CCL2, CXCL8, CXCL9 and CXCL10 serum levels increase with age but are not altered by treatment with hydroxychloroquine in patients with osteoarthritis of the knees

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Abstract

Aim: Osteoarthritis (OA) is a major cause of morbidity and incapacity in the elderly. This study evaluates serum levels of the chemokines CCL2, CXCL8, CXCL9, and CXCL10 in 16 patients with primary OA of the knees, and investigates how treatment with hydroxychloroquine (HCQ) for 4 months affects these chemokine levels.

Method: Thirteen elderly patients received a placebo. Healthy control groups consisted of 10 elderly individuals (age > 60 years) with no clinical or radiological evidence of OA (CT-O), and 10 young adult individuals, (CT-Y group, age < 40 years).

Results: The CT-Y group presented lower levels of all chemokines studied, in comparison to the other groups. HCQ treatment did not alter the serum levels of CCL2 (P = 0.80), CXCL8 (P = 0.76), CXCL9 (P = 0.95) and CXCL10 (P = 0.74) in OA patients.

Conclusion: Hydroxychloroquine treatment did not alter the serum levels of CCL2, CXCL8, CXCL9 or CXCL10 in patients with OA of the knees, although increased serum levels correlated with aging for all subjects, including controls.

Key words: aging, arthritis, chemokines, hydroxychloroquine, pain.

INTRODUCTION

Osteoarthritis (OA) is considered to be the most important rheumatic illness, not only because of its prevalence but also because of its strong socioeconomic impact. It is one of the principal causes of morbidity and incapacity in elderly people. The disease can provoke pain, rigidity of the joints involved and significant functional limitation. OA is characterized by a complex interaction between cartilage, bone and synovia involving degradation and repair. Several hypotheses have been proposed regarding the etiopathogenic mechanisms involved in OA, but its etiology remains unknown. Mechanical, biochemical, inflammatory, immunological, genetic and metabolic factors are considered to be of importance.1–3

The current pharmacological treatment of OA is principally directed at the treatment of the symptoms. Analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) are the most common medications used for
this condition. Even though drugs exist which are aimed at delaying the progress of OA, there is a lack of evidence in clinical trials demonstrating the effectiveness of these medications.4–6 Little evidence exists regarding the clinical response of patients to the slow-acting anti-rheumatic drugs such as antimalarials.7 In contrast, clinical trials have shown hydroxychloroquine (HCQ) to be an effective treatment in the therapy of some rheumatic illnesses such as systemic lupus erythematosus (SLE).8 The mechanism of action of the antimalarials, although not fully understood, is thought to involve inhibition of antigen–antibody interactions, inhibition of interleukin-1 (IL-1) synthesis and subsequent cartilage degradation induced by this cytokine, and inhibition of lysosomal functions of macrophages.8 Antimalarial drugs also inhibit endosomal Toll-like receptor signaling, which limits B-cell and dendritic cell activation.9

Chemokines are a large family of cytokines secreted by a variety of cell types (mainly monocytes, macrophages and endothelial cells) that play a major role in attracting cells to the site of tissue lesion or inflammation. To date, approximately 50 human chemokines and 20 receptors have been identified. Based on the number and spacing of cysteine residues in the amino-terminal region of the hitherto identified chemokines, they have been categorized into four subfamilies defined as C, CC, CXC and CX3C chemokines.10 A number of chemokines and their receptors have been explored as therapeutic targets. These targets are subjects of interest in different areas of biomedical and pharmaceutical research. Their role in OA has been studied in recent years. It has been suggested that monocyte chemoattractant protein 1 (CCL2), interleukin-8 (CXCL8), monokine induced by interferon-gamma (CXCL9) and interferon-gamma-inducible protein 10 (CXCL10) may participate in the process of the induction of biomechanical stress within the joint cartilage and in the bony lesion in OA.11–14 This study evaluates the effect of HCQ treatment on serum levels of CCL-2, CXCL8, CXCL9 and CXCL10 in patients with primary OA of the knees.

MATERIALS AND METHODS

This study was approved by the Committee of Ethics in Research of the Federal University of Juiz de Fora and all patients signed informed consent, in conformity with the Declaration of Helsinki. Twenty-nine patients aged 60.8 ± 9.2 years with at least a 1-year history of primary symptomatic OA of the knees were evaluated. The diagnosis of OA was based on the criteria of the American College of Rheumatology (ACR 2).15 All patients presented clinical evidence (knee pain for at least 6 months and on most of the days during the previous month) and radiographic evidence (Kellgren and Lawrence’s grade 2 or 3) of OA.16 The patients studied belonged to the classes I, II and III of the American Rheumatism Association, now known as ACR I.17 The characteristics of the patients with OA are shown in Table 1. Patients with a history of other joint diseases, joint trauma, or autoimmune diseases were excluded from the study. None of the patients had been treated with intra-articular hyaluronic acid or corticosteroids. Patients were allowed to use only acetaminophen for symptom relief during the study period. Prior to the beginning of this study, patients had used chondroitin sulfate, glucosamine, diacerein, NSAIDs or anti-inflammatory doses of corticosteroids; however, they were out of these medications for at least 12 months. All patients had erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels within normal ranges. All patients were subjected to an ophthalmologic evaluation before initiating the study.

Two control groups were included in the study. One group consisted of 10 individuals older than 60 years (CT-O group) and the other of 10 individuals younger than 40 years (CT-Y group). Old and young controls were defined as subjects who met the following criteria: (i) no clinical symptoms of chronic inflammatory

| Table 1 Characteristics of the osteoarthritis (OA) patients |
|----------------|----------------|
| Number of patients | 16 | 13 |
| Female gender | 13 (81.3) | 13 (100) |
| Race | | |
| Caucasian | 6 (37.5) | 5 (38.5) |
| Non-caucasian | 10 (62.5) | 8 (61.5) |
| Functional class, ARA§ | | |
| I | 1 (6.3) | 0 |
| II | 7 (43.7) | 9 (69.2) |
| III | 8 (50) | 4 (30.8) |
| Kellgren and Lawrence system | | |
| Grade 2 | 8 (50) | 6 (46.2) |
| Grade 3 | 8 (50) | 7 (53.8) |
| Knees with OA | | |
| Bilateral | 14 (87.4) | 8 (61.5) |
| Right | 1 (6.3) | 4 (30.8) |
| Left | 1 (6.3) | 1 (7.7) |

†Sixteen OA patients were treated with 400 mg/day of HCQ for 4 months. ‡Thirteen OA patients received placebo. §ARA, American Rheumatism Association (now called American College of Rheumatology). HCQ, hydroxychloroquine.
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Hydroxychloroquine treatment was well-tolerated overall by the 16 patients and all of them completed the study. All patients required the use of paracetamol during the treatment period. The paracetamol consumption, expressed as number of 500 mg tablets used per day, was 1.2 ± 1.8 in the group that used HCQ and 1.2 ± 2.0 in the group that used placebo (P = 0.53). The age of the OA patients treated with HCQ varied from 48 to 77 years (60.8 ± 9.2), with 13 of the patients being female (81.3%). In the placebo group the mean age was 60.5 ± 10.4 years (P = 0.924 vs. HCQ group), being all female. In the CT-O group the age varied from 61 to 76 years (68.4 ± 5.3), with seven of the subjects being female (70%). In the CT-Y group the age varied from 21 to 39 years (25.1 ± 6.1), with seven of the subjects being female (70%). The majority of the OA patients were non-Caucasian and most belonged to the functional classes II and III, which can be characterized as moderate disease (Table 1).

The serum chemokine levels of OA patients prior to the treatment with HCQ or with placebo did not differ significantly (59.8 ± 43.7 pg/ml and 47.4 ± 30.6, respectively for CCL2; 6.9 ± 3.8 and 6.21 ± 1.93, respectively for CXCL8; 1370 ± 702.5 and 1525 ± 626.5, respectively for CXCL9; 287.2 ± 211.4 and 268.7 ± 173.8, respectively for CXCL10). After 4 months of HCQ treatment in OA patients the chemokine levels still showed no significant difference from the placebo-treated group (74.1 ± 65.1 and 58.9 ± 42.2, respectively for CCL2; 8.08 ± 5.33 and 6.49 ± 1.94, respectively for CXCL8; 1409 ± 684.6 and 1556 ± 681.2, respectively for CXCL9; and 288.6 ± 261.9 and 188.8 ± 97.1, respectively for CXCL10).

For all chemokines studied in OA patients, the differences between the basal chemokine levels prior to treatment and the chemokine levels after 4 months of treatment with HCQ were not statistically significant (Fig. 1). The chemokine levels measured in the CT-O group were 39.1 ± 10.2 for CCL2, 9.9 ± 6.2 for CXCL8, 2282.2 ± 293.8 for CXCL9 and 294.2 ± 112.1 for CXCL10. There was no significant difference between the CT-O group and the OA patient group in CCL2 (P = 0.75), CXCL8 (P = 0.26) and CXCL10 (P = 0.56) levels. The chemokine levels measured in the CT-Y group were 23.7 ± 26.8 for CCL2; 2.9 ± 0.4 for CXCL8; 601.2 ± 438.7 for CXCL9 and 156.8 ± 46.4 for CXCL10. The CXCL9 serum levels were higher in the CT-O group than in both the OA group (P = 0.001) and the CT-Y group (P = 0.0002) (Fig. 1). The levels of CXCL8 and CXCL10 differed significantly (P = 0.0002 and P = 0.002, respectively) between the CT-O and the CT-Y groups (Fig. 1). The
CT-Y group presented lower levels of CCL2 ($P = 0.04$) and CXCL8 ($P = 0.0002$), in comparison with the OA patients (Fig. 1).

**DISCUSSION**

The present work is the first to study the relationship between knee OA and serum levels of chemokines before and after treatment with HCQ. In the past, OA was considered to be a purely degenerative disease. Today, there is extensive evidence for involvement of an inflammatory process in the progression of the disease with participation of inflammatory mediators, among them chemokines.$^{12}$ The chemokines form a large family of cytokines sharing structural homologies. They are known to act as regulators of leukocyte migration, being potent mediators of inflammation because of their ability to recruit and activate leukocyte subpopulations.$^{12-14}$

There are few reports in the literature on the use of HCQ in the treatment of OA. Bryant et al.$^7$ reported HCQ treatment of eight patients with OA of the hands, obtaining satisfactory results in six of the patients. In the responders improvement was observed between 7 weeks and 7 months after the beginning of treatment. Vuolteenaho et al.$^{18}$ found that HCQ suppresses production of nitric oxide induced by IL-1β in cartilage affected by OA, concluding that HCQ could be useful in the treatment of OA. Recently, a double-blind, placebo-controlled study by Jokar et al.$^{19}$ showed significant improvement in the symptoms of mild to moderate knee OA in patients receiving HCQ.

![Figure 1](image-url) Figure 1 The serum concentrations of chemokines CCL2 (a), CXCL8 (b), CXCL9 (c) and CXCL10 (d) were determined by cytometric bead array before and after treatment with hydroxychloroquine (HCQ) in osteoarthritis (OA) patients. Values are shown with quartile range and extreme values presented by box with whiskers. CT-Y = control young group. CT-O = control old group. OA-BT = OA patients before HCQ treatment. OA-AT = OA patients after HCQ treatment. Bar indicates median value. $^*P < 0.05$. 

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In this study, serum levels of CCL2, CXCL8, CXCL9, and CXCL10 did not increase in patients with OA of the knees and they did not change significantly after HCQ use. However, we were not able to exclude the possibility that these chemokines participate in the pathogenesis of OA. It may be explained by the fact that the patients participating in this study did not show prominent inflammatory alterations. It is possible that patients during periods of disease flare-ups with intense inflammatory alterations would show increased serum levels of the chemokines studied. Recently Hulejová et al.\textsuperscript{20} found increased expression of several cytokines in different joint tissues affected by OA and that CXCL8 was significantly upregulated in OA synovium and in the subchondral bone region. Endres et al.\textsuperscript{21} showed increased levels of CCL2 in OA sinovial fluid patients, and lower levels of CXCL10. Wozniacka et al.\textsuperscript{22} found reduction of IL-6, IL-18 and tumor necrosis factor (TNF-\(\alpha\)) after 3 months of chloroquine treatment in patients with SLE. The fact that we did not demonstrate a change in chemokine levels in the serum after treatment with HCQ does not exclude the possibility of an influence of the drug on the disease through another mechanism.

There is a link between advanced age and the presence of inflammatory mediators.\textsuperscript{23–28} The serum levels of several pro-inflammatory cytokines such as IL-1, IL-6, TNF-\(\alpha\) and CXCL8 are higher in healthy elderly individuals compared to healthy young individuals.\textsuperscript{27,28} In this study, the serum levels of the chemokines studied were higher in the elderly individuals in both the CT-O and OA groups in comparison to the younger CT-Y group. Other authors found similar results.\textsuperscript{23,24,27,28} Inaderia et al.\textsuperscript{23} reported an increase of CCL2 in elderly patients and attributed this elevation to the higher prevalence of atherosclerosis in this population. CCL2 plays an important role in atherogenesis by attracting monocytes to blood vessel walls.\textsuperscript{29}

The increase of CXCL9 serum levels observed in the individuals of the old control group in comparison with the other two groups may be due to aging. Our results are in agreement with Shurin et al.\textsuperscript{24} who were the first to show that increase of CXCL9 is associated with aging. The authors studied the influence of age on the production of cytokines and chemokines in 397 healthy individuals between 40 and 80 years of age, and found increased serum levels of CXCL9 and CXCL10, which were associated with the most advanced age in both males and females.

Antonelli et al.\textsuperscript{25} evaluated 164 healthy individuals between 10 and 79 years of age, and found that the levels of both Th1 (CXCL10) and Th2 (CCL2) chemokines were increased in older individuals. The change of Th1 and Th2 chemokines levels with age is controversial. Some authors suggest that the robustness of the Th1 response declines with age and that of the Th2 response increases with age, while others argue the opposite.\textsuperscript{30–35} CCL2 plays an important role in the development of the Th2 adaptive response, influencing the differentiation of Th0 into Th2 cells \textit{in vitro}.\textsuperscript{36} In this study, an increase in the serum levels of CCL2 was observed in the patients with OA in comparison with the young control individuals. It is possible that there is a prevalence of the Th2 response in these patients. However, the molecular mechanism for this possible chronic inflammatory condition associated with aging is not yet known. It was demonstrated by Klimiuk et al.\textsuperscript{37} that leflunomide reduces the serum levels of RANTES (regulated upon activation, normal T cell expressed and secreted), CCL2 and CXCL8 in patients with rheumatoid arthritis. This reduction was associated with improvements in the disease parameters.

As a study limitation, most of the patients had long-standing disease with chronic symptoms, but few signs of inflammatory activity. In addition, patients had markers of inflammation such as ESR and CRP within normal limits, which may have excluded patients with more intense inflammation, precisely those who would have more chances to show changes in circulating levels of chemokines.

In conclusion, HCQ treatment used for 4 months did not significantly alter the serum levels of CCL2, CXCL8, CXCL9 or CXCL10 in patients with symptomatic OA of the knees. Studies of patients with OA of the knees presenting clinical and laboratory evidence of active inflammation, including synovial fluid analysis, could furnish more information about the relationship between this disease and chemokine serum levels. The factors that influence the prevalence of OA in the age group above 60 years and the factors that lead to the increase of serum levels of chemokines during aging deserve further attention.

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