The Role of Chloroquine Phosphate on Acute Phase Reactant Proteins in Patients with Knee Osteoarthritis

Eeman S. Saleh*,1, Kismet M.Turki**, and Mohammed H. Al-Osami***

*Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
**College of Medicine, University of Baghdad, Baghdad, Iraq.
***Department of Rheumatology and Rehabilitation, Medical Baghdad City, Baghdad, Iraq.

Abstract
The acute phase response is a major pathophysiological phenomenon that accompanies inflammation whether acute or chronic. Complement (C3 and C4) and C-reactive protein (CRP) are positive acute phase proteins (+ve APPs). Their production take place in hepatocyte and the blood concentration of these parameters are increased in osteoarthritis (OA). Chloroquine (CQ) is a dihydropic weak base traditionally used to treat malaria. Recently the phosphate salt of CQ is used to decrease this type of (+ve APPs). In this study, patients who suffered from knee osteoarthritis (KOA) are treated with oral dosage form of chloroquine phosphate (CQP) for one month, twice daily. Our results demonstrate that CQP improves the patient status by decreasing complement and C-reactive protein in blood.

Key words: Chloroquine, knee osteoarthritis, acute phase proteins, complement, C-reactive protein.

Introduction:
Osteoarthritis (OA) is the most common disease in the world and a major cause of pain and disability which usually develops in distal interphalangeal (DIP) joints of finger, the weight bearing joints of leg and the movable portion of spine (1). It is associated with a breakdown of cartilage in any joint in the body (2). Pathologically, OA was defined as a gradual loss of articular cartilage combined with thickening of subchondral bone/ bony out growth (osteoaphy) at joint margins with mild to chronic non specific synovial inflammation (3). CQ is 4-aminoquinoline approved for treatment and prophylaxis of malaria. Recently CQ is used by some authors in the OA as disease modifying anti-rheumatic drug (DMARD) (4), claimed to cause lowering of blood level of proinflammatory mediators (5). Our study shows the effect of CQP on the serum concentration of CRP, C3 and C4 in patients with KOA. C-reactive protein is a laboratory marker that is important in the assessment of inflammation, serve as a predictor and indicator of response to therapy and overall outcome in various disorders (6). The major function of CRP is to bind phosphocholine, this is by permitting recognition of foreign pathogens and phospholipid constituents of damaged cells (7), so the activation of complement system and / or binding to phagocytic cells will take place, and to initiate elimination of targeted cells by interaction with both humoral and cellular afrecter system of inflammation, as a result CRP is a component of innate immune response (8), and useful in early detection of low grade inflammation (9).

*Based on oral presentation in the seventh scientific conference of the College of Pharmacy /University of Baghdad held in 26-27 November 2008.
1 Corresponding author E-mail: www.Faith_1960@yahoo.com
Received: 31/12/2008
Accepted: 28/3/2009
C3 and C4 serve proinflammatory roles, including chemotaxis, plasma protein exudation at the site of inflammation and opsonization of infectious agents and damaged cells\(^{(9)}\). C3 is beta-2 protein 180 KDa, cleave by C3 convertase into C3a and C3b. C3b reacts with factor B to produce more C3 convertase and activate C5, so the serum level of C3 is increased in acute inflammation\(^{(11)}\). C4 is beta-1 protein, 210 KDa cleaved by C1s to produce C4a and C4b. C4b interact with C2b to activate classical pathway C3 convertase.\(^{(12)}\)

**Patients and method**

Fifty healthy people (30 female and 20 male) as control and seventy-four patients (45 female and 29 male) are selected randomly from the Out Patient Clinic in Baghdad Teaching Hospital / Medical City / Baghdad from January to September 2008 with inflammatory KOA diagnosed according to American College of Rheumatology\(^{(13)}\). All patients were assessed by Kellegren and Lawrence grading criteria for radiographic severity of knee osteoarthritis with different signs and symptoms such as joint pain, stiffness, bony enlargement, bony tenderness and crepitus\(^{(14)}\). Their ages are ranged from (45 to 78) years with mean value ± standard error of mean (55.07 ± 6.18). Chloroquine phosphate tablet (Medoquine 250mg/150mg CQ base), was prescribed by Rheumatologist as twice daily after meal for one month, because CQP is 4-aminoquinoline derivative (CQ+phosphate), it absorbed completely and rapidly from GIT, its mean absorption half-life is four hours with a lag time slightly more than 30 minutes\(^{(15,16)}\), and its duration of action is extended from sixteen to forty five days\(^{(17)}\). The serum analysis of all patients and control was done in the General Health Laboratory Center / Baghdad, before using this drug and one month later in order to estimate the level of C3, C4 and CRP. C3 and C4 are determined by Radial Immune Diffusion\(^{(11)}\) while CRP is assessed turbidometrically by antigen antibody reaction technique\(^{(17)}\). Results were analysed by statistical package for social science (SPSS).

**Results**

The presented data in this study showed the comparisons between level of C3, C4 in microgram per milliliters (μg/ml) and CRP in milligram per liter (mg/l) in healthy and patients before the treatment as well as after one month of using chloroquine phosphate are depicted in table (1), figures (1,2,3). There were a significant decrease in C3, C4 and CRP compared to their level at baseline and control.

**Table (1): The serum level of C3, C4 and CRP before using CQP at baseline and after one month of the treatment.**

|          | Control  | Baseline | P value Pre-post-treatment |
|----------|----------|----------|---------------------------|
| **C3 (μg/ml)** |          |          |                           |
| T        | 950.1±21.1 | 1794.4±34.2 | S p>0.01                  |
| M        | 877.3±17.3 | 1788.2±48.8 |                           |
| F        | 987.4±20.2 | 1798.4±47.16 |                           |
| **C4 (μg/ml)** |          |          |                           |
| T        | 320.23±12.2 | 396.08±41.6 | S p>0.01                  |
| M        | 318.1±15.7  | 367.7±20.2  |                           |
| F        | 307.2±13.4  | 414.3±19.9  |                           |
| **CRP (MG/l)** |          |          |                           |
| T        | 1.08±0.1  | 4.3±0.3  | S p>0.01                  |
| M        | 1.1±0.12  | 3.8±0.6  |                           |
| F        | 2.09±0.17  | 4.63±0.4  |                           |

SEM: Standard error of mean.
Significant P value : (P<0.05), (P>0.01).
Figure (1): The level of serum C-reactive protein at baseline (pre-treatment), after one month of using chloroquine phosphate (post-treatment) and control.

Figure (2): The level of serum complement three at baseline and one month later of using chloroquine phosphate.

Figure (3): The level of serum complement four at baseline and one month later of using chloroquine phosphate.
Discussion

C3, C4 and CRP are components of +ve APPs , their production are increased by hepatocyte (18). The elevation of these proteins are detected in patient with OA which is due to releasing of inflammatory molecules (cytokines)(19). Jawad et al in 2004(20) demonstrated that the using of CQP as DMARD for three months in patients with KOA, lead to decrease the serum CRP level. In our study the presented data shows a significant decrease in this parameter after one month of using CQP (p<0.05), table (1), figure (1), so our result is in agreement with all explanation and findings. Chloroquine phosphate decreases serum level of CRP, C3 and C4 depending on it's ability to enter lysosomes and all acidic compartments of the cells (lysosomotropic effect) (21). It interferes with intracellular processing, receptor recycling (21) and the secretion of proteins which lead to decrease the production of cytokines and other inflammatory mediators decreases lymphocyte proliferation as an immune effect (22). Non - lysosomotropic effect of CQP includes the inhibition of phospholipase, antagonization of prostaglandin stabilization of lysosomal membrane in synoviocytes (23,24,25). In 2006 , Numman et al used silymarin to treat patients with KOA instead of CQP(26). Silymarin is a plant (mixture of flavolignans),isolated from the ripe seeds of Silybum marianum (Milk Thistle), it proved to have effective inhibitory effects on cyclooxygenase and 5- lipoxygenase in vitro and experimental animals (27). Their results showed that this drug when prescribed for two months in Koa, it decreases the serum level of C3 and C4 significantly. Our study shows a significant decrease in C3 and C4 at the end of trial ( p<0.05) table (1), figure (2) and (3). All findings, trials in addition to the mode of action of CQP are supported the results.

Conclusion:

CRP, C3 and C4 are decreased after using CQP for one month in patients with KOA.

Recommendation

Further study is needed to asss other parameters in serum and synovial fluid.

References

1. Morehead K , Sack KE . Osteoarthritis : What therapies for this disease of many causes ? Postgraduate Med. 2003;114, 5.
2. Brandt KD .Osteoarthritis. In: Kasper DL(ed) .Harrison's Principles of-Internal-Medicine,6th , ed .McGraw-Hill Companies,2005;2036-45.
3. Berenbaum F. Osteoarthritis: A. Epidemiology, Pathology and Pathogenesis . In: Klippel JH, Crofford LJ, Stone JH, Wiegand CM (eds). Primer on rheumatic disease 12th ed. Atlanta, Georgia. Arthritis Foundation, 2001; P.285-89.
4. Jawad HM, Salman S ,Mohammed L. The effect of chloroquine phosphate as a disease modifying agent in osteoarthritis . Ph D thesis submitted to University of Baghdad/College of Medicine and the committee of postgraduate studies in Clinical Pharmacology, 2004.
5. Jelab A, Jawad HM, Salman S, Mohammed L: The effect of chloroquine phosphate on pro-inflammatory interleukins in osteoarthritis. MSc thesis submitted to University of Baghdad/College of Medicine and the committee of postgraduate studies in Clinical Pharmacology.2007.
6. Baddour VT , Bradley JD . Clinical assessment and significance of inflammation in knee osteoarthritis. Curr Rheumatol Rep,1999;1:59-63.
7. Volanakis JE. Acute phase protein in rheumatic disease. In: Koopman WJ (ed.).Arthritis and allied condition. A Textbook of Rheumatology. Williams Wilkins, Baltimore 1997; P.505.
8. Hoffman JA, Kafatos FC, Janeway CA, Ezekowitz RA.Phylogenetic perspective in innate immunity.Science,1999;248,1313.
9. Visser M, Bouter LM,McQuillan GM,et al. Elevated C- reactive protein levels in over weight and obese adult JAMA,1999;282, 2131-35.
10. Molenaar ET,Voskuyl AE,et al. Complement activation in patients with rheumatoid arthritis mediated in part by C-reactive protein. Arthritis Rheum, 2001;44, 997.
11. Feldkamp CS. Immunochemical techniques. In: Kaplan LA, Pesce AJ, Kazmierzak SC (eds). Fourth Edition. Textbook of Clinical Chemistry , Mosby USA,2003. P.227-45.
12. Kyle RA. Classification and diagnosis of monoclonal gamma pathies. In: Rose NR, Friedman H and Fahey JL (eds.). Manual of clinical laboratory immunology (3rd ed.). Washington DC ; American Society of Microbiology 1986; P.152.
13. Recommendation for the medical management of osteoarthritis of the hip and knee.Update. American College of
Chloroquine and knee osteoarthritis

Rheumatology, Subcommittee on Osteoarthritis Guidelines. Arthritis Rheum, 2000; 43: P. 1905.

14. Kellgren HJ, Lawrence JS. Radiologic assessment of osteoarthritis. Ann Rheum Dis, 1957; 16: 494-501.

15. Smith DG, Aronson JK. Drug for arthritis. In: Grahame-Smith DG (ed.). Textbook of clinical pharmacology and drug therapy. 3rd ed. Oxford University Press, 2002; P. 355-356, 509-510.

16. Furst DE. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. Lupus, 1996; 5: 11.

17. Black S, Kushner I, Samols D. Serum C-reactive protein. J Biol. Chem., 2004; 279: 479-484.

18. Morley JJ, Kushner I. Serum C-reactive protein level in diseases. Ann NY Acad. Sci., 1982; 389: 406.

19. Gabay C, Kushner I. Acute phase protein and other systemic responses to inflammation. N Engl. J Med., 1999; 340: 440-448.

20. Petri M. Hydroxychloroquine use in Baltimore Lupus Cohort: Effect on lipid, glucose and thrombosis. Lupus, 1996; 5: 16.

21. Rynes RI. Anti-malarial drug. In: Kelley WN, Harris ED JR, Ruddy S, Sledge CB (eds.). Textbook of Rheumatology, 5th ed. WB Saunders. Philadelphia, 1997; P.59-1.

22. Karres I, Kremer JP, Dietle I, Steckholzer U, Jochum M, Ertel W. Chloroquine inhibits proinflammatory cytokines release into human whole blood. Am J Physiol/Regul Integ Comp Physiol, 1998; 274, 1058-64.

23. Fox R. Anti-malarial drug. Possible mechanism of action in autoimmune disease and prospects for drug development. Lupus, 1996; 5: 4.

24. Wallace DJ. Anti-malarial therapies. In: Wallace DJ, Hahu BH (eds.). Dubois lupus erythematosus (5th ed.) Williams Wilkins, Baltimore, 1997; P. 1117.

25. Stuhmeier KM. Mepacrine inhibits matrix metallo proteinase-1 (MMP-1) and (MMP-9) activation in human fibroblast-like synoviocyte. J Rheumatol, 2003; 30: 2330.

26. Numan IT, Hussain SA, Al-Ani TA. Evaluation of the clinical use of silymarin in knee osteoarthritis: Application of dual inhibition concept of cyclooxygenase and 5-lipoxygenase. Ph D thesis submitted to University of Baghdad/College of Pharmacy and the committee of postgraduate studies in Pharmacology, 2006.

27. Pepping J. Milk thistle: Silybum marianum. Am J Health Syst Pharm, 1999; 56: 1195-97.