

The Effects of Chloroquine Phosphate on the Serum Level of Proinflammatory Interleukins in Patients with Knee Osteoarthritis

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Summary:

Background: Osteoarthritis is the most common joint disorder. Treatment is usually limited to short term symptom relived and is by no means satisfactory. chloroquine phosphate has shown promising results as a disease modifying osteoarthritis drug.

Methods: Eighty-four patients with primary knee osteoarthritis were enrolled in this study .Patients were selected according to osteoarthritis diagnosis of the American College of Rheumatology. Blood samples were taken from patients at first visit for later measurement of IL-1 beta,IL-6 &IL-8 levels .Each patient was given chloroquine phosphate 250 mg to be taken twice daily for two months duration. After this period blood samples were also taken for measurement of the same parameters above.

Results: In patients who completed the trial, there was significant lowering of interleukins level (namely interleukin-6 & interleukin-8). The mean level of IL-6 changed from 19.85 ± 4.36 picogram (pg)/ ml at baseline to 11.09 ± 2.57 pg/ml (p value < 0.0001) and IL-8 from 3.14 ± 1.36 pg/ml to 1.70 ± 0.90 pg/ml (p value 0.029). There was no significant lowering of IL-1 β .

Conclusions: Chloroquine phosphate can significantly reduce serum level of detectable pro-inflammatory interleukins namely IL-6 & IL-8 in patients with knee osteoarthritis.

Keywords: Chloroquine Phosphate, Proinflammatory Interleukins, Osteoarthritis.

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Introduction:

Osteoarthritis (OA) is a joint disorder characterized by progressive deterioration of the articular cartilage, combined with thickening of the subchondral bone; bony outgrowths (osteophytes) at joint margins, and mild, chronic non-specific synovial inflammation. It is the most common joint disease affecting > 80% of those who reach 70 years (1). Osteoarthritic cartilage is characterized by an increase in anabolic and catabolic activity. In early stages, the synthesis of collagens, proteoglycans, hyaluronun is increased and chondrocytes tend to replicate forming broad clusters. At the same time, synthesis of degradative enzymes such as the matrix metalloproteinases (MMPs) family of collagenases, gelatinases, hyaluronidase and stromolysins and other enzymes including lysosomal proteases (cathesins) is increased while some of the substances that inhibit cartilage destruction [such as tissue inhibitors of metalloproteinases (TIMPSs)] are themselves destroyed or inhibited (2).

Although OA often is classified as non-inflammatory disease, numerous studies have shown that inflammatory cytokines provide essential biochemical signals that

stimulate chondrocytes to release cartilage degrading – enzymes. In addition the inflammatory changes in the synovial membrane in patients with OA are, on occasion, almost indistinguishable from those in patients with an inflammatory arthritis such as rheumatoid arthritis (3). IL-1 β , IL-6 & IL-8 contribute to osteoarthritic process by increasing number of inflammatory cells in synovial tissue, stimulating the proliferation of chondrocytes and inducing an amplification of the IL-1 effects on the increased synthesis of MMPS and inhibiting proteoglycan production(4). Chloroquine is a synthetic 4-aminoquinolone formulated as the phosphate salt for oral use(5).It is used as antimalarial by causing interference with plasmodial DNA replication and possibly through metabolic effects on plasmodia (6).It also has anti-inflammatory effects which can be direct action by stabilizing lysosomes or through decreasing leukocyte chemotaxis and trapping free radicals and reducing cytokine mRNA expression (7,8).It has also immunoregulatory potency through suppressing the responsiveness of T-lymphocytes to mitogens (7). Interleukins are soluble mediators that control many critical interactions among cells of the immune system (9). There are until now more than 25 of them and are collectively branches from a large family called «cytokines»(10). The cytokines are secreted by particular cell types in response

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to a variety of stimuli and produce characteristic effects on the growth, mobility, or function of target cells. They serve as chemical messengers within the immune system, they also play a significant role in driving hypersensitivity and inflammatory responses (11). Most cytokines are normally present in undetectable amounts in the blood, unless produced in excess by pathology (12). Proinflammatory cytokines are believed to play a pivotal role in the initiation and development of osteoarthritic process. IL-1 beta, 6 & 8 are among the important proinflammatory cytokines that mediate cartilage destruction and development of acute and chronic inflammatory processes (13). Interleukin-1 beta is secreted by monocytes and tissue macrophages and in small amounts from B&T lymphocytes (10, 14). It plays major role in the pathogenesis of rheumatoid arthritis, chronic diabetes mellitus & periodontal diseases. Elevated levels of IL-1 beta have been reported in the circulation of febrile or septic patients, in patients with Crohn's disease and during graft rejection (15). Interleukin-6 is produced by various cells, including T and B-lymphocytes, monocytes fibroblasts, keratinocytes, endothelial cells and several tumor cells (16). IL-6 is a major inducer of the acute phase reactions in response to inflammation and tissue injury. IL-6 also promotes the growth and differentiation of B cells increasing immunoglobulin synthesis and also stimulating T-cell growth and cytotoxic T-cell differentiation. Elevated IL-6 concentrations have been documented in the serum and synovium of patients with rheumatoid arthritis (9, 17). Interleukin-8 (also known as NAP for neutrophil-activating peptide) has potent activating effects on neutrophils including chemotaxis, degranulation and release of lysosomal enzymes, and release of reactive oxygen metabolites (18). It is found in synovial fluid of patients suffering from rheumatoid arthritis, osteoarthritis and gout & high level correlates with mortality in patients with septic shock (19). The aim of the study is to evaluate serum levels of the proinflammatory interleukins in patients with osteoarthritis before and after two-month treatment with chloroquine phosphate.

Patients & methods:

This study was carried out in the outpatient clinic of the department of rheumatology of Baghdad Teaching Hospital in Baghdad, Iraq from December 2005 to June 2006. Eighty-four patients with primary knee osteoarthritis were enrolled in this study. Patients were selected according to osteoarthritis diagnosis of the American College of Rheumatology (20). Detailed history of the patient including age, sex, duration of disease, and the use of any non-steroidal anti-inflammatory drug (NSAID) was obtained. At first visit blood samples were taken from each patient. The samples

were centrifuged and the sera were separated and frozen for later measurement of IL-1 beta, IL-6 & IL-8 levels. Each patient was given chloroquine phosphate 250 mg to be taken twice daily for two months duration. After this period blood samples also were taken for measurement of the same parameters above. Interleukin 1 β , 6 & 8 concentrations were detected in the pre- and post-treatment serum samples through ELISA ((Enzyme – Linked Immunosorbent Assay) method which is a specific and highly sensitive method for quantitative measurements of cytokines in solutions and the procedures for cytokine ELISA are essentially the same (21,22). The ELISA kits used were purchased from BioSource Europe S.A (Nivelles, Belgium) for IL-1 β & IL-8 and Immunotech SAS (Marseille, France) for IL-6. Minimum detectable concentrations or sensitivity for IL-1 β , IL-6 & IL-8 were 0.35 pg/ml, 3.00 pg/ml and 0.70 pg/ml respectively. Results were expressed as mean change $\pm$ SEM, with P. values based on paired t-test. All reported P. values are two sided with $\alpha = 0.05$; $P < 0.05$ was considered statistically significant.

Results:

Eighty four patients with knee osteoarthritis were enrolled in the study. Of these patients only 34 patients (40%) completed the trial and came after the 2 month period. The comparisons between level of interleukin 1- β , 6 & 8 in pictogram/ml (pg/ml) before and after treatment are depicted in Tables (1,2 & 3) and Figures (1,2 & 3) respectively. There was a significant lowering in IL-6 level (which was the most ELISA detectable interleukin in sera of patients with OA) and IL-8 as compared to IL-1 β (which was mostly undetectable in sera of patients with OA) after the two month treatment.

Table 1 Interleukin-1 β level at baseline and after two months of treatment

	Baseline	After 2 months	P
Interleukin-1 β level (pg/ml)	2.90 $\pm$ 0.90	1.94 $\pm$ 0.61	0.249 (Non Significant)

Table 2 Interleukin-6 level at baseline and after two months of treatment

	Baseline	After 2 months	P
Interleukin-6 level (pg/ml)	19.55 $\pm$ 4.36	11.09 $\pm$ 2.5*	<0.0001 (Significant)

Table 3 Interleukin-8 level at baseline and after two months of treatment

	Baseline	After 2 months	P
Interleukin-8 level (pg/ml)	3.14 $\pm$ 1.36	1.70 $\pm$ 0.90	0.029 (Significant)

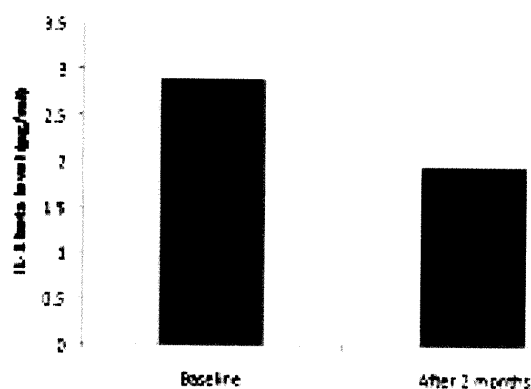
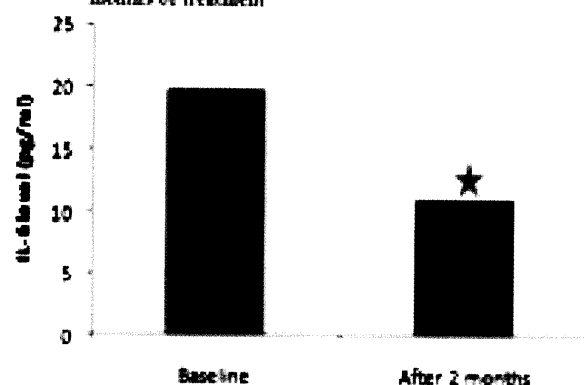
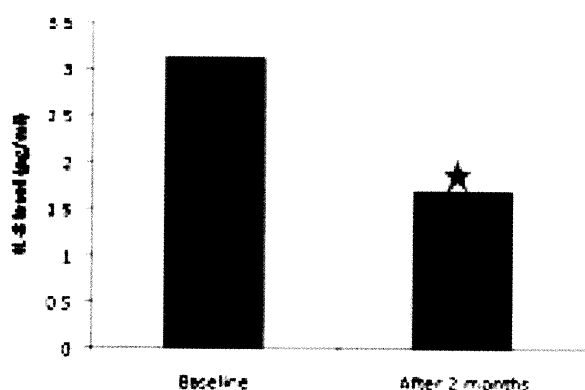


Figure 1 The relation between IL-1 β level at baseline and after two months of treatment



★ $P < 0.0001$

Figure 2 The relation between IL-6 level at baseline and after two months of treatment



★ $P < 0.029$

Figure 3 The relation between IL-8 level at baseline and after two months of treatment

Discussion:

The present study indicates that administration of chloroquine phosphate over 2 months can affect the level of inflammatory markers such as proinflammatory interleukins in blood of patients with OA. Proinflammatory

interleukins (cytokines in general) are believed to play a pivotal role in the initiation and development of this disease process (23). IL-1 beta is extremely important to cartilage destruction and can induce joint articular cells to produce other cytokines such as IL-6 & IL-8(24). IL-6 can increase the number of inflammatory cells in synovial tissue, stimulating the proliferation of chondrocytes and inducing an amplification of IL-1 β effects (25) IL-8 is a potent chemotactic cytokine for polymorpho-nuclear neutrophils (PMN), stimulating their chemotaxis and generating reaction oxygen metabolites (26). Over the last decade OA research has increasingly focused on drugs that not only improve patient symptoms, but additionally are capable of altering the course of osteoarthritic development and consequently postpone or even prevent the need for surgery, the so called disease modifying osteoarthritis drugs (DMOAD) (27). We previously studied the effect of chloroquine phosphate as a disease modifying agent in osteoarthritis (28). Previous studies demonstrated that chloroquine reduces the responsiveness of peripheral blood mononuclear cells to mitogens (29), thus inhibiting T-cell proliferation and suppressing the generation of immunoglobulin-secreting cells. These effects of chloroquine were more explained by a reduced production of lymphocyte – stimulating factor IL-1 β (30). In addition, In vitro studies using monocyte monolayers showed a dose-dependent decrease in IL-6 secretion after treatment with chloroquine (31). In our study, we have measured the effect of chloroquine phosphate on serum level of interleukins IL-1 β , IL-6 & IL-8 at baseline and after the two-month trial period. IL-6 was the most detectable interleukin in sera of patients and this may be related to the role of interleukin-6 in induction of acute phase response, there was significant lowering in interleukin-6 level in patients who completed the trial and this goes with previous In vitro findings (31). Interleukin-1 β , although represents the main interleukin in the pathophysiology of OA, was mostly undetectable in majority of patients. this can be explained by the fact that the measurement involved the serum rather than the synovial membrane interleukin as synovial membrane is the site of major actions of this interleukin so it is present in large amounts in synovial fluids. The precise mechanism of action of chloroquine as an anti-inflammatory agent has not been fully elucidated yet. One of the most important anti-inflammatory actions of chloroquine phosphate and hydroxy-chloroquine is their effect on lysosomes and stabilization of lysosomal membranes (32). The lysosomotropic agent chloroquine phosphate has been effective in the treatment of diseases associated with increased secretion of pro-inflammatory cytokines such as malaria or rheumatoid arthritis (3). Chloroquine has a weak base effect. The fact that weak

bases other than chloroquine are able to down-regulate synthesis of proinflammatory cytokines provides evidence that the anti-inflammatory effect of chloroquine may be due to an intracellular effect on lysosomes with an elevated lysosomal pH and a decreased intralysosomal enzyme activity (33). Studies found that there is a dose-dependent down regulatory or inhibitory effect of chloroquine on proinflammatory cytokine synthesis through inhibition of mRNA expression of IL-1 β , IL-6 & TNF- α at transcriptional level due to its weak base effect (3). Other anti-inflammatory actions of chloroquine may include suppression of the responsiveness of T-lymphocytes and decreasing polymorphonuclear cell chemotaxis (3), direct inhibition of lysosomal phospholipase A and phospholipase C, decreasing total prostaglandin production and membrane phospholipids turn over (34) and trapping of free radicals (3).

Conclusion:

Chloroquine phosphate can significantly reduce serum level of detectable pro-inflammatory interleukins namely IL-6 & IL-8 in patients with knee osteoarthritis.

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