Scandinavian Journal of Rheumatology

ISSN: 0300-9742 (Print) 1502-7732 (Online) Journal homepage: http://www.tandfonline.com/loi/irhe20

Anti-inflammatory effect of dialysable leucocyte extract in a rat model of osteoarthritis: histopathological and molecular characterization

P Acosta, N Pérez, E Pérez, B Correa, C Pérez, C Gómez, V Sánchez & DG Pérez

To cite this article: P Acosta, N Pérez, E Pérez, B Correa, C Pérez, C Gómez, V Sánchez & DG Pérez (2016): Anti-inflammatory effect of dialysable leucocyte extract in a rat model of osteoarthritis: histopathological and molecular characterization, Scandinavian Journal of Rheumatology, DOI: 10.3109/03009742.2016.1153140

To link to this article: http://dx.doi.org/10.3109/03009742.2016.1153140

Published online: 21 Apr 2016.

Anti-inflammatory effect of dialysable leucocyte extract in a rat model of osteoarthritis: histopathological and molecular characterization

P Acosta[1], N Pérez[1], E Pérez[2], B Correa[3], C Pérez[3], C Gómez[1], V Sánchez[1], DG Pérez[1]

[1]Laboratory of Molecular Biomedicine, National School of Medicine and Homeopathy (ENMH), National Polytechnic Institute (IPN), [2]Hospital Dr Victorio de la Fuente Narváez, and [3]Bioexport, SA de CV, Mexico City, Mexico

Objectives: To evaluate the effect of dialysable leucocyte extract (DLE) on pro- and anti-inflammatory profiles in a rat model of osteoarthritis (OA).

Method: Forty-eight male Wistar rats were divided into three groups: normal rats without treatment, OA rats treated with placebo, and OA rats treated with DLE. After treatment, the animals were killed to obtain cartilage for histological analysis and to determine the expression of pro- and anti-inflammatory cytokines by reverse transcription multiplex polymerase chain reaction (RT-MPCR) and immunohistofluorescence analyses.

Results: Histological analysis revealed that OA cartilage from rats treated with DLE displayed similar characteristics to non-OA cartilage from the control group. The OA cartilage treated with placebo showed alterations in the cellular architecture and in chondrocyte cluster formation. Analysis of cytokine expression by RT-MPCR showed that OA cartilage from DLE-treated rats expressed platelet-derived growth factor (PDGF), interferon (IFN)-γ, and fibroblast growth factor (FGF)-2, similar to non-OA cartilage from the control group. However, OA cartilage from rats treated with placebo expressed interleukin (IL)-1, PDGF, and I kappa B (IκB). Confocal immunodetection of FGF-2, PDGF, and non-phosphorylated IκB showed that they were distributed in the cytoplasm of most chondrocytes in OA cartilage from DLE-treated rats whereas no nuclear factor kappa B (NF-κB) expression was observed in the nuclei. Instead, in OA cartilage from the placebo group, only weak FGF-2 staining was observed, PDGF and IκB were not detected, and NF-κB was strongly observed in both cytoplasm and nuclei.

Conclusions: Our findings suggest that DLE treatment modifies the OA process, promoting the expression of anti-inflammatory cytokines and diminishing the inflammatory effects, avoiding the nuclear translocation of NF-κB in chondrocytes.

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive and irreversible loss of cartilage with subchondral area remodelling and inflammation of the synovial membrane (1). Clinically, OA has a multifactorial aetiology and is characterized by stiffness and joint pain, limited movement, crepitus, and varying degrees of local inflammation (2, 3).

In the pathophysiology of OA, catabolic and anabolic factors that trigger excessive degradation and loss of articular cartilage are altered, generating in chondrocytes the inability to maintain a homeostatic balance between synthesis and degradation of the extracellular matrix (ECM), an increase in pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF)-α, and an increase in cartilage catabolism, favouring the synthesis of anabolic factors such as transforming growth factor (TGF)-β, leading to a deregulation metabolic pattern, and promoting collagen and proteoglycan degradation (4–7). The goal of current treatments is to reduce pain and improve joint function; however, these treatments are insufficient to halt the progressive loss of cartilage (8).

Dialysable leucocyte extract (DLE) contains molecules of low molecular weight of about 10 000 Daltons that can modulate the immune response because of its ability to increase the synthesis of molecules such as interferon (IFN)-γ and IL-2. It also increases activation and chemotaxis of macrophages and natural killer cells (9–13). DLE has been clinically useful in several diseases and has also been used as an adjuvant treatment for rheumatoid arthritis (14–24). There is evidence that DLE modulates inflammation and improves mobility in articular pathologies (23); however, the molecular action exerted by this therapy in OA has not yet been tested. In the current study, we investigated the molecular and histopathological effects of DLE in an experimental OA rat model.

David Guillermo Pérez, National School of Medicine and Homeopathy (ENMH), National Polytechnic Institute (IPN), Guillermo Massieu Helguera 239, Col. La Escalera, 07320 Mexico City, Mexico.
E-mail: ishiwaramx@yahoo.com.mx

Accepted 8 February 2016

DOI: 10.3109/03009742.2016.1153140

Method

Overall study design and treatment

All experiments were performed using protocols approved by the ethics committee of the National School of Medicine and Homeopathy (ENMH) of the National Polytechnic Institute (IPN), Mexico. Forty-eight male Wistar rats weighing between 130 and 150 g or 60–80 days old (HARLAN Laboratories HsdHan®: WIST, Mexico City, Mexico) were divided into three groups: a control group composed of normal rats without treatment; a placebo group composed of OA rats administered with 0.5 mL of placebo (15% glycine), three times a week for 4 weeks; and a DLE group composed of OA rats administrated with 0.5 mL of DLE (33% DLE), three times a week for 4 weeks. OA was induced by partial meniscectomy (25); the surgical procedure was performed under ketamine 0.7 mL (Anesket®; Pisa, Mexico City, Mexico) and xylazine 0.3 mL (Procin®; Pisa) intraperitoneal anaesthesia. Trichotomy was performed on the joint area (right leg) after previous asepsis and antisepsis of the region. An anteromedial incision was made on the inside of the right knee, parallel to the longitudinal axis; the tissues were dissected in planes to address the medial compartment of the joint and between 30 to 50% of the lateral meniscus was sectioned. Two days after surgery, the rats were subjected to high-impact exercises for 20 days. After treatment, the femoral condyle and the tibial plateau of the affected joints were obtained for morphological, histological, immunohistofluorescence, and molecular analyses.

Clinical evaluation schedule

All groups were assessed clinically to determine changes in pain and mobility. Clinical signs were documented by a qualitative measure assigning a score as shown in Table 1.

Morphological and histological analyses

The tibial tray and femoral condyles were extracted to perform comparative morphological analyses. For the histological analysis, the articular cartilage explants from the loading areas (femoral condyles and tibial plateau) were fixed in 10% formaldehyde in phosphate-buffered saline (PBS) buffer and Bouin solution. The explants were processed as follows: dehydrated with increasing alcohol concentrations of 70, 90, and 100% for 10 min; then rinsed three times with xylene for 10 min; and finally infiltrated in paraffin at 60°C. Sections (3-μm-thick) were cut using a microtome (American Optical rotary microtome 820, San Marcos CA, USA). For histological evaluation, haematoxylin and eosin (H&E) staining was used (Sigma Aldrich, St Louis, MO, USA); the proportion of proteoglycans was assessed by safranin O staining (Sigma Aldrich) and evaluation of collagen was performed using Masson's trichrome stain (Sigma Aldrich).

Table 1. Classification of clinical signs.

Score	Clinical signs
1	Healthy, without signs of illness
2	Piloerection
3	Lethargy + piloerection
4	Walking slowly + lethargy + piloerection

Expression of anti- and pro-inflammatory cytokines by reverse transcription multiplex polymerase chain reaction (RT-MPCR)

Total RNAs were extracted from cartilage using Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). RNAs concentrations were determined spectrophotometrically and were RQ1 RNAse-free DNAse treated (Promega, Madison, WI, USA) to avoid genomic DNA contamination. cDNAs were then synthesized using the Super Kit III First-Strand Scrip Synthesis Supermix (Invitrogen). Using the synthesized cDNAs, we evaluated the expression of pro-inflammatory [I kappa B (IκB), TNF-α, IL-1, IL-6, and nuclear factor kappa B (NF-κB)] and anti-inflammatory cytokines [IFN-γ, platelet-derived growth factor (PDGF), FGF-2, IL-4, IL-2, and IL-10) using the MPCR kits for rat inflammatory and pro-inflammatory cytokine genes (Maxim Biotech Inc, San Francisco, CA, USA), respectively. Reaction mixtures for both kits were placed in a thermocycler (Applied Biosystems, Carlsbad, CA, USA) with the following cycling conditions: 96°C for 1 min; 64°C for 4 min, for two cycles; then 40 cycles of 94°C for 1 min and 64°C for 4 min; and finally incubating at 70°C for 10 min. The amplification products were separated on 12% polyacrylamide (acrylamide/bisacrylamide 29:1) gels in TBE buffer (tris–HCl 100 mm boric acid, 2 mm EDTA). Electrophoresis was performed at 1000 V for 1.5 h. The gels were stained with ethidium bromide (0.5 μg/mL), visualized in a UV transilluminator (UVP PhotoDoc-It Imaging Systems, Upland, CA, USA), and photo-documented.

Localization of PDGF, FGF-2, IκB, and NF-κB cytokines in cartilage by immunofluorescence

Section slides of cartilage from the three groups were incubated separately with 50 μL of the primary antibodies against the cytokines PDGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA), FGF-2 (Santa Cruz Biotechnology), IκB (Abcam, Cambridge, MA, USA), and NF-κB (Abcam) overnight at 4°C. All antibodies were diluted in a 1:50 ratio. This was followed by incubation with fluorescein isothiocyanate (FITC)-

tagged rabbit anti-goat immunoglobulin (Ig)G antibody (1:60; Jackson Immuno Research, West Grove, PA, USA) for 1 h at room temperature. Nuclei were counterstained with propidium iodide for 5 min (1:1000; Vector Laboratories, Burlingame, CA, USA). The procedure was concluded with Vectashield mounting and sections were sealed with nail polish, keeping cuts at 4°C for microscopic analysis. Immunolabelled sections were viewed through a confocal laser scanning confocal microscope (LSM 710, Carl Zeiss, Germany) using a 100× oil-immersion plan Apochromat objective (numerical aperture 1.4). The resulting stack of images was projected and analysed on a two-dimensional (2-D) plane using a pseudo colour display green (FITC) and red (propidium iodide). Fluorochromes in double-labelled samples were excited at wavelengths of 488 nm (for FITC) and 554 nm (for propidium iodide).

Results

Clinical evaluation scores based on the scale described in the method are summarized in Figure 1. Clinical evaluation of the rats showed that physical activity in the control group was not affected. On day 5, rats treated with placebo showed a significant decrease in physical activity, along with piloerection; by day 10 the rats had displayed slow movements and the piloerection had increased; and by day 15 the rats displayed lethargy and changes in gait (stiffness of the affected joint while walking). Instead, rats treated with DLE also presented piloerection on day 5 but physical activity was similar to that of the rats in the control group. Of note, the rats maintained increased mobility during high impact exercises until day 15; however, on day 20 they began to display lethargy, piloerection, and some alterations in gait (stiffness of the affected joint while walking).

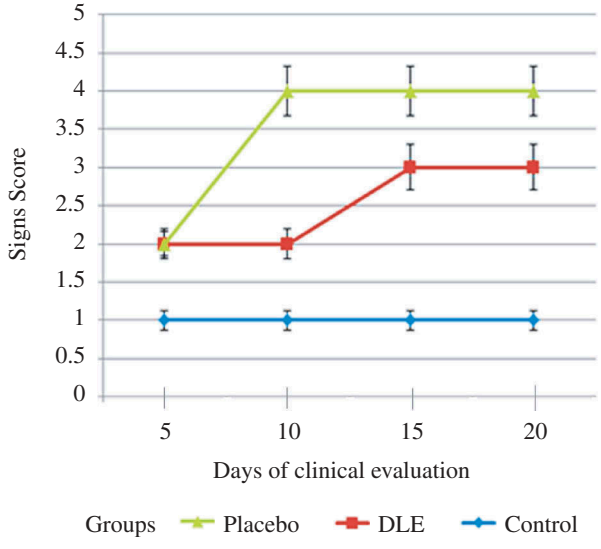

Figure 1. Clinical evaluation schedule. The rats in the placebo group showed more clinical signs of OA than those treated with DLE.

Morphological analysis

Morphological study of the joints of the control group found that the surface of the tibial and femoral articular cartilage showed normal characteristics such as a pearly bluish colour with a firm consistency (Figures 2A and 2B). In knees from OA rats treated with placebo, the femoral condyles showed erosions and partial thickening. There was no evidence of joint thickening of the tibial plateau but there was significant fibrosis in both samples, and the cartilage was seen to be more opaque in comparison to the joints of the control group (Figures 2C and 2D). By contrast, analysis of the knees from animals treated with DLE showed that the medial femoral condyles were equally affected by erosion and thickening, although in the tibial plateau we observed less fibrosis in comparison to the placebo group (Figures 2E and 2F).

Histological analysis

Histological analysis (H&E staining) of samples from the control group showed flat characteristics typical of healthy articular cartilage, preserving the architecture of the tissue, as well as the morphology and disposition of chondrocytes in the tissue. The superficial zone contained numerous elongated and flattened chondrocytes, the intermediate zone contained round chondrocytes distributed randomly within the matrix, and the deep zone was characterized by small round chondrocytes that were arranged in short columns, perpendicular to the free surface of the cartilage (Figure 3A). By contrast, the articular cartilage of the rats treated with placebo showed focal discontinuity, disorientation of chondron columns, chondrocyte proliferation (clusters) in the middle zone, and cell hypertrophy as well as focal expansion and loss of chondron orientation (Figure 3B). While the cartilage of the rats treated with DLE showed a smooth surface, the chondrocytes were organized in zones, appropriately oriented; no enlargement or distortion of chondrons and no proliferative changes in the chondrocytes were found. The morphology and orientation of the superficial chondrocytes, as well as the perpendicular columns, were very similar to those of normal cartilage and no chondrocyte clustering was observed (Figure 3C).

Safranin O staining for proteoglycans revealed, in the control group, apparently healthy sham cartilage that had intact superficial, middle, and deep zones with a deep red stain. The image also showed that the chondrocytes were arranged in columns (Figure 3D). By contrast, OA samples from the placebo group showed greater heterogeneity in the entire thickness of the cartilage, predominantly in the superficial and middle zones, also showing zones of fibrocartilage, suggesting further proteoglycan depletion (Figure 3E). By contrast, samples from the DLE group displayed bright red staining in the middle and deep zones, showing pale areas on the surface layer and several zones of fibrocartilage, which denotes a decrease in the proportion of proteoglycan, compared with the homogeneous staining throughout the cartilage

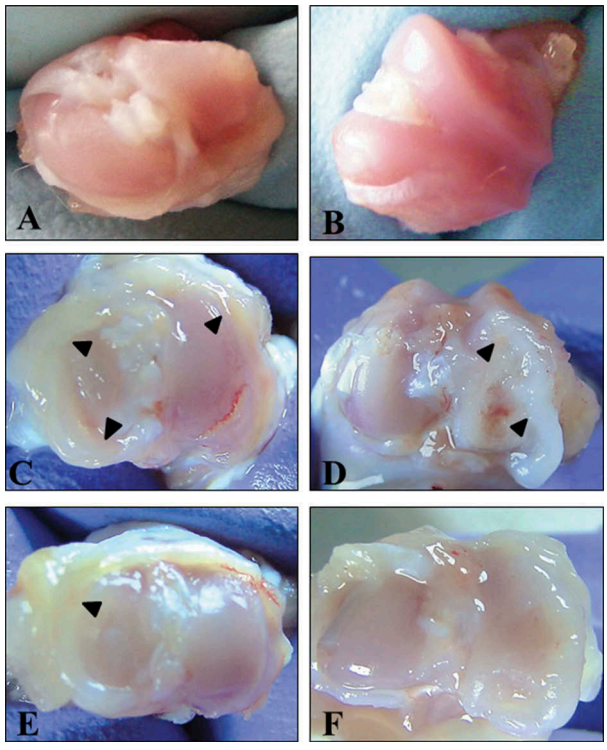

Figure 2. Macroscopic analysis of the joint. (A) Tibial plateau and (B) femoral condyles from rats in the control group; (C) tibial plateau and (D) femoral condyles from rats treated with placebo; and (E) tibial plateau and (F) femoral condyles from rats treated with DLE. Arrowheads show the areas of fibrosis.

thickness in the control group, suggesting that in the DLE group there was also a partial proteoglycan depletion (Figure 3F).

Masson trichrome staining allowed us to specifically inspect the collagen fibres. The samples obtained from cartilage explants of the control group showed a homogeneous blue stain indicating normal quantities of collagen (Figure 3G). The OA cartilage samples obtained from rats treated with placebo showed significant collagen degradation, evidenced by the heterogeneous red stain affecting all layers of cartilage, showing focal rarefaction and condensation of collagen fibres in the superficial and middle zones (Figure 3H). By contrast, samples from the DLE group showed a slight decrease in collagen fibres, predominantly in the middle areas of the cartilage (Figure 3I).

Expression of anti- and pro-inflammatory cytokines by RT-MPCR

We evaluated the expression of six anti-inflammatory cytokines IL-10, IL-4, IL-2, PDGF, IFN-γ, and FGF-2 by RT-MPCR and found that IFN-γ, FGF-2, and PDGF were expressed in the control group (Figure 4). Samples obtained from rats treated with placebo only showed expression of PDGF whereas samples obtained from rats treated with DLE displayed an expression pattern similar to that of the control group; of note, the expression of PDGF was higher (Figure 4). We also evaluated the expression of the pro-inflammatory cytokines IL-1, TNF-α, and IκB. No basal expression of pro-inflammatory cytokines in normal cartilage was found (Figure 4); as expected, samples obtained from rats treated with placebo showed expression of IL-1 and Iκ-B whereas samples obtained from rats treated with DLE only expressed IL-1 (Figure 4).

Immunohistofluorescence location of FGF-2, PDGF, IκB, and NF-κB in chondrocytes

Taking into consideration the RNA expression profiles of anti- and pro-inflammatory cytokines in OA rats treated with DLE, we looked for their location using specific antibodies. In healthy articular cartilage, FGF-2 and PDGF anti-inflammatory cytokines were localized in the cytoplasm of most chondrocytes from all cartilage layers (Figure 5), while weak and diffused expression of IκB was detected and no expression of NF-κB was seen. By contrast, in chondrocytes from the placebo group, FGF-2 was detected on a granular pattern in the cytoplasm of most chondrocytes, while no PDGF was detected (Figure 5). With regard to the expression of pro-inflammatory cytokines IκB and NF-κB, NF-κB immunoreactivity was observed in the cytoplasm and nucleus of several chondrocytes whereas no staining for IκB was found. By contrast, analysis of the DLE group showed that FGF-2 and PDGF were localized mainly in the cytoplasm of chondrocytes from the middle and deep zones (Figure 5). Regarding the pro-inflammatory cytokines, we observed that non-phosphorylated IκB immunoreactivity was weakly localized in certain areas of the cytoplasm of some chondrocytes distributed throughout the cartilage. However, very weak or no expression for NF-κB was found in the cartilage tissue after DLE treatment, similar to that observed in healthy chondrocytes (Figure 5).

Discussion

OA is a chronic degenerative disease and is considered to be the most common joint disease in people aged > 65 years. DLE is a biological agent that has been clinically useful for several pathologies (13), probably through an immunomodulatory action, regulating the expression of anti-inflammatory or pro-inflammatory cytokines (14, 19). The present study documented the histopathological and molecular effects of DLE in an OA rat model.

In the current study, clinical signs indicate that, in contrast to the OA rats, animals without treatment show a decrease in physical activity, piloerection, and lethargy. Rats treated with DLE initially displayed physical activity similar to the healthy animals, suggesting

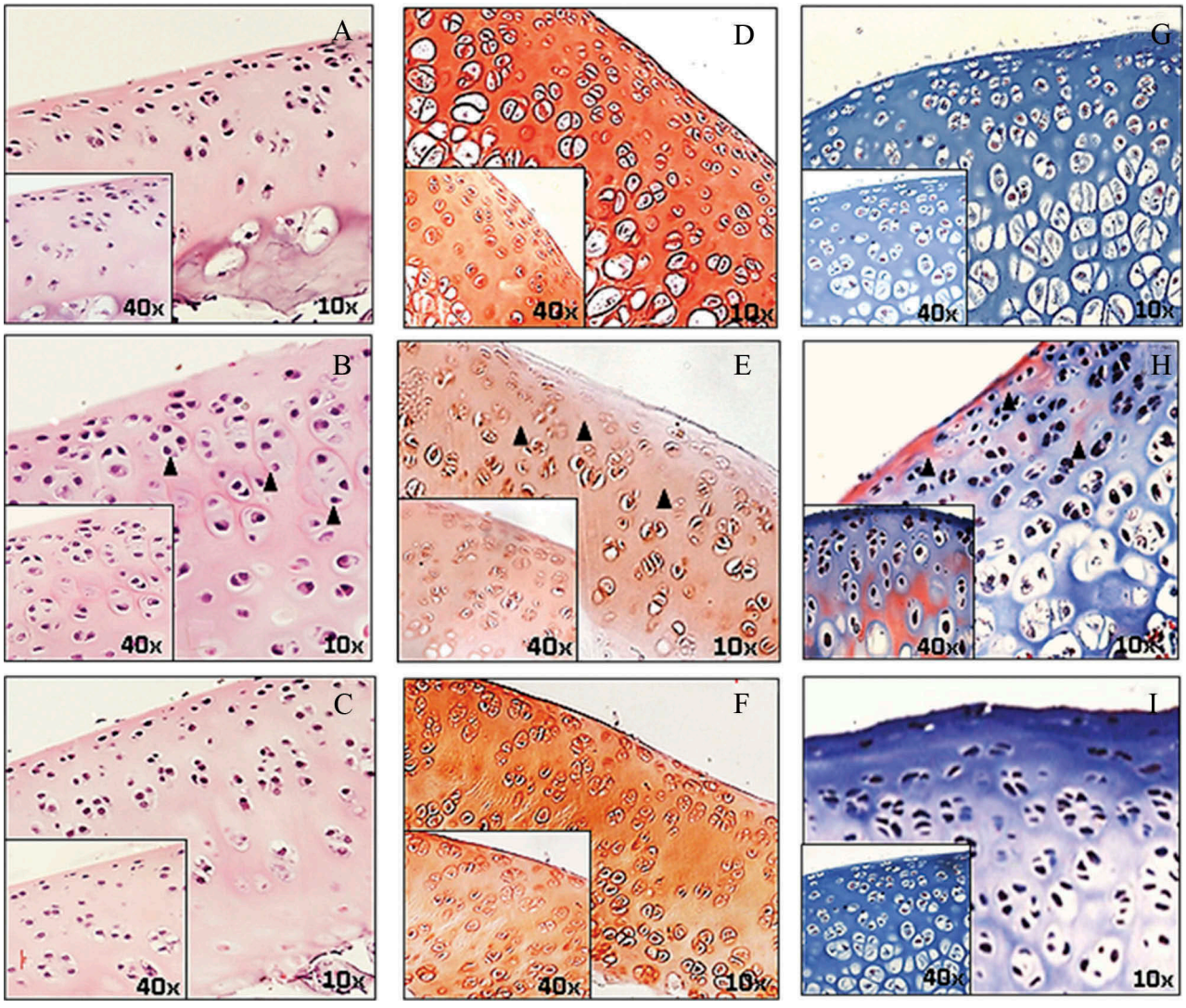

Figure 3. Histological analysis of samples from the control, placebo, and DLE groups stained with H&E (A, B, and C, respectively), safranin O (D, E, and F, respectively), and Masson's trichrome (G, H, and I, respectively). Representative cartilage section images from the different treatments are shown. Arrowheads show rarefaction and condensation of collagen fibres.

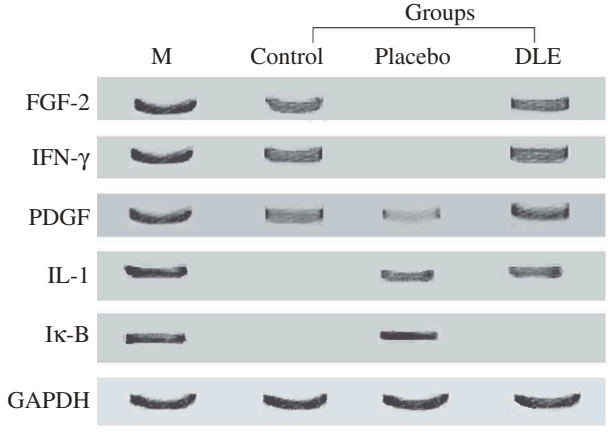

Figure 4. Representative RT-MPCR of pro- and anti-inflammatory cytokines from cartilage obtained from control, placebo, and DLE groups, respectively. Glyceraldehyde 3-phosphate dehydrogenase (GADPH) was included as internal control.

that DLE treatment diminished pain and improved physical activity. Macroscopic analysis of the joints showed a slight decrease in fibrosis in those animals treated with DLE and, histologically, the cartilage explants in general showed the cellular architecture preserved, similar to that of normal articular cartilage (26). A study by Pritzker et al showed that, during the degenerative process of the ECM in OA, the cartilage loses up to 75% proteoglycan content in the superficial, middle, and deep zones (27). In our study we observed that, in OA rats without treatment, the proteoglycan degradation spanned multiple zones of the cartilage. By contrast, proteoglycans in cartilage from rats treated with DLE showed homogeneous staining, particularly in the surface area, suggesting less degradation. In healthy cartilage, the collagen fibres have different orientations according to the tissue zone (28). In OA, fibrosis occurs in the surface region, with fragmentation and degradation of collagen fibres in

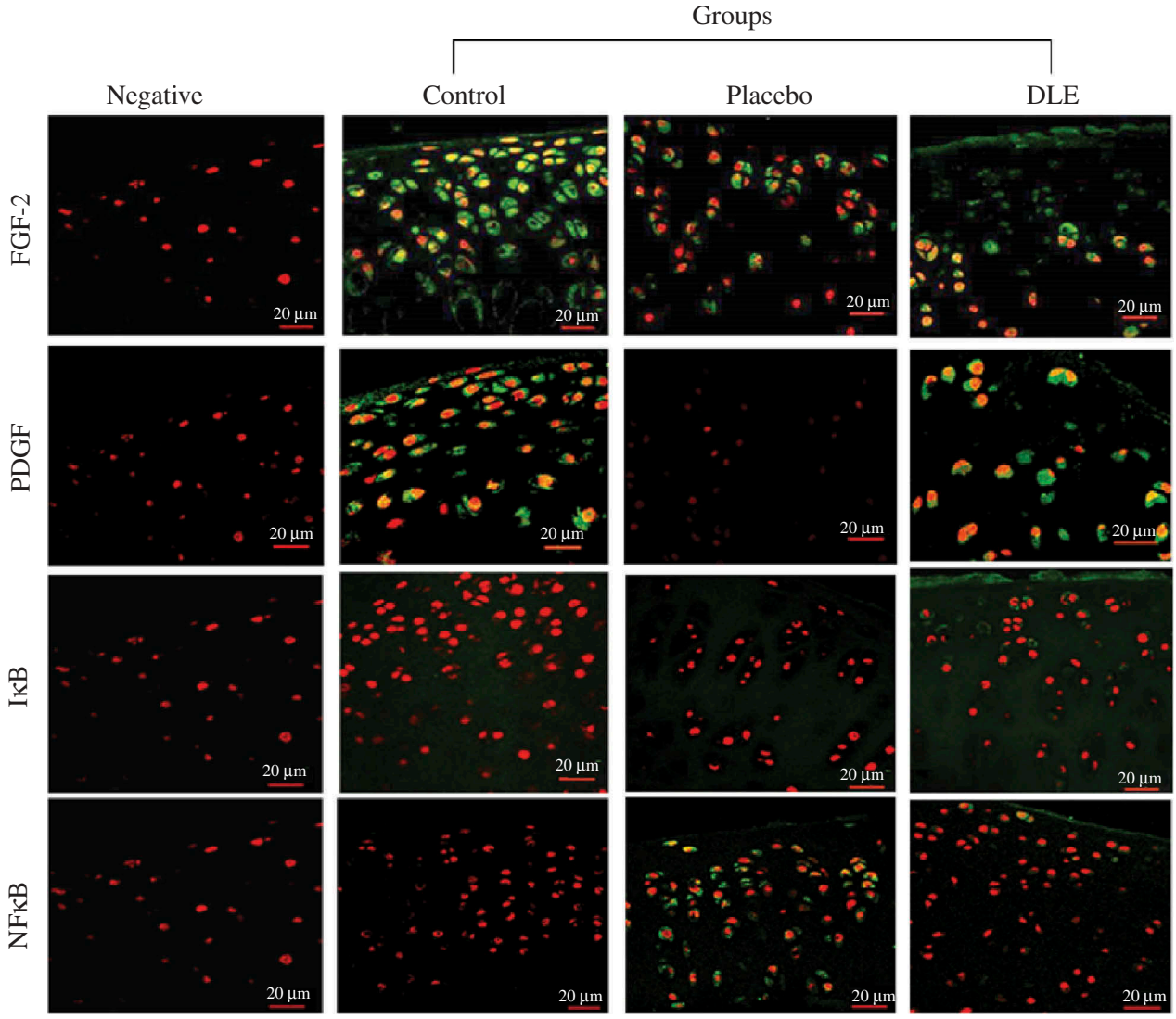

Figure 5. Immunohistochemical staining for FGF-2, PDGF, IκB, and NF-κB. Representative images from different treatment groups are shown.

the four layers of articular tissue (27). These findings were observed on the explants from OA untreated animals, where collagen degradation was evident in all areas of the cartilage. By contrast, lower degradation was observed mainly in the surface region of cartilage. Similar results were obtained in a study by Boileau et al, using an unsaponificable avocado fraction in OA induced in dogs (29).

The structural changes observed after DLE treatment suggest regulation of pro- and/or anti-inflammatory processes. Evaluation of gene expression of pro- and anti-inflammatory cytokines by multiplex RT-MPCR showed that PDGF and FGF-2 were expressed basally in chondrocytes from normal cartilage, similar to findings described previously by Blom et al (6). We also detected the expression of IFN-γ, which has not been considered as an anabolic factor but is known to inhibit the reabsorption of articular cartilage and regulate TNF to modulate the process of cartilage degradation (30). Cartilage from OA animals without treatment showed no expression of FGF-2 and IFN-γ and a low expression of PDGF. However, when animals were treated with DLE, the expression of PDGF, FGF-2, and IFN-γ increased significantly. This effect was also observed by Guillen and Mirabeth, suggesting that the increase in the expression of PDGF on cartilage increases the synthesis of proteoglycans, promotes collagen formation and the induction of tissue repair (31). Similarly, Grassi et al found that the expression of IFN-γ inhibits chondrocyte apoptosis through regulation of caspase 8 and caspase 3 and the activation of the anti-apoptotic protein FLIP. These authors suggested that IFN-γ and CD95 were involved in the apoptosis of human chondrocytes (32). Muddasani et al showed that FGF-2 promotes the synthesis of collagen and proteoglycans to regulate the production of catabolic enzymes by activating factors such EIK-1, mitogen-activated protein kinase (MAPK), and NF-κB (33). These authors also observed that low levels of

FGF-2 reduce the synthesis of bone morphogenetic proteins (BMPs) and insulin-like growth factor (IGF)-1, and simultaneously induce the synthesis of matrix metalloproteinase (MMP)-13 (33).

Considering the various effects found for PDGF, IFN-γ, and FGF-2 in the anabolic process of cartilage, the overexpression of these factors in response to DLE treatment suggests that this therapy could promotes the synthesis of proteoglycans and collagen, counteracting the degradation of the ECM during the OA induced, as well as the inhibition of apoptosis of chondrocytes. These events correlate with the histopathological analysis and the clinical signs developed, suggesting that the development of OA is retarded significantly.

Pro-inflammatory cytokines were not expressed in healthy cartilage; however, in untreated OA rats and in OA rats treated with DLE we found similar levels of expression of IL-1. These results are consistent with data reported by Blom et al, showing that OA processes always increased the expression of catabolic factors such as IL-1 (6). Thus, treatment with DLE did not modify the expression of IL-1. Of interest, we found that IκB is not basally expressed in healthy cartilage and also it was not expressed in cartilage of OA rats treated with DLE, but it was strongly detected in OA rats without treatment. These results suggest that the NF-κB signalling pathway could be involved in the pro-inflammatory events during OA. Immunohistofluorescence analysis of PDGF, FGF-2, IκB, and NF-κB suggested that FGF-2 was located in the cytoplasm of chondrocytes from normal cartilage and in cartilage from the DLE group, correlating with RT-MPCR expression. However, it should be noted that although FGF-2 was not detected by RT-MPCR, it was observed in chondrocytes from the placebo group. This may be because, during the pathophysiology of OA, the *de novo* synthesis of FGF-2 RNA could be inhibited, while pre-existing FGF-2 RNA could synthesize sufficient amounts of protein.

PDGF is a potent mitogen and chemotactic factor for cells of mesenchymal origin, including fibroblasts, osteoblasts, and chondrocytes. Thus, it could be capable of enhancing tissue regeneration and repair promoting angiogenesis and chemotaxis of fibroblasts and collagen synthesis (34). In cartilage from the normal and DLE-treated groups we observed an intense and homogeneous distribution of PDGF fluorescence in chondrocytes in all zones and, by contrast, the absence or very low amounts of PDGF protein in chondrocytes from cartilage obtained from the placebo group. Some in vitro and in vivo studies support the putative therapeutic use of PDGF in cartilage repair (35, 36).

Analysis of the unphosphorylated IκB protein showed that the protein was diffusely detected in normal chondrocytes and not detected in chondrocytes from non-treated OA rats, while in OA rats treated with DLE a more evident staining for IκB was observed in most of chondrocytes. These results suggest that, in OA untreated animals, IκB is in its phosphorylated form interacting with NF-κB, allowing its translocation to the nucleus to carry on the transcriptional activation of pro-inflammatory genes. However, in animals treated with DLE, IκB was in its unphosphorylated form, avoiding NF-κB translocation (37). These results are also in concordance with the gene expression findings observed by RT-MPCR, in which even though IκB was expressed in OA untreated animals, most of the protein was phosphorylated and was not detected by the antibodies, whereas in OA rats treated with DLE, the IκB gene expression was inhibited, and most of the pre-synthesized protein could be unphosphorylated, diminishing the pro-inflammatory effects.

Conclusions

Our results suggest that DLE treatment of OA rats ameliorated the clinical manifestations of the disease, diminishing both fibrosis and the destruction of the EMC. Molecular and immunofluorescence analysis strongly suggests that DLE treatment induces in chondrocytes the expression of FGF-2 and PDGF anti-inflammatory cytokines, and the reduction of pro-inflammatory events, probably avoiding the translocation of NF-κB to the nucleus, interfering with the NF-κB inflammatory activation pathway.

Acknowledgements

This work was supported by SIP-IPN (SIP-20100654) and CONACyT grants given to DGP. We thank Maria del Jesus Perea Flores of the Center for Nanoscience and Nanotechnology of IPN for technical assistance.

References

1. Martel-Pelletier J, Boileau C, Pelletier J-P. Cartilage in normal and osteoarthritis conditions. Best Pract Res Clin Rheumatol 2008; 22:351–84.
2. Arden N, Nevitt N. Osteoarthritis, epidemiology. Best Pract Res Clin Rheumatol 2006;20:3–25.
3. Lories R, Luyten F. The bone–cartilage unit in osteoarthritis. Nat Rev Rheumatol 2011;7:43–9.
4. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011;7:33–42.
5. Martel-Pelletier J. The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type I receptor and analysis of binding kinetics and biologic function. Arthritis Rheum 1992;35:530–40.
6. Blom AB, van der Kraan PM, van den Berg WB. Cytokine targeting in osteoarthritis. Curr Drug Targets 2007;8:283–92.
7. Shimoaka T, Ogasawara T, Yonamine A. Regulation of osteoblast, chondrocyte, and osteoclast functions by fibroblast growth factor (FGF)-18 in comparison with FGF-2 and FGF-10. J Biol Chem 2002;277:7493–500.
8. Michael JW, Schlüter-Brust KU, Eysel P. The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. Dtsch Arztebl Int 2010;107:152–62.
9. Fudenberg H, Pizza G. Transfer factor 1993, new frontiers. Prog Drug Res 1994;42:309–400.
10. Kirkpatrick CH, Gallin JI. Suppression of cellular immune responses following transfer factor: report of a case. Cell Immunol 1975;15:470–4.

11. Kirkpatrick CH, Rozzo SJ, Mascali JJ. Murine transfer factor. III. Specific interactions between transfer factor and antigen. J Immunol 1985;135:4027–33.
12. Kirkpatrick CH. Transfer factors, identification of conserved sequences in transfer factor molecules. Mol Med 2000;64:332–41.
13. Ojeda MO, Van't Veer C, Fernández CB, Araña MJ, Buurman WA. Dialyzable leukocyte extract differentially regulates the production of TNFalpha, IL-6, and IL-8 in bacterial component-activated leukocytes and endothelial cells. Inflamm Res 2005;5:74–81.
14. Pineda B, Estrada-Parra S, Pedraza-Medina B, Rodriguez-Ropon A, Pérez R, Arrieta O. Interstitial transfer factor as adjuvant immunotherapy for experimental glioma. J Exp Clin Cancer Res 2005;24:575–83.
15. Pizza G, Chiodo F, Colangeli V, Gritti F, Raise E, Fudenberg HH. et al. Preliminary observations using HIV-specific transfer factor in AIDS. Biotherapy 1996;9:41–7.
16. Pizza G, De Vinci C, Cuzzocrea D, Menniti D, Aiello E, Maver P. et al. A preliminary report on the use of transfer factor for treating stage D3 hormone-unresponsive metastatic prostate cancer. Biotherapy 1996;9:123–32.
17. Pizza G. In vitro studies during long-term oral administration of specific transfer factor. Biotherapy 1996;9:175-85.
18. Cordero M, Flores G, Orea M, Estrada S, Serrano E. Safety and efficacy of treatment for severe atopic dermatitis with cyclosporin A and transfer factor. Rev Alerg Mex 1999;46:49–57.
19. Dvoroznáková E, Porubcová J, Sevcíková Z. Immune response of mice with alveolar echinococcosis to therapy with transfer factor, alone and in combination with albendazole. Parasitol Res 2009;105:1067–76.
20. Espinosa S, Orozco S, Plaza A, Estrada S, Estrada I, et al. [Effect of transfer factor on treatment with glucocorticoids in a group of pediatric patients with persistent moderate allergic ashma], in Spanish. Rev Alerg Mex 2009;56:67–71.
21. Cabezas Quiroga R, Estrada Parra S, Padierna L, Padierna J, Fernández C, López P. [Immunotherapy with transfer factor in patients with herpes zoster], in Spanish. P. Biotechnol ap 1990;7:52–7, 1990.
22. Estrada-Parra S, Nagaya A, Serrano E, Rodriguez O, Santamaria V, Ondarza R, et al. Comparative study of transfer factor and acyclovir in the treatment of herpes zoster. Int J Immunopharmacol 1998;20:521–35.
23. Georgescu C. Effect of long-term therapy with transfer factor in rheumatoid arthritis. Med Interne 1985;23:135–40.
24. Stanciková M, Rovenský J, Pekárek J, Orviský E, Blazícková S. et al. Influence of various forms of dialyzable leukocyte extracts on rat adjuvant arthritis. Arch Immunol Ther Exp 1994;42:295–9.
25. Lozoya K, Kouri J. A novel rat osteoarthrosis model to assess apoptosis and matrix degradation. Pathol Res Pract 2000;196:729–45.
26. Montrull H, Brizuela N, Demurtas S, Spitale L, Meirovich C. Structure and secretory activity of cultured chondrocytes from patients with osteoarthritis. Biocell 2005;29:163–7.
27. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
28. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health 2009;6:461–8.
29. Boileau C, Martel-Pelletier J, Caron J, Msika P, Guillou GB, Baudouin C, et al. Protective effects of total fraction of avocado/soybean unsaponifiables on the structural changes in experimental dog osteoarthritis: inhibition of nitric oxide synthase and matrix metalloproteinase-13. Arthritis Res Ther 2009;11:1–9.
30. Bunning RA, Russell RG. The effect of tumor necrosis factor alpha and gamma-interferon on the resorption of human articular cartilage and on the production of prostaglandin E and of caseinase activity by human articular chondrocytes. Arthritis Rheum 1989;32:780–4.
31. Guillen S, Mirabeth L. [Chondrocyte differentiation in rich plasma platelet growth factors for articular chondroplasty], in Spanish. Patología del aparato locomotor 2005;3:13–23.
32. Grassi F, Piacentini A, Cristino S, Toneguzzi S, Facchini A, Lisienoli G. et al. Inhibition of CD95 apoptotic signaling by interferon-gamma in human osteoarthritic chondrocytes is associated with increased expression of FLICE inhibitory protein. Arthritis Rheum 2004;50:498–506.
33. Muddasani P, Norman JC, Ellman M, van Wijnen AJ, Im HJ. Basic fibroblast growth factor activates the MAPK and NFkappaB pathways that converge on Elk-1 to control production of matrix metalloproteinase-13 by human adult articularchondrocytes. J Biol Chem 2007;282:31409–21.
34. Civinini R, Nistri L, Martini C, Redl B, Ristori G, Innocenti M. Growth factors in the treatment of early osteoarthritis. Clin Cases Miner Bone Metab 2013;10:26–9.
35. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clin Orthop Relat Res 2011;469:2706–15.
36. Kwon DR, Park GY. Intra-articular injections for the treatment of osteoarthritis: focus on the clinical use of several regimens. In: Chen Q, editor. Osteoarthritis – diagnosis, treatment and surgery. InTech, 2012;67–100 (http://cdn.intechopen.com/pdfs-wm/30683.pdf). Accessed 24 February 2016.
37. Saklatvala J. Inflammatory signaling in cartilage, MAPK and NF-κB pathways in chondrocytes and the use of inhibitors for research into pathogenesis and therapy of osteoarthritis. Curr Drug Targets 2007;8:305–13.