI for 6 h increased COX-2 protein expression. As expected, this inhibitor had no effect on the expression of the constitutive isoform COX-1 (Fig. 1A). Also, as seen in Fig. 1C, mitochondrial dysfunction due to OLI treatment for 4 h caused a dose-dependent increase in mRNA COX-2 expression. Results of parallel studies evaluating the production of PGE2 in synoviocytes treated for 9 h with OLI were consistent with those of protein and mRNA COX-2 expression studies (Fig. 1D). After the treatment of cells with the COX inhibitor diclofenac (10 μM), the PGE2 levels drastically decreased (Fig. 1E).

Mitochondrial dysfunction synergizes with the cytokine IL-1β to induce inflammatory response in cultured normal human synoviocytes

Pretreatment of synoviocytes with OLI synergistically intensified COX-2 protein and mRNA expression induced by a low concentration of IL-1β (0.1 ng/ml) at 6 and 4 h, respectively (Fig. 2A and C, respectively). OLI pretreatment reproduced the effect of a 10 times greater concentration of IL-1β, being the percentage of increase higher at the lower IL-1β concentration (Fig. 2B). Finally, a combination of mitochondrial dysfunction and low doses of IL-1β also resulted in a synergistic induction of PGE2 production at 9 h (Fig. 2D).

IL-8 is a major player in arthritis progression. As seen in Fig. 3A, mitochondrial dysfunction produced a dose-dependent increase in IL-8 mRNA expression in synoviocytes stimulated for 4 h. We also showed that sensitizing these cells with OLI caused a significant increase in IL-8 mRNA and protein expressions induced by IL-1β (Fig. 3B and C, respectively).

Mediators involved in the inflammatory response after impairment of mitochondrial function in normal human synoviocytes

Because increased oxidative stress may lead to the up-regulation of redox-sensitive proinflammatory transcription factors, we investigated the role of ROS in the proinflammatory response induced by OLI. Synovial cells were first pre-incubated with the mitochondria-targeted antioxidant mitoTEMPO (50 μM). By inhibiting mitochondrial ROS production, IL-8 production induced by OLI or by the combination of OLI and IL-1β was significantly reversed (Fig. 4A and B). In addition, the general ROS scavenger NAC significantly decreased the COX-2 protein expression that was increased by the combination of IL-1β and OLI (Fig. 4C).

Because IL-1β and oxidative stress have been reported to induce activation of NF-κB, we investigated whether this transcription factor is involved in the synergistic effect induced by mitochondrial dysfunction and IL-1β. Subjecting synovial cells to pretreatment with BAY, an NF-κB inhibitor, significantly reversed COX-2 protein expression, as well as PGE2 and IL-8 levels, synergistically increased by IL-1β (Fig. 5A-C, respectively). When we analysed NF-κB translocation to the nucleus in normal human synoviocytes by p65 immunofluorescence (Fig. 5D), we found that OLI-treated synovial cells had
Fig. 1 Oligomycin induces COX-2 expression and PGE$_2$ production in cultured normal human synoviocytes

(A) Representative flow cytometry of COX-1 and COX-2 protein expression after 6 h of incubation with IL-1$\beta$ or OLI.
(B) Quantification of COX-2 protein expression expressed as MFI relative to the basal condition ($n$ = 9).
(C) COX-2 mRNA quantification after 4 h of stimulation expressed as the fold change relative to the basal condition ($n$ = 5).
(D) PGE$_2$ determination after 9 h of treatment ($n$ = 5 in duplicate).
(E) Effect of diclofenac in OLI-induced PGE$_2$ production. *$P$ < 0.05 vs OLI.

Data are mean (s.e.m.). Unless otherwise specified, *$P$ < 0.05, **$P$ < 0.01 and ***$P$ < 0.001 vs basal. COX-2: cyclooxygenase-2; PGE$_2$: prostaglandin E$_2$; OLI: oligomycin; FI: fluorescence intensity; MFI: median fluorescence intensity.
small amounts of activated nuclear NF-κB and that the response to a low concentration of IL-1β (0.1 ng/ml) was slightly increased when cells were also pre-incubated with OLI. Consistent results were obtained when we analysed NF-κB binding activity by EMSA under the same conditions (Fig. 5E).

Resveratrol protects synovial cells from inflammation induced by mitochondrial dysfunction

Because the obtained data suggested that ROS released by mitochondria during mitochondrial dysfunction and activation of NF-κB are implicated in COX-2 and IL-8 induction, we examined whether the natural anti-inflammatory and antioxidant compound resveratrol is able to attenuate this effect.

First, we confirmed that resveratrol is able to decrease OLI-induced ROS production in synoviocytes as shown by DHE staining (Fig. 6A). Second, pretreatment with resveratrol reduced the expression of COX-2, PGE2 and IL-8 exacerbated by IL-1β in our model of mitochondrial dysfunction (Fig. 6B-D). Also, treatment with resveratrol caused a decrease in the nuclear translocation and binding activity of NF-κB induced by IL-1β + OLI (Fig. 6E and F, respectively).

**Fig. 2** Mitochondrial dysfunction strongly modulates COX-2 expression and PGE2 production induced by IL-1β in synoviocytes

(A) Representative cytometry showing COX-1/COX-2 expression after 30 min of incubation with OLI before treatment with IL-1β for 6 h (n = 7). (B) COX-2 protein quantification at 6 h under basal conditions or in the presence of increasing concentrations of IL-1β either alone or in combination with OLI (n = 6). *P ≤ 0.05 vs IL-1β 0.1 ng/ml. (C) COX-2 mRNA determination at 4 h. Values are the fold change vs basal (n = 6). *P ≤ 0.05, **P ≤ 0.01. (D) PGE2 determination at 9 h (n = 7 in duplicate). Data are expressed as mean (S.E.M.). **P ≤ 0.01, ***P ≤ 0.001. COX-2: cyclooxygenase-2; PGE2: prostaglandin E2; OLI: oligomycin.
Fig. 3 IL-8 is up-regulated by mitochondrial dysfunction in normal human synoviocytes

(A) IL-8 mRNA quantification after 4 h of incubation. Values are the fold change vs basal, expressed as mean (S.E.M.) (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 vs basal.

(B) IL-8 mRNA quantification after 4 h of exposure to OLI + IL-1β. Values are the fold change vs basal, expressed as mean (S.E.M.) (n = 10). *P < 0.05, ***P < 0.001.

(C) IL-8 protein release determination in synoviocytes pre-incubated with OLI before addition of IL-1β (9 h). Data are the mean of IL-8 released per 50,000 cells (S.E.M.) (n = 4 in duplicate). *P < 0.05, ***P < 0.001. OLI: oligomycin.

Fig. 4 Role of ROS in the inflammatory response induced by mitochondrial dysfunction

(A) IL-8 production in synoviocytes pre-incubated with the mitochondria-targeted antioxidant mitoTEMPO for 1 h before treatment with OLI for 9 h. Data are mean (S.E.M.) of the percentage compared to OLI (n = 6). (B) IL-8 quantification in synoviocytes pre-incubated with mitoTEMPO before treatment with IL-1β + OLI. Data are mean (S.E.M.) of the percentage compared to IL-1β + OLI (n = 5). (C) COX-2 expression was assayed in synoviocytes pre-incubated for 30 min with NAC before treatment for 6 h with IL-1β + OLI (n = 6). Data are mean (S.E.M.) of the percentage compared to IL-1β + OLI. *P < 0.05. ROS: reactive oxygen species; OLI: oligomycin; COX-2: cyclooxygenase-2; NAC: N-acetylcysteine.
FIG. 5 Role of NF-κB in the inflammatory response induced by mitochondrial dysfunction plus IL-1β

(A) COX-2 protein quantification in synoviocytes pre-incubated for 30 min with BAY before treatment for 6 h with IL-1β + OLI (n = 6). *P ≤ 0.05 vs IL-1β + OLI without BAY. (B and C) PGE2 and IL-8 determination in synoviocytes pre-incubated with BAY before treatment for 9 h with IL-1β + OLI (n = 3 in duplicate). *P ≤ 0.05 vs IL-1β + OLI without BAY. (D) Immunofluorescence of p65 under basal conditions, IL-1β (20 min), OLI (40 min) and IL-1β + OLI (20 min with OLI followed by IL-1β for 20 min) (n = 3). (E) Representative EMSA under the above conditions (n = 6). NF-κB: nuclear factor-kappa B; COX-2: cyclooxygenase-2; OLI: oligomycin; BAY: BAY-117085; PGE2: prostaglandin E2; EMSA: electrophoretic mobility shift assay.
Discussion

In the last few years, numerous studies have strongly suggested that mitochondrial dysfunction plays a role in rheumatoid disorders [11, 15, 21–24]. Relevant to RA, the involvement of mitochondrial damage in different aspects of RA [25–27], e.g., autoimmunity [28, 29] or the hypoxic state of synovial tissue [10], has been pointed out. There is also accumulating evidence for a connection between mitochondrial dysfunction and inflammation [30, 31]. Our present findings in synoviocytes have implications for the pathology of RA that have never been described before to our knowledge. Synoviocytes are one of the main secretory cells in the articular joint, having a great impact on the pathogenesis of the disease [2]. Since mitochondrial dysfunction has been found in RA synoviocytes, we think it is of great relevance to investigate what the consequences of this mitochondrial dysfunction are in terms of inflammation, which is the main feature of RA pathogenesis [1, 3]. The results of this work constitute the first evidence of mitochondria as organelles involved in the proinflammatory response of synoviocytes. In particular, we demonstrated that mitochondrial dysfunction induces a low-grade inflammatory response in normal human synoviocytes and sensitizes these cells, causing a significant increase in the inflammatory response induced by cytokines. ROS generation and NF-κB activation are involved in this process. In addition, we showed that resveratrol significantly reduced this inflammatory response.

COX-2, one of the major players involved in the inflammatory process, is up-regulated in inflamed joint synovial tissue and is responsible for elevated PGE2 production, a key mediator in different forms of inflammatory arthritis [32, 33]. COX-2 overexpression is likely induced by pro-inflammatory mediators such as IL-1β and TNF-α [33, 34]. PGE2 is involved in inflammation, apoptosis, angiogenesis and possible structural changes that characterize arthritic diseases and contribute to tissue oedema and hyperalgesia [35, 36].

In our present study, when mitochondrial dysfunction was induced in normal synoviocytes by OLI, a commonly used inhibitor of mitochondrial ATP synthase [18, 19], an increase in COX-2 mRNA and protein expression levels was observed. The increase in COX-2 expression was accompanied by a dose-dependent increase in PGE2 release. These data are strengthened by those obtained in our previous work in which we showed that mitochondrial dysfunction produced a slight increase in COX-2 expression and PGE2 production in chondrocytes [18]. Released PGE2 may contribute to the generation of positive feedback mechanisms [37]. Mitochondrial dysfunction has also been reported to induce PGE2 release through 4-hydroxy-2-nonenal (4-HNE), a lipid peroxidation by-product that is increased in the synovial tissue of patients with inflammatory arthritis and associated with a higher frequency of mtDNA mutations [5, 38].

Next, we evaluated whether mitochondrial dysfunction could aggravate the inflammatory response induced by cytokines in normal human synoviocytes. For this, we assessed whether mitochondrial dysfunction enhances COX-2 expression induced by cytokines. Among the cytokines implicated in the pathogenesis of RA, IL-1β is considered one of the most powerful mediators, being found in the affected joints in the range 10–100 pg/ml [20]. OLI treatment in human synoviocytes resulted in a synergistic increase in the inflammatory response induced by IL-1β. In OLI-treated synoviocytes, an otherwise less efficient concentration of IL-1β was as effective as a 10 times greater concentration of IL-1β in the absence of pretreatment with OLI. Again, this increase in COX-2 protein expression was accompanied by increased COX-2 mRNA expression and PGE2 release. Interestingly, recent data have shown that PGE2 promotes IL-1β expression in articular chondrocytes, thus amplifying the local inflammatory process and synergistically accelerating the expression of pain-associated molecules, including nitric oxide synthase and IL-6 [39]. These findings confer even more physiological relevance to our findings regarding the effects of mitochondrial dysfunction.

Furthermore, it has previously been reported that the decrease in synovial tissue partial oxygen pressure of RA-affected joints directly correlates with increased disease activity and mitochondrial dysfunction [10]. The hypoxic state of arthritic joints in vivo exacerbates the inflammatory response in synoviocytes by increasing COX-2 expression, PGE2 production and release of MMPs in response to IL-1β [40]. These findings support our hypothesis that synoviocytes with mitochondrial dysfunction are more responsive to cytokines in relation to inflammatory response. Moreover, preliminary data of our group show that mitochondrial dysfunction promotes VEGF production and MMP-1 and MMP-3 release, probably leading to an inflammatory response as well as synovial angiogenesis and joint destruction.

Chemokines are considered important molecules in RA pathology, which is characterized by infiltration of inflammatory cells. High levels of IL-8 are present in both the synovial tissue and fluid of RA patients and is known to have angiogenic activity in the RA joint [32]. Our results showed that OLI-induced mitochondrial dysfunction in synoviocytes significantly induced IL-8 expression. In addition, the combined treatment of OLI and IL-1β also resulted in a synergistic effect on the production of IL-8.

The MRC is one of the most important sites of ROS production. As shown in supplementary Fig. S1, available at Rheumatology Online, inhibition of mitochondrial ATP synthase with OLI induced both cytosolic and mitochondrial ROS production in cultured human synoviocytes. Also, when synoviocytes were treated with the mitochondria-targeted antioxidant mitoTEMPO or the general ROS scavenger NAC, IL-8 production induced by OLI or the combination IL-1β + OLI was significantly decreased. These findings confirm ROS, and specifically mitochondrial ROS, as a key mediator in the inflammatory pathway. In other cell types, mitochondrial dysfunction also increased the generation of ROS, resulting in increments of cytotoxicity and inflammatory mediators or accumulation of inflammatory cells [18, 41, 42].

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**Fig. 6** Modulatory effects of resveratrol on the inflammatory response induced by mitochondrial dysfunction plus IL-1β

(A) Representative images of ROS detection using DHE in OLI-treated synoviocytes with or without resveratrol pretreatment and quantification expressed in AU relative to OLI (n = 2). (B–D) COX-2 protein expression (6 h, n = 7), PGE₂ and IL-8 production (9 h, n = 3 in duplicate) in synoviocytes treated with or without resveratrol before stimulation with IL-1β + OLI. *P ≤ 0.05 vs control without resveratrol. (E) p65 immunofluorescence in (1) synoviocytes stimulated with OLI + IL-1β and (2) synoviocytes pre-incubated with resveratrol for 30 min before IL-1β + OLI treatment (n = 3). (F) Representative EMSA under the conditions described above (n = 5). ROS: reactive oxygen species; DHE: dihydroethidium; OLI: oligomycin; AU: arbitrary units; COX-2: cyclooxygenase-2; PGE₂: prostaglandin E₂; EMSA: electrophoretic mobility shift assay.
Because it controls the transcription of a number of proinflammatory genes, the NF-κB pathway is considered to be a key regulator of tissue inflammation, including the induction of COX-2 and IL-8 expression in several cell types, e.g. rheumatoid synovial fibroblasts [34]. NF-κB is a redox-sensitive transcription factor that can be activated under oxidative stress conditions by the so-called atypical pathway [43]. ROS production by dysfunctional mitochondria could be an intermediate step in COX-2/NF-κB signalling [44]. In this study we found that mitochondrial dysfunction induced a slight increase in NF-κB activation. Also, pharmacological inhibition of NF-κB with BAY significantly prevented up-regulation of COX-2 expression, PGE₂ production and IL-8 synthesis induced by mitochondrial dysfunction in combination with IL-1β. This finding suggests that NF-κB sites may be essential for the inflammatory response induced by mitochondrial dysfunction in synoviocytes. Other groups have obtained similar results, reporting enhanced sensitivity to activation of NF-κB under conditions of mitochondrial dysfunction [41, 49]. Other redox-sensitive transcription factors or post-transcriptional regulations might have to be considered when interpreting our results [37, 46]. Furthermore, we cannot rule out that inhibition of oxidative phosphorylation could also have an effect on the complex interaction between mitochondrial pathways and inflammatory response, e.g. by interfering with Ca²⁺ exchange. It is worth noting that the results obtained in this study are not specific for the ATP synthase inhibitor OLI. Other compounds that induce mitochondrial dysfunction, such as the inhibitor of oxidative phosphorylation AA or the mitochondrial pro-oxidant PQ, have similar effects on the inflammatory response of normal human synoviocytes (see supplementary Fig. S2, available at Rheumatology Online).

It is well known that oxidative stress and inflammation play a role in the pathology of different types of inflammatory arthritis, e.g. RA. Hence there are high expectations for studies that examine therapeutic strategies offered by natural compounds that exhibit anti-inflammatory properties. Resveratrol, a natural polyphenol found in high concentrations in grape skin and red wine, has been widely recognized for its anti-inflammatory, antioxidant, anticancer and anti-ageing properties. In animal models it has been shown that resveratrol protects from the development of age-related diseases and improves mitochondrial function [47]. Furthermore, preliminary results obtained by our group demonstrated that dietary resveratrol diminishes the histological damage and cell recruitment in antigen-induced arthritis in rats (unpublished results). In several cell types, resveratrol has been shown to have a number of beneficial effects, i.e. by reducing NF-κB activation, PGE₂ production and free radical formation; by blocking mitochondrial membrane depolarization and ATP depletion or by inducing mitochondrial biogenesis and protecting against chondrocyte apoptosis [14, 48]. It is the current belief that resveratrol may inhibit NF-κB signalling and inflammation by up-regulating the enzyme adenosine monophosphate kinase (AMPK) [49]. However, despite the many studies that highlight the beneficial effects of resveratrol on health, controversial results have also been reported [50].

In our study, resveratrol was effective in preventing inflammation after treatment of synoviocytes with a combination of IL-1β and OLI. In fact, resveratrol reduced PGE₂ production to basal levels and dramatically decreased COX-2 expression and IL-8 production. This anti-inflammatory effect is mediated by decreased ROS production and NF-κB activation. Furthermore, we also found that resveratrol decreased COX-2, PGE₂ and IL-8 expressions in RA synoviocytes (unpublished results). Taken together, our results indicate that resveratrol may represent an encouraging strategy for controlling the inflammatory response in synoviocytes.

We conclude that mitochondrial dysfunction can induce a low-grade inflammatory response by itself and synergistically intensify the expression of inflammatory mediators induced by cytokines in normal human synoviocytes. The synergistic up-regulation of inflammatory mediators in the articular environment would be of great physiological relevance, aggravating oxidative stress and the vicious cycle of inflammation and contributing to joint structure alteration, swelling and pain. ROS generation and activation of transcription factors such as NF-κB are key factors involved in the inflammatory response modulated by mitochondrial dysfunction. These findings may provide a better understanding of the underlying mechanisms of inflammatory arthritis. Future studies should elucidate the preservation of mitochondrial activity with natural anti-inflammatory and antioxidant compounds, e.g. resveratrol, as a potential strategy for controlling the inflammatory response in synoviocytes.

### Rheumatology key messages

- Mitochondrial dysfunction stimulates an inflammatory response and also exacerbates IL-1β-induced inflammation in normal human synoviocytes.
- Reactive oxygen species production and nuclear factor-κB activation are involved in the inflammatory response induced by mitochondrial dysfunction.
- The polyphenol resveratrol can modulate the inflammation induced by mitochondrial dysfunction in normal human synoviocytes.

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Conflicts of interest.

The authors have declared no disclosure statement.

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Supplementary data

Supplementary data are available at Rheumatology Online.

References


Mitochondrial damage aggravates inflammation


