

The role of chloroquine phosphate on tumor necrosis factor - alpha and interleukin-one in knee osteoarthritic patients

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الخلاصة:

مرض الفصال العظمي للركبة يتضمن سلسلة من الفعاليات الالتهابية التي تؤثر على مستوى محركات الخلايا الابتدائية مثل (انترلوكين واحد وعامل الورم التغفني نوع الفا) في الخلايا وأنسجة مرضى الروماتيزم. هذه العوامل تلعب دوراً مهماً في تأثيرها على مرضيات العضال العظمي. عقار فوسفات الكلوراكوين له القابلية على دخول أجزاء الخلية ذات الطبيعة الحامضية مثل اللايسوسوم، اندوسوم ومعقدات كولجي بهذا سيكون تأثيره على طريق سير الالتهاب بواسطة تحفيزه مناعة الجسم والتأثير الثاني على الأنزيمات التي تساعد على تآكل الغضاريف والمفاصل وتنشأ هذه الحالة المرضية. أجريت تجربة عشوائية على 74 مريضاً (45 أنثى و 29 ذكر) و 50 شخصاً من الإحصاء (30 أنثى و 20 ذكر) في مستشفى مدينة الطب العيادة الاستشارية (من شهر تموز 2006 إلى آب 2007) ثم تشخيصهم من قبل الطبيب الاختصاصي حسب طريقة الكلية الأمريكية لمبحث الرثية، وتحريز الأعراض فكان حسب مؤشر كليكرين ولورنس للفصال العظمي بالإضافة إلى صور شعاعية أمامية خلفية لمفصل الركبة. وصف للمرضى عقار فوسفات الكلوراكوين بجرعة 250 ملغم يومياً مرتين بعد الأكل لمدة شهر. سحبت عينة من دم الأصحاء والمرضى الذين استعملوا هذا العقار لغرض قياس نسب مستوى المحركات الابتدائية وقد لوحظ انخفاض معنوي لهذه العوامل للمرضى بعد مقارنة نتائجهم مع الإحصاء ومقارنة المرضى قبل وبعد العلاج .

Abstract:

Osteoarthritis (OA) is a condition characterized by a series of inflammatory processes. Various pro inflammatory cytokines like interleukin-one (IL-1) and tumor necrosis factor-alpha (TNF- α) are present in synovium and tissue of rheumatic patients, so these agents play an essential role in pathogenesis of joint diseases. Chloroquine phosphate (CQP) interferes the cellular function of acid microenvironments such as lysosome, endosome and Golgi's complex, as well as affects pathways of inflammation, immune cascade and enzyme activity which ultimately resulted in cartilage destruction in knee joint with OA.

Introduction:

OA is the most common musculoskeletal disorder world - wide. Aging, genetic, hormonal and mechanical factors are the major contribution to its onset and progression^[1]. Articular cartilage, subchondral bone and synovial membrane are the sites of abnormalities in disease processes, so OA can be described as degradation and loss of articular cartilage accompanied by hypertrophic bone changes with osteophyte formation, subchondral bone thickening and at clinical stage, the inflammation of synovial membrane takes place^[2].

IL - 1 has a pivotal role in the evolution of joint inflammation^[3]. A high level of IL -1 beta (IL-1 β) are found in synovial fluid (SF) of arthropathies which characterized by a strong erosive activity^[4]. TNF- α has important role not only in inflammatory arthritis but in degenerative joint disease^[5]. It controls the homeostasis of matrix synthesis and matrix degeneration in articular cartilage in concert with other cytokines such as IL -1 and insulin growth factor-1(IGF-1), so in OA the increasing of TNF production by activated synoviocyte P₅₅ TNF receptor expression on chondrocyte imply the contribution of TNF mediated matrix degradation to disease pathogenesis^[6]. Chloroquine (CQ) has been used successfully, in the treatment of inflammatory disease such as rheumatoid arthritis (RA) and discoid lupus erythematosus (DLE)^[7]. Previous studies demonstrated that CQ reduces the responsiveness of peripheral blood mononuclear cells to mitogen^[8], so inhibiting T-cell proliferation and suppressing the generation of immunoglobulin secreting cells. Finally it affects the production of pro inflammatory mediators, as result controls the progression of OA^[9].

Patients and Methods:

Seventy - four patients [forty - five female 60% and twenty - nine male 40%) and 50 healthy people (30 female, 20 male) are selected randomly from the Out Patient Clinic in Baghdad Teaching Hospital/Medical City/Baghdad. From July 2006 to August 2007 the ages of individual are ranged from (45-78) years with mean value (55.07 \pm 6.18).

All patients have symptomatic and radiologic evidence of knee osteoarthritis (KOA) with different signs and symptoms. All patients were assessed by kellegren and lawerence grading criteria for radiographic severity of KOA^[10].

CQP tablets were prescribed by a Rheumatologist as (Medoquine 250 mg/ Medochem) twice daily after meal for one month and all patients.

Venous blood sample was drawn from healthy and patients before using the drug and one month later.

Serum was analyzed in General Health Laboratory Center to asses TNF- α , IL-1 (α and β) by ELISA technique.

Results:

The presented data in this study showed the comparison between level of TNF- α , IL-1 α and IL-1 β in pecto gram per milliliters (pg/ml) in healthy and patients before and after one month of using CQP are depicted in table (1), figures (1,2,3). The data shows significant decreases ($p<0.05$) in these pro inflammatory mediators.

The serum level is calculated as mean \pm standard error of mean by using paired t-test.

Pro inflammatory mediators	Control		Baseline	P value control-baseline	After one month	P value pre-post treatment	
	T	M	F		T		M
TNF α (pg/ml)	T	102.75 \pm 9.01	376.45 \pm 26.85	P<0.05	T	112.55 \pm 10.93	P<0.05
	M	109.84 \pm 11.24	380.44 \pm 37.25		M	117.69 \pm 16.12	
	F	98.31 \pm 7.05	373.8 \pm 37.45		F	109.24 \pm 14.79	
IL-1 α (pg/ml)	T	4.08 \pm 0.28	12.21 \pm 0.26	P<0.05	T	7.86 \pm 0.3	P<0.05
	M	6.59 \pm 0.3	13.72 \pm 1.79		M	8.51 \pm 0.65	
	F	3.84 \pm 0.15	11.23 \pm 0.29		F	7.44 \pm 0.26	
IL-1 β (pg/ml)	T	5.01 \pm 0.99	32.26 \pm 8	P<0.05	T	7.79 \pm 1.53	P<0.05
	M	9.89 \pm 1.05	43.61 \pm 13.65		M	11.32 \pm 361	
	F	3.78 \pm 0.84	24.94 \pm 9.75		F	5.52 \pm 0.87	

Table-1: The level of TNF- α , IL-1 α and IL-1 β in sera at baseline and after one month of treatment by CQP.

T: total patients, M: male, F: female

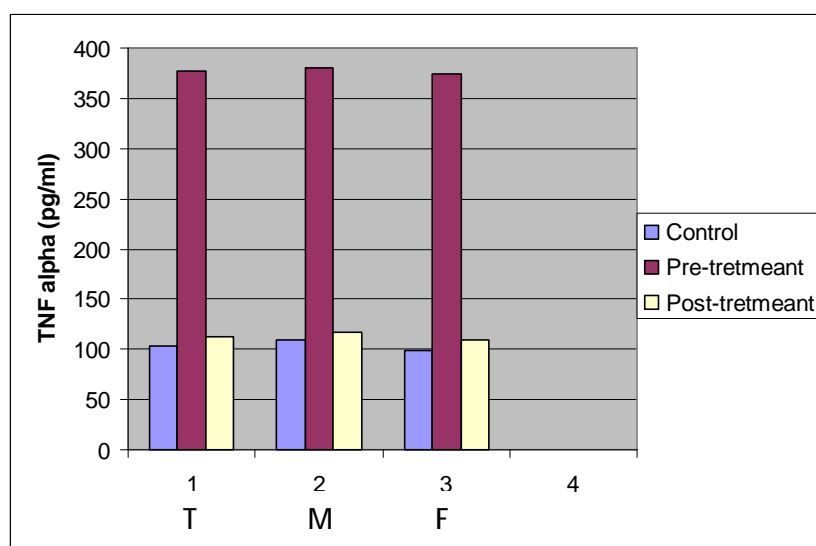


Figure-1: The level of serum TNF α in control and KOA patients

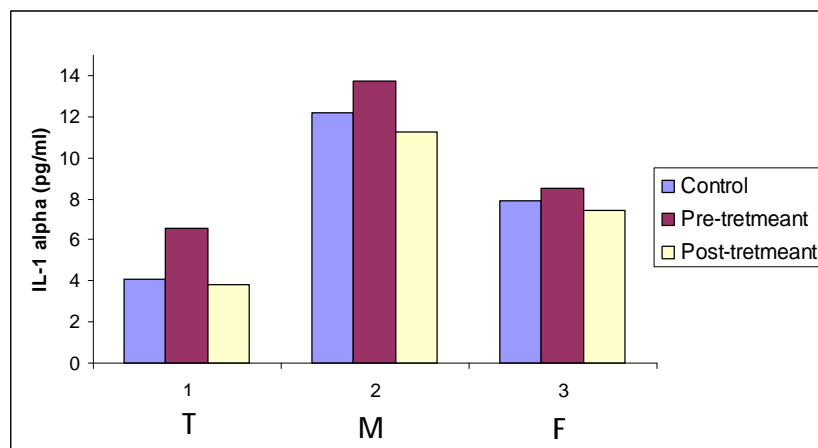


Figure-2: The level of serum IL -1 α in control and KOA patients

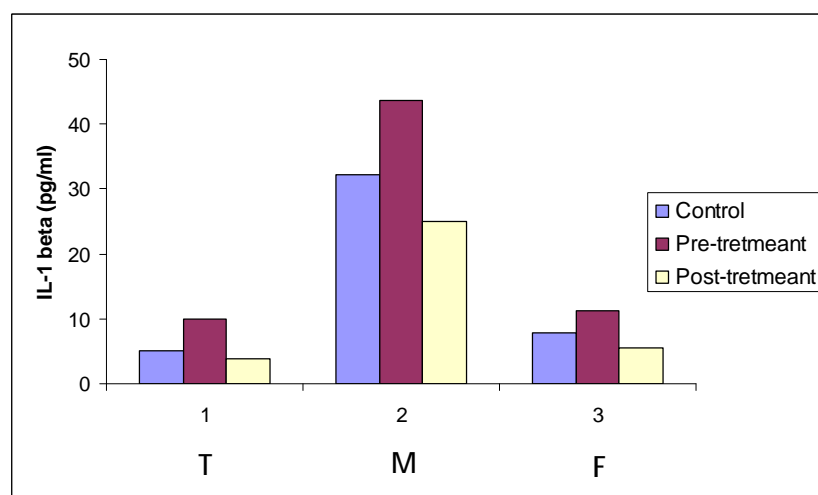


Figure-3: The level of serum IL -1 β in control and KOA patients

Discussion:

IL-1 and TNF- α are pro inflammatory cytokines, they act as activators in the production of the degenerative enzyme^[11,12], IL-1 with other cytokines were principally synthesized by leukocyte and primarily acted on others. The precursor of IL-1 β is produced mainly by synoviocyte but has an autocrine activity of chondrocyte^[13].

TNF - α induces resorption of cartilage^[14], perhaps acting synergistically with IL - 1 β ^[15], its precursor is also secreted by chondrocytes and activated at the cell surface by TACE^[16]. The TACE gene expression is up regulated in human osteoarthritic cartilage^[17]. In this study, our results showed a significant decrease in IL-1(α , β) and TNF- α serum patients level who completed the trial ($p < 0.05$), table (1) and figures (1, 2, 3). These findings are in agreement with the fact that CQ has the ability to suppress the production of pro inflammatory mediators and control the progression of this joint disease^[9]. Ghigo et.al. in 1998

demonstrated that CQ increase nitric oxide (NO) production by enhancing nitric oxide synthase (NOS) activity in murine, porcine and human endothelial cells^[18], thus lead to inhibit interferon (INF) gamma and Lipopolysaccharide (LPS) stimulated inducible NOS expression in macrophage^[19,20] as well as LPS-induced IL-1 β and TNF- α release in monocyte THP-1. In addition CQ inhibits the synthesis of pro inflammatory cytokines especially TNF - α and INF -gamma in macrophage^[21]. Karres et al at the same time observed that CQ at a high doses significantly decreased LPS - induced release of TNF- α , IL-1 β and IL - 6 in human peripheral blood mononuclear cells (HPBMC)^[22]. At 2000, Weber and Levitz reported that CQ at therapeutically attainable concentration exhibited dose-dependant inhibition of LPS - induced pro inflammatory cytokines release by HPBMC. (10 or 100) micromole of CQ are used, these doses are completely abrogated release of TNF - α and IL - 1 β from this cells^[23]. Hong et al at 2004, demonstrated the ability of CQ to inhibit the release of TNF - α , IL-6 and IL - 12 *in vivo* from murine macrophage ANA-1 cells that previously induced by LPS^[24]. The recent study of Jelab et al showed the effects of CQP on IL-1 β , IL-6 and IL-8 in patients with KOA, their finding records a decrease in these mediators after two month of using this drug^[25].

Numan et al 2006 tried to determine the serum level of IL-1 α also in patients had KOA but treated with silymarin (plant material) instead of CQP their result showed a decrease in this mediator^[26].

As a result all these trials that discussed above are in agreement with this study and support it.

In conclusion CQP decreases the serum level of IL-1 α , β and TNF- α in Iraqi selected KOA patients when used for one month and leads to ameliorate the signs and symptoms of this joint disease.

References:

- 1 - Ghosh, P. and Cheras, P.A. (2001). Vascular mechanisms in osteoarthritis. *Best Practice and Research Clinical Rheumatology*, 15: PP. 693-710.
- 2 - Pelletier J.P., Matrel-Pelletier J., Howell D.S. (1997): Etiopathogenesis of osteoarthritis in: Koopman, W.J. (ed). *Arthritis and Allied Condition. A textbook of Rheumatology*, 13th ed. Baltimore Williams and Wilkins, P.1969 - 84.
- 3 - Arend, W.P. and Dayer, J.M. (1990). Cytokines and cytokine inhibitors or antagonists in: *Rheumatoid arthritis. Arthritis Rheum*, 33: PP.305-313.
- 4 - Wood, D.D.; Ibrie, E.J.; Dinarello, C.A. and Cohen, P.L. (1983). Isolation of an interleukin-1 like factor from human joint effusions. *Arthritis Rheum*, 26 : PP.975 - 983 .
- 5 - Westacott, C.I. and Sharif, M. (1996). Cytokines in osteoarthritis: Mediators and markers of joint destruction? *Semin Arthritis Rheum*, 25:PP. 254-75.

- 6 - Fernandes, J.C.; Matrel-Palletier, J. and Pelletier, J.P. (2002). The role of cytokine in osteoarthritis pathophysiology. *Biorheology*, 39:PP.237-46.
- 7 - Dubois, E.L. (1978). Anti malarials in the management of discoid and systemic lupus erythematosus . *Semin Arthritis Rheum*, 8 :PP.33-51.
- 8 - Salmeron, G. and Lipsky, P.E. (1983). Immunosuppressive potential anti malarials. *Am J Med*, 75: PP.19 - 24.
- 9 - Szekanecz, Z. and Koch, A.E. (2001). Cytokines in: *Kelley's Textbook of Rheumatology 6th (ed.)*. Ruddy S, Harris ED, Sledge CB, Sergent JS and Budd RC (eds.) Saunders Company,P. 155.
- 10 - Kellgren, H.J. and Lawrence, J.S. (1957): Radiologic assessment of osteoarthritis. *Ann Rheum Dis*. 16, PP. 494-501.
- 11 - Szekanecz, Z.; Koch, A.E. and Kunkel, S.L. et.al. (1998). Cytokines in rheumatoid arthritis: Potential target for pharmacological intervention. *Drugs Aging*, 12:PP.377.
- 12 - Koch, A.E.; Kunkel, S.L. and Strieter, R.M. (1995). Cytokines in rheumatoid arthritis. *J Invest Med*. 43:PP.29.
- 13 - Ollivierre, F.; Gubler, V. and Towel, C.A. et.al. (1986). Expression of IL-1 gene in human and bovine chondrocytes. A mechanism for autocrine control of cartilage matrix degradation, *Biochem Biophys Commun*; 141:PP. 904 - 911.
- 14 - Saklavata, J. (1986). Tumor necrosis factor - alpha stimulates resorption and inhibit synthesis of proteoglycan in cartilage, *Nature*, 322:PP.547-549.
- 15 - Henderson, B. and Pettipher, F.R. (1989). Arthritogenic actions of recombinant IL-1 and tumor necrosis factor alpha in the rabbit: Evidence for synergistic interaction between cytokines in vivo. *Clin Exp Immunol*; 75:PP.306 - 310.
- 16 - Shinmei, M.; Masuda, K. and Kikuchi, T. et.al. (1991). Production of cytokines by chondrocytes and it's role in proteoglycan degradation. *J Rheumatol*, 27: PP.89 - 91.
- 17 - Amin, A.R. (1999). Regulation of tumor necrosis factor-alpha and tumor necrosis factor converting enzyme in human osteoarthritis, *Osteoarthritis Cartilage*, 7: PP.392 - 394.
- 18 - Ghigo, D.; Aldier, E.; Todde, R. and Costamagna, C. et.al. (1998). Chloroquine stimulate nitric oxide synthesis in murine, porcine and human endothelial cell. *J Clin Invest*; 102: PP.595-605.
- 19 - Hrabak, A.; Sefrioui, H.; Vercruysse, V. and Temesi, A. et.al (1998). Action of chloroquine on nitric oxide production and parasite killing by macrophages. *Eur J Pharmacol* ,354: PP.83 - 90.
- 20 - Park, Y.C.; Pae, H.O.; Yoo, J.C. and Chi, B.M. et.al. (1999). Chloroquine inhibits inducible nitric oxide synthase expression in murine peritoneal macrophage. *Pharm Toxicol*, 85: PP.188-91.

- 21 - Seite, M.; Valbracht, J.; Quach, J. and Lotz, M. (2003). Gold sodium thiomalate and chloroquine inhibit cytokine production in monocytic THP-1 cells through distinct transcriptional and post translational mechanism. *J Clin Immunol*, 23: PP.477-84.
- 22 - Karres, I.; Kremer, J.P. and Dielte, I. et.al. (1998). Chloroquine inhibits pro inflammatory cytokine release into whole blood. *Am J Physiol*, 274: PP.1058 - 64.
- 23 - Weber, S.M. and Levitz, S.M., (2000). Chloroquine interferes with lipopolysaccharides- induced TNF - alpha gene expression by non lysosomotropic mechanism. *J Immunol*, 165: PP.1534- 40.
- 24 - Hong, Z.; Jiang, Z. and Liangxi, W. et.al. (2004): Chloroquine protect mice challenge with CPG ODN and LPS by decreasing pro inflammatory cytokine release. *International Immune Pharmacology*, 4: PP.223-234.
- 25 - Jelab, A.; Jawad, H.M. and Mohammed, L. (2007). Evaluation the effects of chloroquine phosphate on pro inflammatory interleukins (IL-1 β , 6 and 8) in knee osteoarthritis. A MSc thesis submitted to Baghdad College of Medicine and the committee of postgraduate studies in Clinical Pharmacology.
- 26 - Numman, I.T.; Hussain, S.A. and Al-Ani, T.A. (2006). Evaluation of the clinical use of silymarin in knee osteoarthritis: Application of dual inhibition concept of cyclo oxygenase and 5-lipoxygenase. A PhD thesis submitted to Baghdad College of Pharmacy and the committee of postgraduate studies in pharmacology.