Clarithromycin in rheumatoid arthritis patients not responsive to disease-modifying antirheumatic drugs: An open, uncontrolled pilot study

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Abstract

Objective

In 1996 we found by serendipity that 2 patients with rheumatoid arthritis (RA) who were taking clarithromycin (CM) to eradicate Helicobacter pylori experienced a regression of their RA symptoms. Following this observation, we tested the hypothesis that this reduction in symptoms could have been caused by CM administration.

Methods

We performed a 6-month, open, uncontrolled pilot study on 18 patients (14 females and 4 males, mean age 62 yrs.) with RA who had previously received DMARDs (mean 2.6) and discontinued the treatment at least one month earlier because lack of efficacy or severe side effects. Patients were treated with CM at the dose of 500 mg twice per day for the first 10 days, followed by a daily maintenance dose of 250 mg twice per day.

Results

4/18 patients did not complete the treatment, 2/18 were not responsive to the treatment and 2/18 discontinued the treatment. Following ACR criteria the improvement was: 10 patients ACR 20; 6 patients ACR 50; and 2 patients ACR 70. The remaining 4 patients did not reach ACR 20 since either the number of tender or swollen joints was not to the level required. Reductions in PGE2 and soluble phospholipase A2 plasma levels were closely related to CM plasma levels.

Conclusions

Our findings suggest that CM treatment can be beneficial in those patients who are not responsive to or cannot tolerate DMARDs. No definitive conclusions can be drawn based on the present study, due to the small sample size involved.

Key words

Clarithromycin, rheumatoid arthritis, macrolide, antibiotics.

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Received on September 17, 2001; accepted in revised form on February 14, 2002.

Introduction

The cause of rheumatoid arthritis (RA) is still unclear and the aim of therapy is not to eradicate the causative entity but to suppress the persistent inflammation in order to ameliorate the symptoms and reduce the articular damage. However, most of the drugs used in RA were not specifically developed for this purpose, their use being based on clinical evidence. A good example is provided by antimalarial drugs whose empirical use dates from the late 19th century, but only in the 1960s has its effect on RA been evaluated in clinical trials.

By serendipity we found that two patients who were taking CM to eradicate *Helicobacter pylori* (1) had a significant regression of symptoms (2).

Recently it has been raised the question whether antibiotics may play a role in the treatment of RA (3,4). Macrolide antibiotics have been shown to exhibit a broad spectrum of pharmacological effects apart from their antibacterial activity. In particular CM has been shown to modulate the human T lymphocyte response *in vitro* (5,6) and *in vivo* in humans (7) and to have an effect on cytokine production *in vitro* and *in vivo* in experimental models (8).

On the basis of the above findings we have designed a pilot open clinical study aimed to evaluate if CM therapy in RA could display beneficial effects in those patients who are not responders to DMARDs.

Patients and methods

Patients

This study (included patient selection) was conducted by the Rheumatology Unit of the Salvatore Maugeri Foundation in Castel Goffredo (Mantua, Italy). The protocol was approved by the Ethical Committee of the Salvatore Maugeri Foundation in Pavia. All patients were required to give their written informed consent. The eligibility criteria were as follows: age >18 years; RA fulfilling the ACR criteria; duration of the disease ≥ 2 years; ACR functional class 2nd or 3rd; corticosteroid dosage stabilised for at least 1 month prior to study entry (≤ 6 mg prednisolone or equivalent). All patients had previously received DMARDs (mean 2.6) and had discontinued the treatment at least since 1 month because lack of efficacy or severe side effects (Table I). DMARDs had been used also in different combinations.

Intra-articular corticosteroid injections were not allowed in the last month before enrolment in the study. Patients with active disease met at least 4 of the following 7 criteria: erythrosedimentation rate (ESR) ≥ 28 mm/hour, morning stiffness ≥ 45 minutes, ≥ 10 tender joints (mean 12.5), ≥ 1 swollen joint (mean 4.8); health assessment questionnaire (HAQ) score ≥ 1.25; visual analogic scale (VAS) score ≥ 3/10; physician's global assessment of disease ≥ 3/10. Women of childbearing age who were not practising contraception and patients in treatment with carbamazepine and antihistaminics were excluded. Also patients with bacterial diseases were excluded. In particular, serum tests for antibodies anti-*Borrelia burgdorferi* were negative and reactive arthritis were excluded by urine culture for *E. coli* and *Salmonella*, fecal culture for *Salmonella, Shigella, Yersinia* and *Campylobacter* and urethral swab for Chlamydia and Ureaplasma.

Study design

18 Caucasian patients were enrolled as expected: 14 females and 4 males with a mean age of 62 years and a mean disease duration of 12.5 years. 14/18 were sieropositive, and 15/18 had an erosive RA. They all were treated with CM (Macladin, Laboratori Guidotti, Italy) at the dose of 500 mg twice per day for the first 10 days, followed by a daily maintenance dose of 250 mg twice per day. The dose was selected on the basis of a previous evidence (2).

All patients were evaluated prior to the start of the study and after 10, 30, 60, 90 and 180 days of therapy. The following parameters were monitored: number of swollen joints; number of tender joints; left and right hand strength [measured using a sphygmomanometer and calculating the mean of 3 tests following Lee's method (9)]; the

patient's assessment of pain evaluated on a horizontal VAS scale of 10 cm; the physician's global assessment of disease activity evaluated on a horizontal VAS scale of 10 cm; the patient's global assessment of physical function using the HAQ; and the duration of morning stiffness (in minutes).

The steroid dose was stabilised 1 month before enrolling the patients and for the entire period of the study. Steroid treatment was: 1/18 no steroids; 2/18 deflazacort 6 mg; 1/18 deflazacort 7.5 mg; 3/18 methylprednisolone (MP) 2 mg; 10/18 MP 4 mg; and 1/18 MP 6 mg. No changes in the steroid dose and no intra-articular injections were allowed during the study period. NSAID therapy was also stabilised and changes in drugs were not allowed.

Methods

At the same time points blood was taken from patients (10 ml) and plasma prepared for centrifugation. Blood samples were divided into 3 aliquots. One was used for the measurement of ESR, C-reactive protein (CRP), hemochrome, platelet count, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, blood proteins electrophoresis, glycemia, serum rheumatoid factor, and creatinine. The other two samples were coded and kept frozen at -20°C. Samples coded were then analysed blindly at the end of the six months of treatment to determine: TNF , IL-10, IFN , prostaglandin E_2 (PGE_2), soluble type II phospholipase A_2 ($sPLA_2$) and nitric oxide (NO) plasma levels measured as nitrite + nitrate.

The major endpoint was to evaluate the effectiveness and toxicity of CM given for a 6-month period to patients with active rheumatoid arthritis not responsive to other DMARDs even if combined with low doses of steroids.

The measure of disease activity was evaluated following the ACR definition of improvement in rheumatoid arthritis (10,11): tender joints count; swollen joints count, patient's assessment of pain, patient's global assessment of disease activity, physician's global assessment of disease activity, HAQ score, ESR or CRP levels. Patient's global status, patient's pain and physician global assessment was scored using a visual analogic scale (VAS), with 0 representing normal and 10 representing severe problems. Additional evaluations enclosed the hand strength (as before explained).

Cytokine and prostaglandin E2 measurement. Cytokines levels e.g. TNF , IL-10, IFN (R&D ELISA kits, Milan, Italy) and PGE_2 (ELISA kit Cayman, France) were measured in plasma with ELISA kits according to manifacturer's instructions. The results are expressed as pg/ml and they represent the mean ± s.e.m. of assays were each plasma sample was tested in triplicate.

Human soluble phospholipase A_2 measurement. Soluble PLA_2 was measured as described previously (12). Briefly, plates were coated overnight at 4°C with monoclonal anti-human recombinant sPLA2 antibody BA11 (kindly provided by Dr. Browning, Biogen, USA) at 10 µg/ml. Samples (50 µl) were then added and bound human secretory PLA_2 was detected by incubation with rabbit polyclonal anti-$sPLA_2$ (kindly provided by Dr. Browning, Biogen, USA). Bound rabbit antibody was revealed by incubation with goat anti-rabbit IgG horseradish peroxidase (1: 2,000) and the plates were read at 450 nm.

Clarithromycin plasma levels. CM plasma levels were evaluated as previously described (13). Briefly, 200 µl of plasma were extracted from the appropriate tube after adding 200 µl of bicarbonate buffer containing internal standard erythromycin 1 ng/ml. The tubes were vortex-mixed briefly and allowed to stand at room temperature for 5 min. Diethyl ether / dichloromethane (70/30 v/v; 3 ml) was then added and the samples were vortex-mixed for 30s. The tubes were centrifuged at 2000 rpm for 10 min at 4°C. The upper organic layers were carefully removed and transferred using pasteur pipettes to siliconized test tubes. The solvent was removed by a gentle stream of nitrogen in a dry bath at 37°C and 200 µl of mobile phase were added to the tubes followed by vortex-mixing for 15 s to reconstitute the residue.

The plasma concentrations of clarithromycin were quantified by reversed phase liquid chromatography (HPLC model 1100 system from Hewelett-Packard; USA) coupled to tandem mass spectrometry (LC-MS-MS, Quattro II triple stage quadrupole mass spectrometer Micromass, Manchester, UK).

Toxicity monitoring: All patients were questioned about any adverse event at each follow-up visit. The treating physician could withdraw the patient from the study at any time because of side effects or ineffectiveness of the CM.

Table I. DMARDs previously used by patients enrolled (n = 18), side effects, the patient number that experienced a lack of efficacy. Controindication indicates patients that could not be eligible for that specific treatment.

DMARD	N/18	Side effects		Lack of efficacy	Contro-indication
Methotrexate	11/18	1/11	Liver function disturbances	7/11	3/18
		2/11	Vomit		
		1/11	Bronchiolitis		
Antimalarial	12/18	2/12	Cutaneous rash	10/12	—
Im gold salts	10/18	1/10	Severe dermatitis	8/10	—
		1/10	Proteinuria		
Azathioprine	5/18	——		5/5	3/18
Cyclosporine	2/18	——		2/2	—
Auranofin	3/18	——		3/3	—
Sulfasalazine	3/18	——		3/3	—
Penicillamine	1/18	1/1	General malaise	——	—
Leflunomide	1/18	1/1	Diarrhroea	——	—

Table II. Mean ± s.e.m of clinical and biochemical parameters. * P < 0.05; **P < 0.01 compared with basal values (time 0) before starting CM treatment. HAQ, Health Assessment Questionnaire, VAS, visual analogic scale; ESR erythrosedimentation rate; CRP, C reactive protein (n = 18).

Parameters	Basal	10 days	30 days	60 days	90 days	180 days
HAQ	1.43 ± 0.15	0.89 ± 0.14*	0.84 ± 0.16*	0.66 ± 0.14**	0.61 ± 0.16**	0.57 ± 0.14**
VAS	5.17 ± 0.51	2.89 ± 0.41*	2.50 ± 0.52**	2.75 ± 0.60**	2.67 ± 0.58**	2.22 ± 0.54**
Morning stiffness (min)	73.50 ± 20.0	56 ± 16	30 ± 18	22 ± 15*	9.2 ± 4.8*	18 ± 13**
Hand strength right hand (mmHg)	54.6 ± 8.8	64.2 ± 8.0	66 ± 7.7*	65 ± 8.2*	64 ± 8.7*	67 ± 9.1*
Hand strength left hand (mmHg)	49.4 ± 8.1	62.2 ± 9.2	65 ± 9.0**	63 ± 9.9**	61 ± 9.8*	65 ± 9.3*
ESR	41.0 ± 7.04	29.30 ± 4.94	29 ± 7.51	26.33 ± 5.03	27.2 ± 5.90	25.94 ± 4.9*
CRP	2.41 ± 0.50	1.14 ± 0.22	1.81 ± 0.64	1.32 ± 0.33	1.48 ± 0.39	1.44 ± 0.35*

Statistical analysis

Results concerning the effect on biochemical parameters were analysed by using ANOVA for multiple comparisons followed by Dunnett post-test. Clinical score and all the non-parametric measures were compared by using Kruskall-Wallis test followed by Dunn's post-test.

Results

Two out of the 18 patients recruited were not responsive and dropped out at the second and third month of the study. Two out of 18 discontinued the treatment due to either an acute erosive gastritis or hyperpyrexia probably not related to CM treatment. ESR and CRP data for day 30 was missing for one patient; no other protocol violation was observed. Corticosteroids and NSAID dosage remained stable during all 6 months of the trial. As can be seen in Table II there was a clear and significant improvement in the parameters VAS, HAQ and hand strength after 30 days. The medical judgement scored at the end of the treatment was not significantly different from that scored from the patients. All the parameters measured were significantly improved after 6 months of treatment. The treatment with CM did not cause any significant side effects on blood biochemical parameters. In particular, aspartate aminotransferase and alanine aminotransferase plasma levels were respectively 16.5 ± 1.51 and 16.07 ± 1.19 at the beginning of the study and 16.2 ± 1.20 (NS) and 18.5 ± 2.31 (NS) at the end of the study, showing that there was no liver toxicity. Rheumatoid factor was not modified by CM treatment; it was 124 ± 29 before starting the study and 117 ± 25 (n = 14; NS) after the six months of treatment. Side effects observed and/or reported from the patients were cephalea (n = 3), dyspepsia (n = 3), dysgeusia (n = 3) and glossitis (n = 2), hyperpyrexia (n = 1).

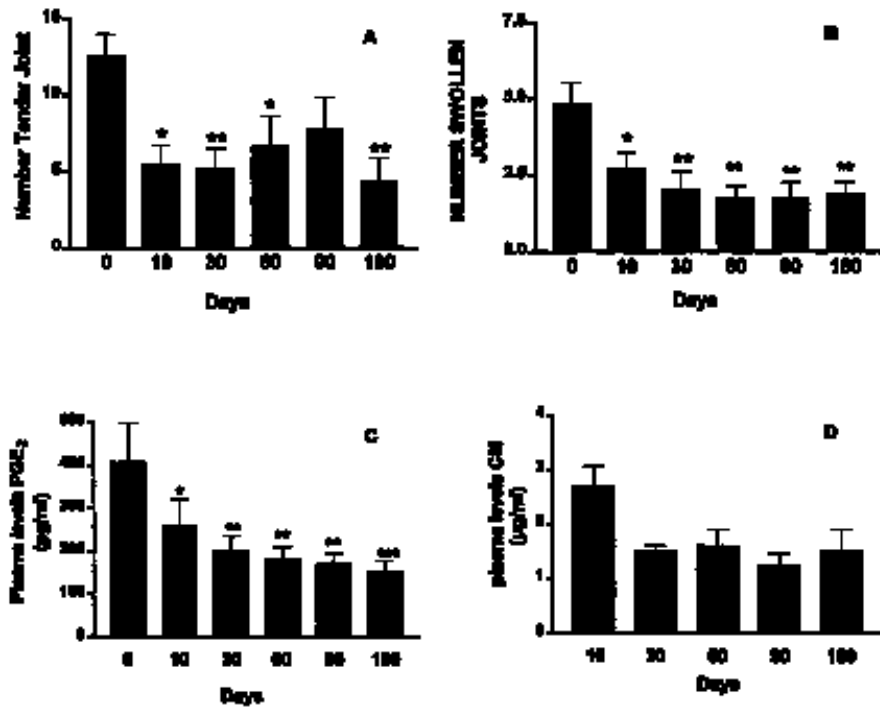

Fig. 1. Effect of CM treatment on tender joints (A) and swollen joints number (B).The reduction in tender and swollen joints paralleled the reduction in PGE2 plasma levels(C) and CM plasma level (D). Data are expressed as mean (s.e.m. *P < 0.05, ** P < 0.01; *** P < 0.001 (n=18).

As shown in Figure 1, the numbers of tender (Fig. 1A) and swollen joints (Fig. 1B) were significantly and strikingly reduced. This reduction in count well correlated with the reduction in PGE_2 levels (Fig. 1C) and CM plasma levels (Fig. 1D). On the basis of the parameters measured, the improvement observed using the ACR criteria was as follows: 10/18 patients showed 20% improvement, 6/18 patients showed 50%, and 2/18 patients showed an improvement of 70%. The remaining 4/18 patients did not reach ACR 20 either for the number of tender joints or for the number of swollen joints.

There were no detectable levels of TNF , IL-10 and IFN in all patients treated before and after the treatment with the exclusion of 2 patients. In these 2 patients TNF levels at the be-

ginning of the treatment were 70 pg/ml and 326 pg/ml and were reduced to 52 pg/ml and 66 pg/ml, respectively. The same patient who showed a reduction in TNF from 70 pg/ml to 52 pg/ml exhibited a parallel increment in IL-10 from 40 pg/ml to 144 pg/ml. Plasma levels of nitrite/nitrate measured as described above were not modified. Interestingly plasma levels of sPLA2 were also significantly reduced after 6 months from 8.63 ± 2.06 ng/ml to 3.7 ± 0.8 ng/ml ($n = 14$; $p < 0.05$).

Discussion

On the basis of an observation made by serendipity (2) we set up a study to evaluate if CM has a potential as treatment for patients unresponsive to DMARDs. For this reason we have designed an open uncontrolled pilot study where we have enrolled a small number of patients.

Here we show that 6-month treatment with CM causes a significant improvement of ACR parameters. In addition, we have also tried to address the possible mechanism through which CM could modulate the disease. Indeed, of the fourteen patients that completed the study 10 patients out of 18 treated (55%) achieved improvement based on ACR parameters ranging between ACR20 and ACR70. Moreover, at the end of the study, after 6 months, all the 14 patients that had completed the study decided to continue the treatment with CM by themselves without any support.

There are several mediators that have been shown to be involved in RA. Among these mediators we have selected IL-10, IFN , TNF , sPLA2, NO and PGE_2 since in the past they have been shown to be inhibited by treatment with CM *in vitro* or in experimental animal models *in vivo*. The patients presented at beginning of the study an elevated plasma level of PGE_2 that was significantly reduced by the treatment with CM. The reduction in PGE_2 levels well correlated with the reduction in swollen ($r^2 = 0.97$; $P = 0.0003$) and tender joints ($r^2 = 0.66$; $P = 0.048$). In addition the CM plasma levels were also strictly related to the reduction in PGE_2 ($r^2 = 0.82$; $P = 0.03$) and swollen joints ($r^2 = 0.89$; $P = 0.0154$) but not with tender joints.

The study on pro-inflammatory cytokines showed that none of the patients studied had detectable IFN plasma levels. Only two patients had elevated TNF plasma levels and CM treatment reduced TNF levels in both cases. IL-10 has been proposed to be anti-inflammatory in RA (14). In all patients studied except one, there were no detectable levels of IL-10. The patient who showed an increased IL-10 plasma levels (about 70%) was the same where TNF levels were reduced of about 30% by CM treatment. Also nitric oxide has been thought to be implicated in RA (15); however nitric oxide levels, measured as nitrite/nitrate, were not modified by CM treatment.

Extracellular $sPLA_2$ can be induced by cytokines (16, 17) can be differentially expressed in RA and osteoarthritis (17) and it is a key enzyme in PGE_2 production. It has been also shown to be elevated in plasma of RA and osteoarthritis patients (18, 19). Following treatment with CM after 6 months also $sPLA_2$ levels were significantly reduced by about 70%.

In conclusion, this study shows that CM treatment in patients not responsive to DMARDs causes a significant amelioration of the disease as evaluated with ACR parameters. Most likely this anti-inflammatory effect can be explained through a reduction of both PGE_2 and $sPLA_2$ plasma levels and it could be linked to the immunomodulatory effects of CM (5-8). The clinical improvement observed was significant for many of the parameters considered already after 10 days of treatment and after six months there was a clear clinical amelioration that well correlated to plasma levels of CM and was significantly related to a reduction in both $sPLA2_2$ and PGE_2 plasma levels. However, a study involving a larger sample of patients is needed in order to draw any definitive conclusions regarding the efficacy of clarithromycin treatment in rheumatoid arthritis.

Acknowledgements

Recruitment of patients and assessments and were performed by Gianantonio Saviola, Lul Abdi Ali, Paola Rossini and clinical analysis were performed by Lorella Campostrini. CM plasma levels were measured by Gilberto de Nucci. Measurement of all mediators were performed by Angela Ianaro, Mariarosaria Bucci and Giuseppe Cirino. Alessandro Coppini and Mario Gori co-ordinated the study and assisted with protocol development. Manuscript was prepared by Giuseppe Cirino with the help of Alessandro Coppini and Gianantonio Saviola.

References

1. MARKHAM A, MCTAVISH D: Clarithromycin and Omeprazole as *Helicobacter pylori* eradication therapy in patients *H. pylori*-associated gastric disorders. *Drugs* 1996; 51: 161-78.
2. SAVIOLA, G, GRIONI G: Possibile efficacia della claritromicina nella artrite reumatoide: relazione su due casi clinici. I*l Reumatologo* 1998; 19: 46-7.
3. VAN VLEM B, VANHOLDER R, DE PAEPE P, VOGELAERS D, RINGOIR S: Immunomodulating effects of antibiotics: Literature review. *Infection* 1996; 4: 275-91.
4. O'DELL JR: Is there a role for antibiotics in the treatment of patients with rheumatoid arthritis? *Drugs* 1999; 57: 279-82.
5. KHAN AA,SLIFER TR,ARAUJO FG, REMINGTON JS: Effect of clarithromycin and azithromycin on production of cytokines by human monocytes. *Int J Antimicrob Agents* 1999; 11:121-32.
6. MORIKAWA K, OSEKO F, MORIKAWA S, IWAMOTO K: Immunomodulatory effects of three macrolides, midecamycin acetate, josamycin and clarithromycin on human T-lymphocyte function *in vitro*. *Antimicrob Agents Chemother* 1994; 38: 2643-7.
7. SCAGLIONE F, FERRARA F, DUGNANI S, DEMARTINI G, TRISCARI F, FRASCHINI F: Immunostimulation by clarythromycin in healthy volunteers and chronic bronchitis patients. *J Chemother* 1993; 5: 228-32.
8. IANARO A,IALENTI A,MAFFIA P *et al.*: Anti-inflammatory activity of macrolide antibiotics. *J Pharmacol Exp Ther* 2000; 292: 156-63.
9. LEE P, BAXTER A, DICK WC,WEBB J: An assessment of grip strength measurement in rheumatoid arthritis. *Scand J Rheumatol* 1974; 3:17-23.
10. FELSON DT, ANDERSON JJ, BOERS M, *et al.*: American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* 1995, 6: 727-35.
11. PREVOO ML, VAN'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LB, VAN RIEL PL: Modified disease activity scores that include twenty-eight joint counts.Development and validation in a prospective longitudinal study of patients with rheumatoid

arthritis. *Arthritis Rheum* 1995; 1: 44-8.

12. CIRINO G, MISASI M, LOFFREDO A, *et al.*: The synovial-like membrane at the bone interface in loose total hip replacements contains high levels of extracellular group II phospholipase A_2. *Life Sci* 1996; 59: PL81-6.
13. LERNER FE, CALIENDO G, SANTAGADA V, SANTANA GSM, MORAES MEA,DE NUCCI G: Clarithormycin bioequivalence study of two oral formulations in healthy human volunteers. I*nt J Clin Pharmacol Ther* 2000; 38: 345-54.
14. ISOMAKI P, LUUKKAINEN R, SAARIO R, TOIVANEN P, PUNNONEN J: Interleukin-10 functions as an antiinflammatory cytokine in rheumatoid synovium. *Arthritis Rheum* 1996, 3: 386-95.
15. MCINNES IB, LEUNG BP, FIELD M, *et al.*: Production of nitric oxide in the synovial membrane of rheumatoid and osteoarthritis patients. *J Exp Med* 1996; 184: 1519-24.
16. BIDGOOD MJ, JAMAL OS, CUNNINGHAM AM, BROOKS PM, SCOTT KF: Type IIA secretory phospholipase A2 up-regulates cyclooxygenase-2 and amplifies cytokine-mediated prostaglandin production in human rheumatoid synoviocytes. *J Immunol* 2000; 165: 2790-7.
17. MASSAAD C, PARADON M, JACQUES C, *et al.*: Induction of secreted type IIA phospholipase A2 gene transcription by interleukin-1beta. Role of C/EBP factors. *J Biol Chem* 2000; 275: 22686-94.
18. JUSTEN HP, GRUNEWALDE, TOTZKE G, *et al.*: Differential gene expression in synovium of rheumatoid arthritis and osteoarthritis. *Mol Cell Biol Res Commun* 2000; 3: 165-72.
19. PRUZANSKI W, VADAS P, STEFANSKI E, UROWITZ M: Phospholipase A2 activity in sera and synovial fluids in rheumatoid arthritis and osteoarthritis. *J Rheumatol* 1985; 12: 211-6.
20. KAISER E. Phospholipase A_2: Its usefulness in laboratory diagnostics. *Crit Rev Clin Lab Sci* 1999, 36: 65-163.