Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2010;25:543-550

Accepted: February 08, 2010

Dehydroxymethylepoxyquinomicin Inhibits Expression and Production of Inflammatory Mediators in Interleukin-1β-induced Human Chondrocytes

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Key Words

Anti-inflammatory effects • Cartilage • Chemokines • Cyclooxygenase • DHMEQ • Nitric oxide

Abstract

The present research was carried out to determine the effects of a nuclear factor-kappaB (NF-κB) inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), derivative of the antibiotic epoxyquinomicin C, on normal human chondrocytes treated with interleukin-1β (IL-1β). This is a cell model particularly useful to reproduce the mechanisms involved in degenerative arthropathies, where oxidative-inflammatory stress determines a progressive destruction of the articular cartilaginous tissue. The expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and inter-cellular adhesion molecule (ICAM)-1 was evaluated through Western blot analysis. The release of chemokines like monocyte chemoattractant protein-1 (MCP-1), regulated upon normal activation T-cell expressed and secreted (RANTES), and interleukin-8 (IL-8) were determined by ELISA assays. DHMEQ acts as a potent inhibitor of iNOS and COX-2 gene expression while also suppressing the production of nitrite in human chondrocytes. In addition, DHMEQ induces a significant dose-dependent decrease in ICAM expression, MCP-1, RANTES, and IL-8 release. DHMEQ helps to decrease the expression and production of pro-inflammatory mediators in IL-1β-induced chondrocytes. DHMEQ may become a therapeutic agent for treatment of chondrodegenerative diseases.

Introduction

The eukaryotic nuclear factor κB (NF-κB) is a transcription factor that is a major regulator of immune responses stimulated by pro-inflammatory agents such as tumor necrosis factor, viruses, interleukin-1, and bacteria. NF-κB normally resides in the cytoplasm bound by an inhibitory protein known as IκB. Phosphorilation of IκB by IκB kinase-β (IKK-β) releases NF-κB, which then moves into the nucleus where it acts in the induction of numerous regulatory genes of the immune system. The products of these genes are pro-inflammatory factors [1]. Five members of the NF-κB family identified are: NF-κB1 (p50/p105), NF-κB2 (p52/p100), ReIA (p65), ReIB, and c-ReI. They share a highly conserved ReI homology domain (RHD), which is responsible for DNA binding, dimerization, and interaction with IκB. The p50/p65

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heterodimer is the major ReI/NF-κB complex in most cells. The functions of NF-κB span diverse cellular processes, including adhesion, proliferation, apoptosis, differentiation, immune regulation, and angiogenesis [2]. The most common conditions associated with NF-κB signaling are chronic inflammatory diseases, such as rheumatoid arthritis, psoriatic arthritis, celiac diseases, ulcerative colitis, and Crohn' disease [2]. The NF-κB pathway may be central to the process of atherosclerosis with regards to inflammation of the vascular endothelium [3].

The pro-inflammatory cytokine interleukin-1β (IL-1β), produced in an arthritic joint by activated synovial cells and infiltrating macrophages, is considered to be one of the most potent catabolic factors in joint diseases [4]. IL-1β induces the production of several mediators of cartilage degradation; it exerts its inflammatory effects by activating a diverse spectrum of signaling cascades in the cells [5, 6] that leads to the induction of inducible nitric oxide synthase (iNOS) and the production of high levels of the second messenger NO in arthritic joints [7]. IL-1β can also mediate its effects through cyclo-oxygenase-2 (COX-2) induction, which produces high levels of prostaglandin E_2 (PGE_2), which mediates cartilage resorption by decreasing proliferation of chondrocytes, enhancing metalloproteases (MMPs)' activity, and inhibiting aggrecan synthesis in chondrocytes [8]. IL-1β triggers the phosphorylation and degradation of IκB, thus permitting the entry of p50/p65 into the nucleus, where p50/p65 activates the transcription of various genes, and two of the many genes are iNOS [9, 10] and COX-2 [11, 12]. Therefore, inhibitors of NF-κB functions should be useful as anti-inflammatory agents.

Recently, a novel NF-κB inhibitor, dehydroxymethyl-epoxyquinomicin (DHMEQ), derivative of the antibiotic epoxyquinomicin C [13], has been found to inhibit tumor necrosis factor (TNF)-α induced activation of NF-κB by suppressing NF-κB nuclear translocation [14]. DHMEQ directly binds to p65 and other Rel family proteins to inhibit DNA binding of NF-κB [15]. DHMEQ inhibited the secretions of inflammatory cytokines from cancer cells and macrophages. It effectively suppressed prostate carcinoma, thyroid carcinoma, breast carcinoma, pancreatic carcinoma, multiple myeloma, adult T-cell leukemia, Hodgkin lymphoma, and AIDS-associated lymphoma in nude or SCID mice without any side effect [16-18].

Thus, the present research was carried out to elucidate the effects of DHMEQ in a cell model particularly useful to reproduce the mechanisms involved in degenerative arthropathies, where oxidative-inflammatory stress determines a progressive destruction of the articular cartilaginous tissue [19]. Chondrocytes are the only cellular component of articular cartilage and are capable of phenotype modulation, making these cells pivotal in the progression of joint diseases.

In the present study, we used DHMEQ to inhibit the expression of NF-κB in cultured IL-1β-induced human chondrocytes, so as to explore the inhibitive effects in transcription activation of NF-κB, and further observe the expression of iNOS, and COX-2. Furthermore, the expression of immuno-modulatory membrane molecules such as inter-cellular adhesion molecule-1 (ICAM-1), and release of chemokines like monocyte chemoattractant protein-1 (MCP-1), regulated upon activation normal T-cell expressed and secreted (RANTES), and interleukin-8 (IL-8) were determined.

Materials and Methods

Chemicals

DHMEQ, kindly provided by Prof. K. Umezawa (Keio University, Kanagawa, Japan), was synthesized as previously described [13]. All chemicals were purchased from Sigma Aldrich Co (Milan, Italy). Antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); ELISA kits were purchased from Amersham Biosciences (Part of GE Healthcare, Zurich, Switzerland) and R&D Systems Europe (Abingdon, Oxfordshire, UK).

Chondrocytes isolation, culture, and treatment

Normal human articular cartilage was obtained at replacement surgery from some patients with femoral neck accidental fractures and that informed consent was obtained [20]. The isolation procedure was conducted under antiseptic conditions. The cartilage was cut into small fragments and carefully washed using Dulbecco's Modified Eagles Medium (DMEM) culture medium containing $NaHCO_3$, 25 mM Hepes, 1 mM sodium pyruvate, 50 μg/ml gentamycin, 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml amphotericin B. Chondrocytes were isolated through three sequential passages of enzymatic digestion of the extracellular matrix: incubation with 0.1% hyaluronidase type III (1 mg/ml for 100 mg of cartilage), for 30 mins at 37 °C; incubation with 0.5% pronase type XIV (5 mg/ml for 100 mg of cartilage), for 60 mins at 37 °C; finally incubation with 0.2% collagenase type IA (2 mg/ml for 100 mg of cartilage), for 45 mins at 37 °C. The obtained cellular suspension was filtered (filters from 100 and 70 μm) to eliminate the residues of the digestion, cellular aggregates and to obtain a monocellular suspension of chondrocytes. This was washed three times with DMEM supplemented with 10% fetal calf serum (FCS), and was subjected to vital coloration method staining with trypan blue in order to determine the number and the vitality of recovered cells. Chondrocytes were plated either in

6 well plates (for ELISA and nitrite tests) or in 100 mm Petri-dishes (for Western blot). After 24 hrs the medium was removed and cells were treated as follows: a) untreated control; b) IL-1β (10 ng/ml); c) IL-1β plus indomethacin (10^{-5} M); d) IL-1β plus DHMEQ (1 μg/ml), e) IL-1β plus DHMEQ (5 μg/ml), and e) IL-1β plus DHMEQ (10 μg/ml). After 48 hrs the supernatants and/or chondrocytes of cultures were collected for the different assays. Cell viability was also determined.

Determination of NO_3^- release

Nitrite concentration in the supernatant was quantified by colorimetric assay based on the Griess reaction [21]. Briefly, 0.1 ml of supernatant from untreated, indomethacin- or DHMEQ-treated cultures was mixed with an equal volume of Griess reagent (1% sulphanylamide and 0.1% naphthyl-ethylenediamine dihydrochloride in 5% of hydrochloric acid, 1:1 vol/vol) at room temperature for 10 mins. The absorbance was measured at λ= 550 nm in a microplate spectrophotometer reader (Titertek Multiskan, DAS, Italy). Sodium nitrite was used as a standard.

Western blot analysis

The expression of iNOS, COX-2, and ICAM was evaluated by Western blot analysis. Briefly, the untreated and treated chondrocytes were washed twice with ice-cold PBS and collected with lysing buffer (10 mM Tris-HCl plus 10 mM KCl, 2 mM $MgCl_2$, 0.6 mM PMSF, and 1 % SDS, pH 7.4). After cooling for 30 mins at 0º C, cells were sonicated. Twenty micrograms of total protein, present in the supernatant, were loaded on each lane and separated by 4-12 % Novex Bis-Tris gel electrophoresis (NuPAGE, Invitrogen, Italy). Proteins were then transferred to nitrocellulose membranes (Invitrogen, Italy) in a wet system. The transfer of proteins was verified by staining the nitrocellulose membranes with Ponceau S and the Novex Bis-Tris gel with Brillant blue R. Membranes were blocked in Tris buffered saline containing 0.01 % Tween-20 (TBST) and 5 % non-fat dry milk at 4°C overnight. Rabbit monoclonal anti-NF-κB p50 (1:1000 dilution) (#04-234, Millipore, CA) antibody, mouse monoclonal anti-COX-2 (1:100 dilution), -iNOS (1:300 dilution), -ICAM-1 (1:200 dilution), (Santa Cruz Biotechnology, Santa Cruz, CA) and mouse monoclonal anti-α-tubulin (1:5000 dilution) (Sigma, Milan, Italy) antibodies were diluted in TBST and membranes incubated for 24 hrs at room temperature. Antibodies were detected with horseradish peroxidase-conjugated secondary antibody using the enhanced chemiluminescence detection Supersignal West Pico Chemiluminescent Substrate (Pierce Chemical Co., Rockford, IL). Bands were measured densitometrically by ImageJ software and their relative density calculated based on the density of the α-tubulin bands in each sample. Values were expressed as arbitrary densitometric units corresponding to signal intensity.

ELISA assays

Monocyte chemoattractant protein-1 (MCP-1), and interleukin-8 (IL-8) were measured on cell-free supernatants collected after 48 hrs treatment by specific sandwich enzyme-linked immunosorbent assay (ELISA) kits (Amersham Biosciences, Switzerland). RANTES antibodies and protein for ELISA were purchased from R&D Systems Europe (Abingdon, Oxfordshire, UK). Chondrocyte cultures were carried out in triplicate for each condition. All assays were performed as specified by the manufactures of respective kits. A standard curve was produced for each assay using known concentrations of MCP-1, RANTES and IL-8 provided in kit. The sensitivity of ELISA kit of MCP-1 was 3.5 pg/ml, of RANTES was more than 5 pg/ml, and of IL-8 was <5 pg/ml. Results are given as mean pg/ml ± SEM.

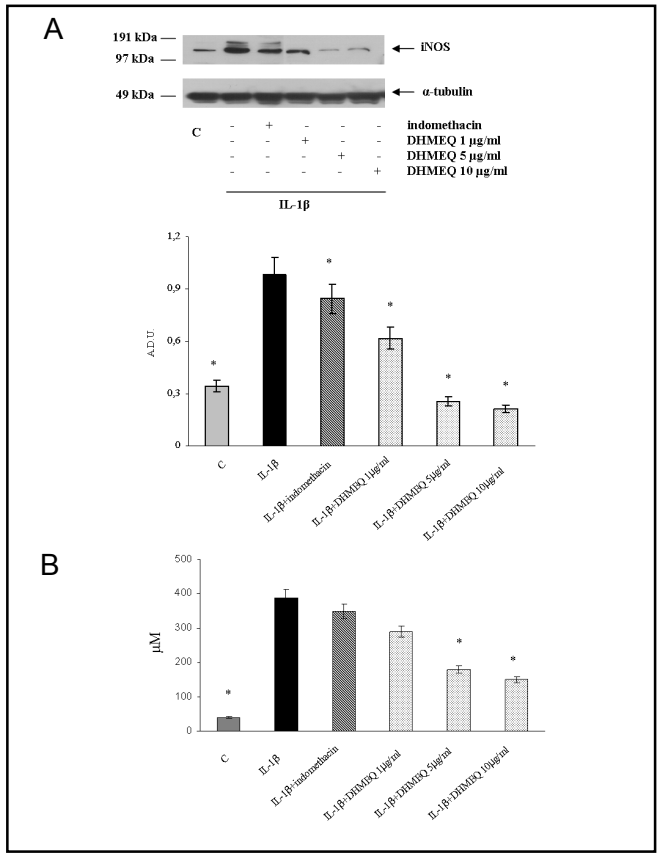


Fig. 1. Effects of dehydroxymethylepoxyquinomicin (DHMEQ) and indomethacin on A) iNOS expression and B) NO release, induced by IL-1β on normal human primary chondrocytes determined by Western blot analysis and Griess assay, respectively. Chondrocytes were treated with IL-1β (10 ng/ml) for 48 hrs in the presence of different concentrations of DHMEQ (1, 5, and 10 μg/ml) or indomethacin (10^{-5} M). Data show the relative expression (mean ± SEM) of iNOS calculated as arbitrary densitometric units (A.D.U.) and production (mean ± SEM) of NO (μM) collected from three independent experiments. *p<0.05 compared with IL-1β-induced iNOS and NO, respectively.

MTT assay for cell viability

After incubation in 96-well plated for 48 hrs, the cell survival was quantified by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (MTT). In brief, cells were incubated with 20 μl of 0.5% MTT in phosphate saline buffer (PBS) for 3 hrs at 37°C in a humidified 95% air/5% CO_2 mixture. The supernatant was removed and 100 μl of dimethylsulphoxide (DMSO) was added. Optical

Fig. 2. Effects of dehydroxymethylepoxyquinomicin (DHMEQ) and hydrocortisone on anti-NF-κB p50 and p105 activation induced by IL-1β on normal human primary chondrocytes determined by Western blot analysis. Chondrocytes were treated with IL-1β (10 ng/ml) for 48 hrs in the presence of different concentrations of DHMEQ (1, 5, and 10 μg/ml) or indomethacin (10^{-5} M). Data show the relative expression (mean ± SEM) of NF-κB p50/p105 calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. *p<0.05 compared with IL-1β-induced NF-κB p50 and p105.

densities at λ = 550 nm were measured with a microplate spectrophotometer reader (Titertek Multiskan, DAS, Italy) using culture medium as a blank.

Statistical analysis

Statistical analysis was performed with statistical software package SYSTAT, version 9 (Systat Inc., Evanston IL, USA). Each result was calculated as a mean value ± standard error (SEM). Evaluation of the statistical significances between paired and unpaired values was performed by Student's t test. Values of p<0.05 were considered to represent statistical significance.

Results

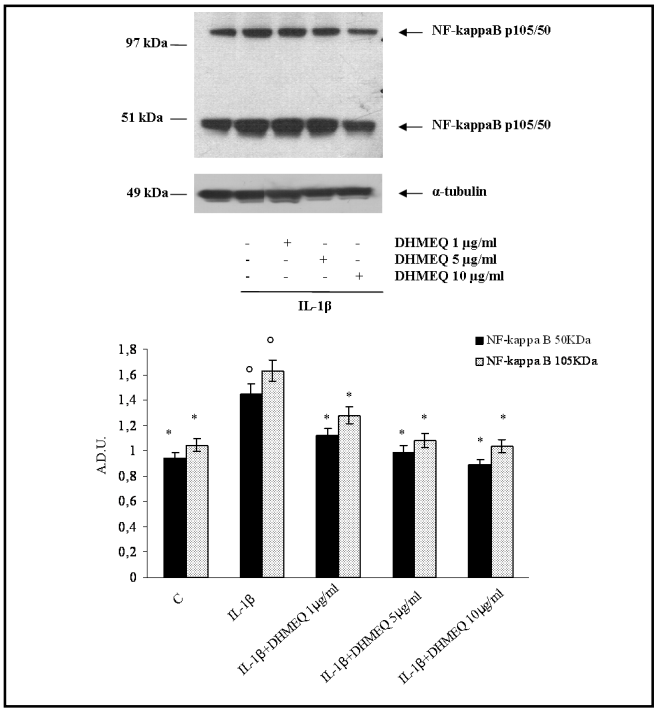
Inducible NO synthase (iNOS) is one of the major inflammatory mediators that contribute to the pathogenesis of cancer and inflammation. As shown in Fig. 1A (upper), the addition of IL-1β led to the expression of iNOS, which expression was inhibited by DHMEQ. DHMEQ inhibited IL-1β induced iNOS expression dose-dependently, as shown in Fig. 1A (lower). Also, DHMEQ dose-dependently reduced NO production, at 10 μg/ml reducing it almost to the control level (Fig. 1B).

The results reported in Fig. 2 by Western blot demonstrated that DHMEQ inhibits NF-κB p50 and p105 nuclear translocation in IL-1β-activated in dose-dependent manner.

As shown in Fig. 3, the IL-1β stimulus induced COX-2 expression. Treatment with DHMEQ resulted in strong dose-dependent inhibition of its expression (Fig. 3).

The results of this research provided evidences that DHMEQ can effectively inhibit the transcription activation of NF-κB and the expression of iNOS, and COX-2 in induced chondrocytes.

In this study, we also examined the capacity of DHMEQ to modulate the production of ICAM-1, MCP-1, RANTES, and IL-8 in human chondrocytes stimulated with IL-1β.



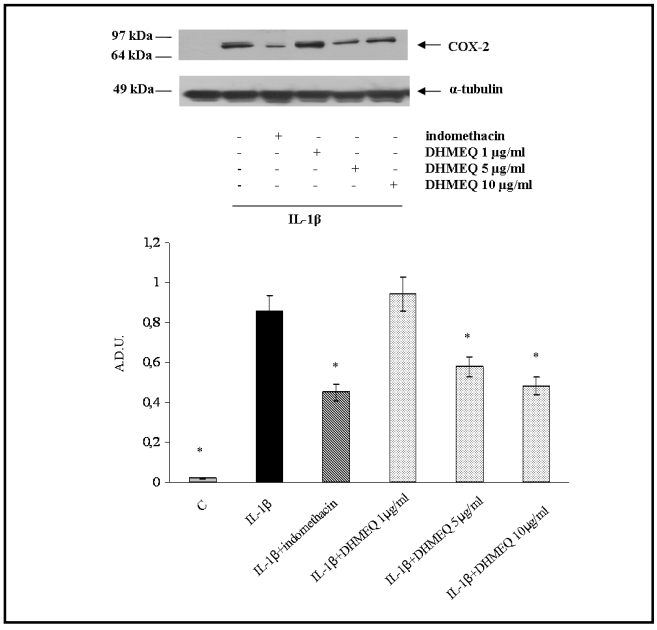


Fig. 3. Effects of DHMEQ (1, 5, and 10 μg/ml) and indomethacin (10^{-5} M) on COX-2 expression induced by IL-1β (10 ng/ml) on primary human chondrocytes determined by Western blot analysis. Data show the relative expression (mean ± SEM) of COX-2 calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. *p<0.05 compared with IL-1β-induced COX-2.

Resting chondrocytes did not express ICAM-1, whereas incubation of cultures with IL-1β for 48 hrs induced a strong expression of ICAM-1 (Fig. 4). Addition of DHMEQ at different concentrations together with IL-

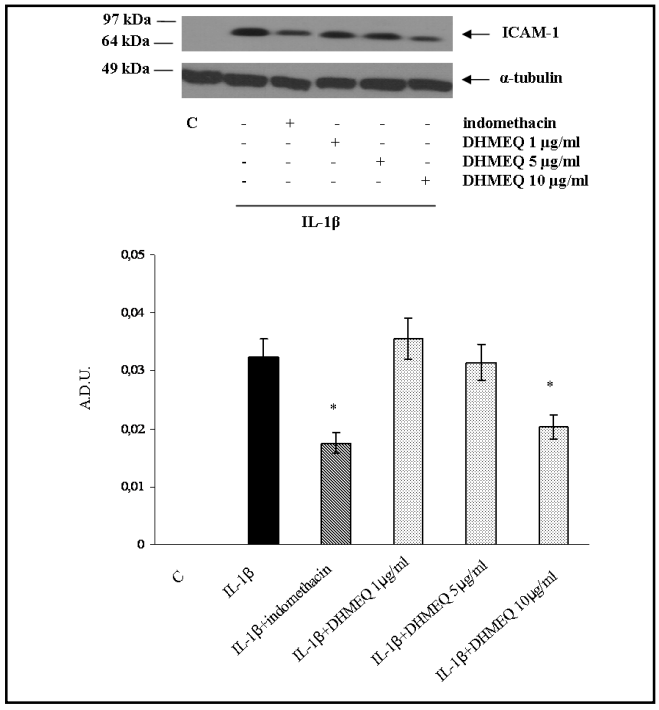


Fig. 4. Effects of DHMEQ and indomethacin on ICAM-1 expression induced by IL-1β on primary human chondrocytes determined by Western blot analysis. Data show the relative expression (mean ± SEM) of ICAM-1 calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. *p<0.05 compared with IL-1β-induced ICAM.

1β induced a dose-dependent inhibition of ICAM-1 expression (Fig. 4). At the highest dose, DHMEQ blocked of 73±4% ICAM-1 expression. Addition of indomethacin led to the reduction of 28±6% of ICAM-1, not significantly different to the decrease induced by 1 µg/ml of DHMEQ.

Regarding MCP-1 release, no amount of MCP-1 was produced by unstimulated chondrocytes, but MCP-1 was potently induced by IL-1β (Fig. 5), which was markedly inhibited by DHMEQ: at lower (1 µg/ml) concentration was present a very significant inhibition (65±5%), that further increased at higher (10 µg/ml) concentration of DHMEQ (80±3%) (Fig. 5). Indomethacin provoked a more modest, but very significant, inhibition (42±6%) on the release of MCP-1 by chondrocytes.

We have also investigated the effects of DHMEQ on the release of IL-8. Negligible amounts of IL-8 was produced by unstimulated chondrocytes (Fig. 6). In contrast, IL-β potently induced IL-8, which was markedly decreased by DHMEQ in dose-dependent manner.

DHMEQ also stimulated a significant dose-dependent decrease in RANTES release on inflamed chondrocytes (Fig. 7). Indomethacin decreased both IL-

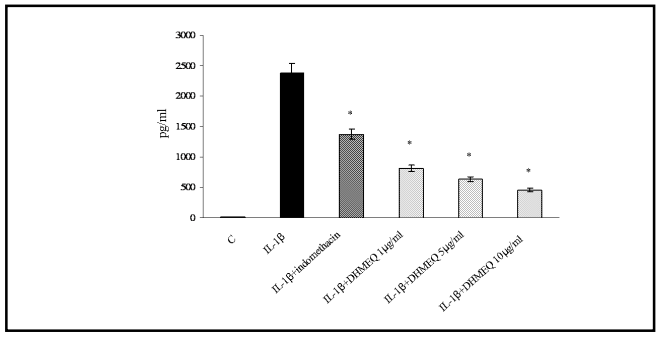


Fig. 5. Effects of DHMEQ and indomethacin on the release of MCP-1 induced by IL-1β on primary human chondrocytes determined by specific enzyme-linked immunosorbent assay (ELISA) kit. The results are expressed as mean pg/ml ± SEM. *p<0.05 when compared to IL-1β-stimulated release.

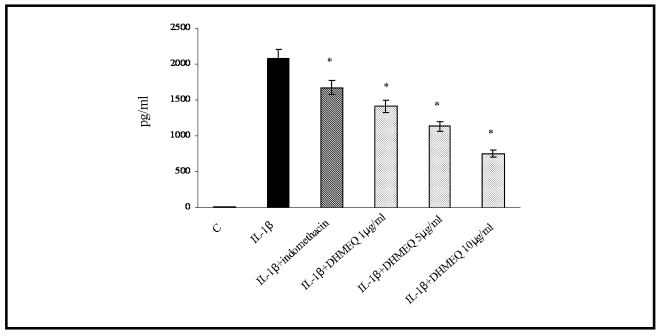


Fig. 6. IL-8 production (means ± SEM) measured in the culture media by enzyme-linked immunosorbent assay (ELISA) from chondrocytes 48 hrs after the addition of DHMEQ and/or indomethacin with IL-1β. Values are expressed as pg/ml. *Significantly different from IL-1β treated samples ($p < 0.05$).

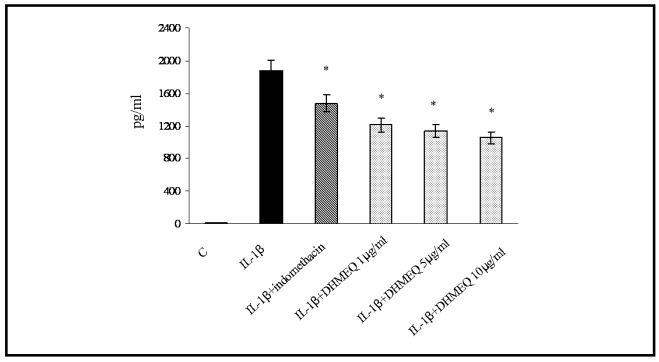


Fig. 7. Effects of DHMEQ and indomethacin on the release of RANTES induced by IL-1β on human chondrocytes. The values are the mean ± SEM of three experiments performed in triplicate. All values with p<0.05 were considered significantly different. *denotes significant difference as compared to IL-1β.

8 and RANTES but, with respect to DHMEQ, showed a lower anti-inflammatory effect (Figs. 6 and 7).

The inhibition of the IL-1β-stimulated expression of proinflammatory molecules in chondrocytes by DHMEQ

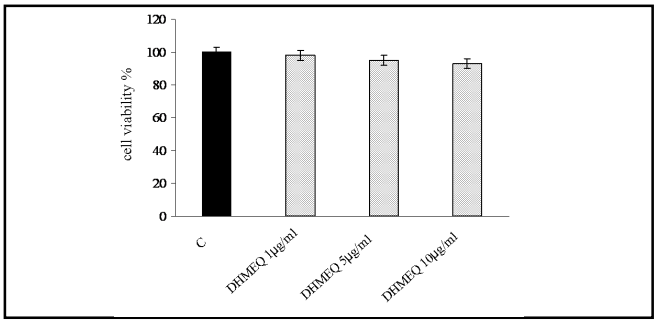


Fig. 8. Human chondrocytes cell viability measured with tetrazolium salt assay (MTT) after 48 hrs of treatment with DHMEQ and/or indomethacin. The values of optical density measured at λ= 550 nm are reported as percentage with respect to the optical density registered for untreated control, the latter considered as 100 % of cell viability. The values are the mean ± SEM of three experiments performed in triplicate.

was not due to DHMEQ cytotoxicity, as assessed by MTT assay (Fig. 8).

Discussion

The results of our study demonstrate that DHMEQ acts as a potent inhibitor of iNOS and COX-2 gene expression while also suppressing the production of nitrite in human chondrocytes. In addition, DHMEQ induces a significant dose-dependent decrease in ICAM expression, MCP-1, RANTES, and IL-8 release induced by IL-1β in human chondrocytes without induction of inhibition of cell viability. These mediators are important in the pathogenesis of osteoarthritis (OA) and the reduction in these mediators have been associated with amelioration of cartilage breakdown.

Dehydroxymethylepoxyquinomicin (DHMEQ) is a new NF-κB inhibitor, and is a derivative of the weak antibiotic epoxyquinomicin C, isolated from the culture broth of *Amycolaptosis* sp. [25]. Most NF-κB inhibitors inhibit IκBα phosphorylation, whereas DHMEQ inhibits nuclear translocation of p65 protein, a component of NF-κB [25, 14].

NO has been shown to mediate some of the catabolic responses induced by IL-1 in chondrocytes [26] and inhibitors of NO synthase, administered to rodents, have been shown to suppress the development of adjuvant-induced arthritis [27, 28]. The iNOS protein synthesis is mainly regulated at the transcriptional level, and the promoter region of the iNOS gene from different species has been reported to contain binding sites for several transcription factors, including NF-κB and activator protein-1 (AP-1) [29-32].

These two transcription factors, NF-κB and AP-1, seem to play major roles in mediating IL-1-induced cellular responses. While activation of NF-κB seems to be an essential step for iNOS induction in most cell types [33], the role of AP-1 and other transcription factors is less clear. The results of this research shows that DHMEQ, specific NF-κB inhibitor, dose-dependently reduces iNOS expression and NO production in stimulated chondrocytes confirming that IL-1β-induced activation of NF-κB lead to iNOS expression.

COX-2 catalyzes the crucial step of the production of prostaglandin E2, which is a principal mediator of joint inflammation such as rheumatoid arthritis and osteoarthritis. Various inflammatory mediators and proinflammatory cytokines, including IL-1β, are responsible for the inflammatory response and upregulate COX-2 expression in arthritic chondrocytes [34, 35]. COX-2 induction by IL-1β in chondrocytes is mediated by NF-κB activation [36]. Recently, Suzuki et al. [37] reported that DHMEQ inhibited lipopolysaccharide (LPS)-induced NF-κB activation in macrophage cell line. It also inhibited the expression of inducible NO synthase (iNOS) and NO production induced by LPS. DHMEQ showed to inhibit LPS-induced secretion of IL-6 and TNF-α. It also inhibited COX-2 expression and prostaglandin E2 production and secretion. Our results provided evidences that DHMEQ can effectively inhibit the transcription activation of NF-κB and decrease the expression of iNOS and COX-2 in induced chondrocytes.

Activation of NF-κB up-regulates the expression of numerous pro-inflammatory genes involved in inflammation such as tumor necrosis factor α (TNF)-α, IL-6, IL-8, and ICAM-1. In particular, recent reports have focused on regulation of IL-8 and ICAM-1 production, as these factors are key mediators of NF-κB-induced inflammation [38, 39]. Increased expression of pro-inflammatory cytokines has been associated with increased expression of ICAM-1. Intercellular adhesion molecule-1 (ICAM-1), a cell surface glycoprotein belonging to immunoglobulin (IgG) super family, participates in inflammatory reactions by interacting with lymphocyte function associated antigen-1 (LFA-1) and Mac-1 expressed in lymphocytes and macrophages, respectively. The interaction of ICAM-1 with LFA-1 results in the adhesion of cells, and consequent activation of signal transduction mechanisms which facilitate the infiltration of leukocytes into the tissues, lymphocyte proliferation, cytotoxic T cell function and T cell mediated B cell activation. Expression of ICAM-1 in affected tissues is upregulated during inflammatory and auto-

immune diseases. *In vitro* cell culture studies demonstrated enhanced expression of ICAM-1 in various cell types upon stimulation with inflammatory cytokines or endotoxins. A soluble form of ICAM-1 was reported to be elevated in the serum of patients with rheumatoid arthritis [40]. Our results showed that IL-1β failed to induce ICAM-1 high expression while NF-κB pathway was blocked by DHMEQ, suggesting that IL-1β induced ICAM-1 expression through the NF-κB pathway.

Dijkgraaf et al. [41] reported that IL-1β stimulates chondrocytes to synthesize IL-8. IL-8 is one of the most important chemotactic factors for neutrophils and T lymphocytes. It also induces neutrophil degranulation, and thus may participate in cartilage degradation [42]. The decrease of IL-8 by DHMEQ may lead not only to a reduction of the number of neutrophils and T-lymphocytes invading the joint cavity but also a decrease of neovascularization of the synovial membrane, as suggested by a paper on the properties of IL-8 as an angiogenic factor [43]. IL-8 gene transcription is generally dependent on the activation of NF-κB for most stimuli. For some stimuli, such as IL-1β, IL-8 transcription is also dependent on AP-1, ERK 1 / 2 and JNK, as demonstrated by the decrease and not inhibition of IL-8 by DHMEQ.

We have also demonstrated that IL-1β-stimulated chondrocytes release MCP-1 and RANTES production. It is noteworthy that the chemokines released by IL-1β-stimulated chondrocytes and synovial fibroblasts can interact with specific receptors present in human chondrocytes. These chemokine receptors have first been described in blood cells involved in immunological responses, where they are responsible for their homing and activation [44]. Subsequently, they have been observed in a variety of cell types. After binding with their receptors, chemokines can activate a catabolic pathway in normal and osteoarthritis chondrocytes through the release of matrix-degrading enzymes and the suppression of proteoglycan synthesis [45].

Finally, compared to indomethacin, used as a reference anti-inflammatory drug, DHMEQ more efficiently blocks the pro-inflammatory actions of Il-1β on chondrocytes, although the levels of these compounds reaching chondrocytes *in situ* may be considerably different from those experienced in the *in vivo* experiments.

Many previous studies have shown that the mediators reported in this research are important in the pathogenesis of osteoarthritis and that the reduction in these mediators have been associated with amelioration of cartilage breakdown. The present *in vitro* study suggests that DHMEQ helps to decrease the expression and production of such inflammatory mediators in chondrocytes.

In summary, this study gives some new insight into the mechanisms of the anti-inflammatory effects of DHMEQ, and this may be a potential mechanism by which DHMEQ prevents stricture formation under inflammatory conditions and becomes a therapeutic agent for treatment of chondro-degenerative diseases.

Acknowledgements

The authors are grateful to Prof. M.L. Carnazza who kindly gave permission to carry out this research at the Department of Anatomy, University of Catania, Italy.

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