

Research Article



THE EFFECT OF GLUCOSAMINE AND CHONDROITIN ALONE OR IN COMBINATION IN TREATMENT OF PEFLOXACINE INDUCED CHONDROTOXICITY IN JUVENILE WISTAR RATS

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Accepted on: 18-02-2011; Finalized on: 28-04-2011.

ABSTRACT

This study attempted to prove our hypothesis that toxicity of pefloxacin can be treated with long term therapy of glucosamine, chondroitin or the combination of these two drugs on wistar rats. Rats were divided in seven groups (eight animals in each group). The rats in the first group were served as normal and received water and food only. The rats in the rest groups received pefloxacin (15 mg/kg body wt) for one month then we divided them as follows, the second group (the control toxic group) was left without treatment. The rats in the third group received pefloxacin for one month then treated with glucosamine (250mg/kg body wt) for three months. The rats in the fourth group received pefloxacin for one month then treated with chondroitin (150mg/kg body wt) for three months. The rats in the fifth group received pefloxacin for one month then treated with glucosamine and chondroitin (125+75mg/kg body wt respectively) for three months. The rats in the sixth group received pefloxacin for one month then treated with magnesium ions (84mg/kg body wt) for three months. The rats in the seventh group received pefloxacin for one month then treated with vitamin E (75mg/kg body wt) for three months. The value of maximum extension of right knee was assayed, the knee joint tissue sections were examined histopathologically, and serum lipid peroxidation (malondialdehyde) was measured by fluorimetric technique. The group treated with Pefloxacin showed more limited knee extension values and high serum levels of malondialdehyde and more degenerative changes in the cartilage. There were significant improvement in the knee extension values and low serum levels of malondialdehyde and less degenerative changes in the cartilage in the groups of glucosamine then chondroitin, the combination group wasn't effective in the treatment. Serum levels of malondialdehyde in the groups of Vitamin E were less than the rest groups in reducing oxidative stress induced by pefloxacin in immature cartilage.

Keywords: Chondrotoxicity, articular cartilage, glucosamine, chondroitin, vitamin E.

INTRODUCTION

Osteoarthritis is a chronic non-inflammatory disease which considered the most common joint disease in the world¹ especially in the aged population but it may affect the kids as a secondary type after treating with some groups of drugs such as quinolones which induce cartilage lesions², this lesions may develop to arthritis with the same symptoms and pathogens to arthritis in elderly³. Quinolones are antibacterial drugs are used against a broad spectrum of bacteria⁴, and they are good tolerance with some limited side effects but the most important side effect in kids is that they may irreversibly damage immature cartilage and cause arthritis⁵. The chondrocyte is the first position which is attacked by quinolone which interact with the component of the extracellular matrix⁶ and damage it according to several theories: Chelating magnesium ions:⁷ so diminished Mg concentration in the extra cellular matrix (EXM) leads to change in the function of B1-integrins (receptors coexist on the surface of the cell)⁴, increases the synthesis of fibronectin fragments which induce MMP (matrix metalloproteinase) enzymes⁶, caspase 8, and as a result inducing apoptosis.⁸ Or quinolone may directly attack Bax (Bcl2 Associated X protein) pathway and this leads to release of cytochrome C to the plasma, cytochrome C combined with CED4 (member of Apaf family) and this induces caspase 9 then apoptosis⁹. Most studies suggest

too that the oxidative metabolism of quinolone forms free radical, and some studies suggests that quinolone works on suppressing topoisomerase of actual nucleus.¹⁵ Finally quinolones may affect directly mitochondria by causing diminishing of mitochondria membrane potential.⁶ From these theories and mechanisms of damaging cartilage we searched for the opposing mechanism for treating these lesions so we suggested for this purpose glucosamine and chondroitin. Glucosamine (GA) is a natural substance participate in proteoglycan manufacturing so it protects the cartilage¹¹ especially in osteoarthritis where the microscopic shows that patients treated with GA have healthy cartilage in comparing with placebo.¹² GA has anti-inflammatory^{1,14} and anti oxidant effects.^{15,16} Chondroitin (CS) is found normally in cartilage, it can improve the motion of osteoarthritis patients and suppresses the progress of the disease and diminishes cartilage erosion¹⁷. CS protects cartilage by enriching it with the normal substances which cartilage needs for repairing. CS suppresses catabolic enzymes¹⁸ and increases the concentration of hyaluronan in cartilage which increases concentration of the fluid in ECM.¹⁹ CS has anti-inflammatory effect too²⁰ by suppression the production of IL-1b, and has anti-oxidative effect.²² Hence, an attempt was made in the present study to elucidate the influence of GA, CS opposite and treat the toxicity effect on cartilage induced by pefloxacin.



MATERIALS AND METHODS

Animals: Healthy male juvenile Wistar rats of same age group (24 ± 3 Days), obtained from Leen institute for experimental animals and animal Facility, Faculty of Pharmacy, Damascus, Syria, were selected for the present study. Animals were housed in an air conditioned animal house facility at $25 \pm 1^\circ\text{C}$, with $50\% \pm 5\%$ relative humidity under a controlled 12 h light/dark cycle. Food and tap water were provided.

Test chemicals: Pefloxacin was purchased from Oubari drug manufactory, Aleppo, Syria and was dissolved in water for oral dosing by incubation tube. Since the first day of the study and for one month with dosage 15mg/kg/day. Glucosamine (GA) and Chondroitin (CS) were purchased from Alfares drug manufactory, Damascus, Syria and was dissolved in water for oral dosing from the day 30 of the study until the fourth month with dosage 250mg/kg/day for GA and 150mg/kg/day for CS. Magnesium ions were purchased from Bahri drug manufactory, Damascus, Syria and were dissolved in water for oral dosing from the day 30 of the study until the fourth month with dosage 84mg/kg/day. Vitamin E was purchased from Asia drug manufactory, Damascus, Syria, and was diluted with olive oil for oral dosing from the day 30 of the study until the fourth month with dosage 75mg/kg/day.

Experimental Design: The rats were divided into 7 groups consisting of 8 rats in each group: the first group (the normal group non treated but was given food and water), the rest groups were given pefloxacin 15mg/kg/day for one month as follows: The second group was left without treatment (and used as toxic control group), the rest groups were treated for three months by giving there drugs orally as the following: The third group was given GA with a dose 250mg/kg/day. The fourth group was given CS with a dose 150mg/kg/day. The fifth group was given GA+CS with a dose 150 mg GA+75mg CS. The sixth group was given Mg^{+2} ions with a dose 84mg/kg/day. And the seventh group was given vitamin E with a dose 75 mg/kg. Drugs were given orally to rats for four months. At the end of the treatment period, the animals (19-weeks-old) were anesthetized with sodium thiobarbital (thiopental) after overnight fasting and killed for measuring Maximum extension angle and doing histological study. Blood was collected via heart puncture into dried tubes and centrifuged, liquating and freezing the plasma at -80°C for subsequent determinations (Determination of serum oxidative stress). All rats were weighed weekly at fixed times throughout the experiments.

Determination of serum oxidative stress: Malondialdehyde (MDA) levels basal and induced were measured by a modified fluorimetric assay. The fluorescence was read at 586 nm according to the direction of oxford kit (USA).

Maximum extension angle: Then we scarified the animals, right knees of all animals were dissected from

the coxofemoral region to the ankle region, leaving the articular capsule intact. After the dissection, the maximum extension angle of each knee was measured with 0 degrees corresponding to the maximum possible extension. In order to minimize any possible bias, all operations and measurements of the extension level were performed by the same surgeon.

Histological study: Immediately after killing, the specimens were fixed in 10% formalin and submitted to histological study. All knees were kept in this solution for 1 day and then demineralized in 7.5% nitric acid for 2 to 3 days. After embedding the tissue in paraffin blocks, Sections at 5 microns were cut with a microtome (Leica RM 2155, Germany) tibial sections were prepared and stained with hematoxylin-eosin and Alcian-blue. The articular cartilage injuries were evaluated and recorded using the Mankin score (table 1). In this system, the higher the score, the higher the level of OA. The entire histological evaluation was performed by a histopathologist who was blind to group distribution when this analysis was made.

Table 1: The Mankin score

Variable	Score
Surface	0=normal 1=irregular 2=fibrillation 3=blisters and erosion
Hypocellularity	0=normal 1=small decrease in chondrocytes 2=large decrease in chondrocytes 3=no cells
Clones	0=normal 1=occasional duos 2=duos or trios 3=multiple nested cells

Statistical Analysis: Data are presented as means \pm SD. The significance of the differences between groups for maximum extension angle, serum MDA levels were estimated by ANOVA test and Turkey's Multiple Comparison test, and for histological study by Kruskal-Wallis test and Multiple Comparison Dunn test. SPSS version 15 for Windows (SPSS, Michigan, IL) was used for the data analyses. $P < 0.05$ was considered statistically significant.

RESULTS

The maximum extension angle: We found that the extension angle of normal group (27.875 ± 5.63 degrees) was statistically different ($p < 0.001$) from the control group (63.5 ± 6.48 degrees) which was given pefloxacin. Whereas there was no significant differences ($p < 0.05$) between the three groups of GA (22.875 ± 2.416 degrees), CS (28 ± 9.03 degrees), GA+CS (31.625 ± 9.53 degrees) and the normal group with advantage to GA group (figure 1).



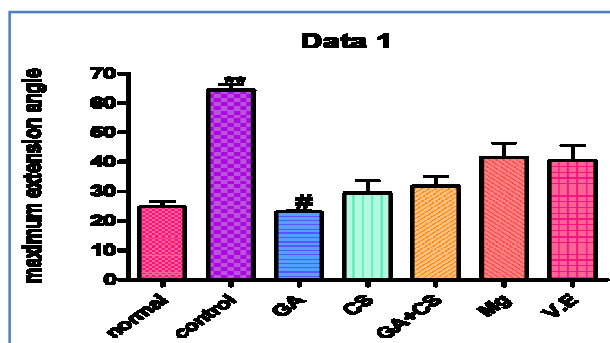


Figure 1: maximum extension angle values at the end of the experiment data are expressed as means \pm S.D (n=8). Statistical significance was analyzed using ANOVAs test (**p<0.01), (#p<0.05).

The histological analysis: using the Mankin score showed high levels of degenerative changes in the control group (6 ± 1.13) in comparison to the normal group (0.625 ± 1.309) ($p<0.001$). The histological scores of the groups receiving GA (1.25 ± 1.388) were not significantly different ($p<0.05$) from the normal group and very different from the control group with a statistically different ($p<0.01$) from the other two groups CS (2.125 ± 1.64), GA+CS (2.625 ± 1.06), Mg (2.75 ± 1.98), vit.E (3.125 ± 1.88) ($p<0.05$) (figure2).

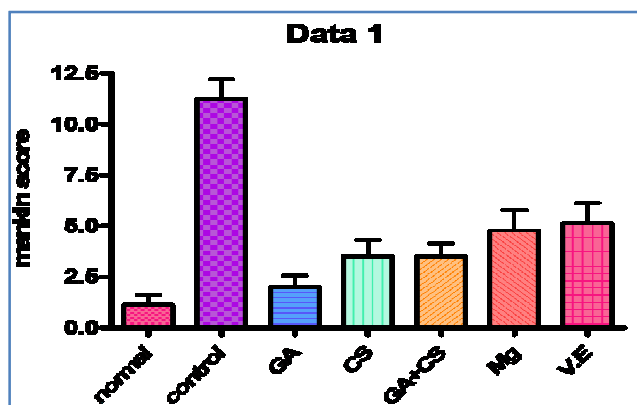


Figure 2: histological changes at the end of the experiment data are expressed as means \pm S.D (n=8). Statistical significance was analysed using Kruskal-wallis statistic test (**p<0.01), (#p<0.05).

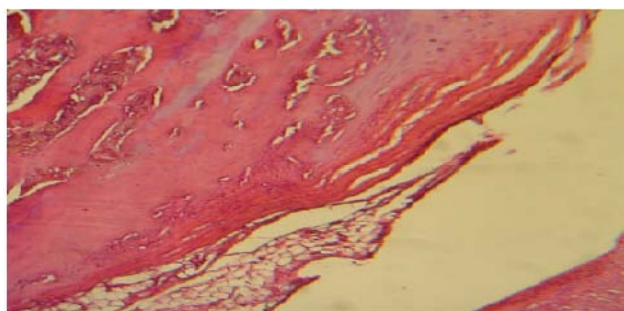


Figure 3: histological photograph of degenerative cartilage from the control group

The values of MDA: We found that the values of MDA in the normal group (0.152 ± 0.0179) was statistically different ($p<0.001$) from the control group (0.583 ± 0.022) which was given pefloxacin, and there was statistically different ($p<0.01$) between the group of pef and the

group of vit E (0.295 ± 0.049). And there Whereas there was no significant differences ($p<0.05$) between the three groups of GA (0.386 ± 0.059), CS (0.449 ± 0.012), GA+CS (and the control group (figure 4).

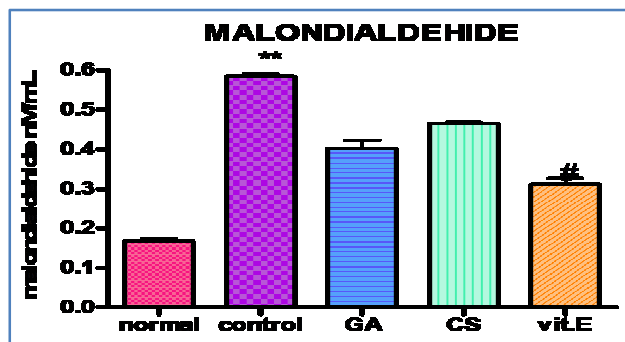


Figure 4: malondialdehyde (indicator of lipid peroxidation) values at the end of the experiment data are expressed as means \pm S.D (n=8). Statistical significance was analyzed using ANOVAs test (**p<0.01), (#p<0.05).

DISCUSSION

In our study we approved the toxic effect of pefloxacin on joint cartilage in juvenile wistar rats, from the results of maximum extension angles which reflect the stiffness degree of joint, In pef group there was significant differences from the normal group, serum MDA levels were higher in significant degree in comparing with the normal group which sign the important effect of the oxidative stress in damaging juvenile cartilage, and histological values reflect the destructive effect of pef on immature cartilage, in this section of study we were in agreement with (Qianqian et al 2009, sheng et al 2007, Simonin et al 2000) and this damage has been demonstrated with multiple species, pefloxacin accumulate in bone or cartilage tissue, revealing significantly higher concentrations in these samples than in plasma. Then it can damage cartilage by many possible mechanisms involves: Impaired function of B1 integrins by chelating magnesium ions⁷, it is well-known that magnesium is an essential mineral that is needed for numerous physiological functions. Changes in magnesium homeostasis concern mainly the extracellular space, impaired function of B1 integrins as a result of magnesium ions deficiency is considered as an initial event resulting in disturbed signal transduction between the extracellular matrix and chondrocytes. Additionally, B1 integrins activate the mitogen-activated protein kinase (MAPK) signal transduction pathway, so Inhibition of the MAPK signal transduction pathway in chondrocytes causes apoptosis⁸, in previous studied it was shown that in rats a magnesium-deficient diet can induce joint cartilage lesions that are identical to quinolone-induced cartilage lesions²⁵. Thus, it is of special interest whether, in reverse, supplementation with magnesium may diminish the chondrotoxic effect of quinolones in vivo, In our study provides data indicating that supplementation with magnesium relevantly diminish joint cartilage lesions induced by quinolones in immature rats, but it can't return to the normal group by comparing its maximum

extension angles and histological values, so magnesium deficiency cannot be the single cause of cartilage lesion. Most studies suggest that the oxidative metabolism of quinolone forms free radicals in the chondrocyte, these radicals attack the lipids and generates more free radicals⁴, which may reach the level that can damage DNA⁴ this damage activate P53 which in turn activate Bax¹⁰. So in our study we added a group of immature rats was received vitamin E which inhibits lipid peroxidation and protects chondrocytes from oxidative stress, and our results indicating that supplementation with vit E relevantly diminish joint cartilage lesions induced by quinolones in immature rats, but (as Mg group) it can't return to the normal group by comparing its maximum extension angles and histological values, although its serum MDA values was very close to the normal group, and this result reflect the ability of vit E in reducing oxidative stress which wasn't the only reason for damaging cartilage. In our study we were the only group who studied the effect of GA, CS, GA+CS, in treating the toxic effect of pef on immature cartilage, for GA, CS there were significant improvement in maximum extension angle and histological values in comparing with the control group and this leads to think of the therapeutic effect of GA, CS and this effect may return to their anti-inflammatory effect of GA^{1,14} and CS²⁰. And for their anti-oxidant effects for GA, CS we found that these two drugs could protect the cartilage from free radicals by chelating Fe ions removing it from the materials susceptible to oxidation like proteins, lipids^{15,22}. In this study we approved the anti-oxidative effect of GA, CS by measuring serum MDA values and the results showed that GA or CS could reduce the values of MDA in comparing with the control group, but they were less effective than vitamin E in reducing oxidative stress. In this field some studies suggest that GA, CS can protect DNA from oxidation by suppressing the emigration of P65, P50 to the nucleus¹⁶. On the other hand GA+CS were less effective than the two drugs alone in reducing the values of maximum extension angle, also in reducing serum MDA values, and finally in improving histological state of cartilage and this sign that GA, CS, were more effective in treating the toxic effect of pef than the combination group and that may be due according to the study of Jacksony et al 2010 which showed decreased absorption of GA when given concurrently with CS which could effectively lower the G blood levels obtained.²⁴ There are also several theories in the ability of pef in damaging immature cartilage like Quinolone works on suppressing and showering topoisomerase of actual nucleus.¹⁵ and it can induce the production of TNF- α ¹⁰ these theories need more researching and these researches must include the ability of GA, CS in reversion these theories for treating immature cartilage also we are looking for application these results in clinical studies on children who have arthritis to make evaluating treatment for these two drugs.

CONCLUSION

We conclude that GA and CS are effective drugs in treatment of damaged immature cartilage in juvenile rats induced by pefloxacin and GA was better than CS, and the combination was less effective.

REFERENCES

1. Hootman JM, Helmick CG, Projections of U.S. prevalence of arthritis and associated activity limitations, *Arthritis Rheum* 54, 2006, 2226-229.
2. Blendele AM. Animal models of osteoarthritis, *J Musculoskelet Neuronal Interact.* 1(4): 2001, 363-76.
3. Underwood J.C.E. General and Systemic pathology, fourth edition, Churchill Livingstone, 2004, p725-726.
4. Qianqian Li, Shuangqing Peng, Zhiguo Sheng and Yimei Wang. Ofloxacin induces oxidative damage to joint chondrocytes of Juvenile rabbits: Excessive production of reactive oxygen species lipid peroxidation and DNA Damage, Evaluation and research center of Toxicology, 2009.
5. Bertram G, Katzung. Basic and Clinical Pharmacology, MC Graw Hill, tenth edition, 2007, p500.
6. Zhiguo Sheng, Shuangqing Perg, Changyong Wang, Qianqian Li, Yimei Wang, Mifeng Liu, Hong-bo-Li, Ravindra K. Hajela, Yan-Sheng Dong and Gang Han. Apoptosis in Microencapsulated Juvenile Rabbit chondrocyte induced by Ofloxacin, Role played by B1 integrin receptors, The journal of pharmacology and experimented therapeutics, vol 322, no 1, 2007, p155-165.
7. Michiyuki Kato. Chondrotoxicity of Quinolone Antimicrobial Agents. *J Toxicol Pathol* 21, 2008, 123-131.
8. Judith Sendzik, Hartmut Lode and Ralf Stahlmann. Quinolone-induced Arthropathy: an update focusing on new mechanistic and Clinical Data, *International Journal of Antimicrobial Agents* volum 33, issue 3, 2009, p194-200.
9. Herold C, Ocker M, Ganslmayer M, Gerauer H, Hahn EG, Schappan D, Ciprofloxacin induces Apoptosis and Inhibits Proliferation of Human colorectal carcinoma Cells, *Br J Cancer* 1; 86(3): 2002, 443-448.
10. Zhiguo Sheng, Xiaojuan Cao, Shuangqing Perg, Changyong Wang, Qianqian Li, Yimei Wang, and Mifeng Liu. Ofloxacin induces Apoptosis in Microencapsulated Juvenil Rabbit Chondrocyte by Caspase8 dependent Mitochondrial Pathway, *Toxicology and Applied Pharmacology*, volume 226, issue 2, 2008, p119-127.
11. Kimble Young, Kradjan, Guglielmo Alldredge. Corelli. *Applied Therapeutics the Clinical Use of Drugs*, eighth edition, Lippincott Williams and Wilkins, 2005, P 35-43.
12. Marc Cohen, Lesley Braun. *Herbs, Natural Supplements*, Elsevier Australia, 2005, p22-123, p214-216.
13. Hua J, Sakamoto K, Nagaoka I. Inhibitory actions of glucosamine a therapeutic agent for osteoarthritis, on the functions of neutrophils. *J Leukoc Biol*, 71(4), 2002, 632-640.
14. Nakamura H, Shibakawa A, Tanaka M, Kato T, Nishioka K, Effects of glucosamine hydrochloride on the production of



- prostaglandin E2, nitric oxide, and metalloprotease by chondrocyte and synoviocytes in osteoarthritis. Clin Exp Rheumatol 22(3), 2004, 293-299.
15. Yang Yan, Lin Wan-shan, Han Bao-Qin and Sun Hai-Zhou. Antioxidative properties of a newly synthesized 2-glucosamine-thiazolidine-n^o-carboxylic acid (Glc NH₂ Cys) in mice, Nutriton Research, volume 26, issue 7, 2006, p369-377.
 16. Francois Rannou, Mathias Francois, Marie-Theres Corvol, and Francis Berenbaum, Cartilage breakdown in Rheumatoid Arthritis, Jone Bone Spine, volume 73, issue 1, 2006, p29-36.
 17. Dechant JE, Baxter.G.M, Ferisbie. D.D, Trotter .G.W, McIlwraith.C.W, Effects of Glucosamine hydrochloride and chondroitin sulphate, alone and in combination, on normal and interleukin-1, conditioned equine articular cartilage explants metabolism, Equine vet J 37(3), 2005, 227-231.
 18. Raoudi M et al, Effect of chondroitin sulphate on hyaluronan synthesis and expression of up d-glucose dehydrogenase and hyaluronan Synthesis in Synoviocytes and articular chondrocytes osteoarthritis cartilage 13 (suppl 1), 2005, 79-80.
 19. Sasada Tadashi, Isouo Tomhiro, Morita Masafumi, Mabuchikiyoshi , Role of chondroitin sulphate on mechanical behavior of articular cartilage, Rep Chiba, Inst Technol (52), 2005, 91-97.
 20. Campo G.M, Arenoso A, Campo S, D' Ascola A, Traina p and Calatroni A, Chondroitin 4 sulphate inhibits NFkB Translocation and Capsase Activation in Collagen induced Arthritis in mice, Osteoarthritis and Cartilage, volume 16, issue, 2008, p1474-1483.
 21. Tovu M, Dumais B. S and P du Souich M. Anti inflammatory activity of CS, Osteoarthritis and Cartilage, 16, 2008, S14-S18.
 22. Ha B J, In Oxidative stress ovariectomy menopause and role of chondroitin sulphate, Arch pharm Res 27(8), 2004, 867-872.
 23. Moti L Tiku, Haritha Narla, Mohit Jain and Pravean Yalamanchili; Glucosamine prevents in vitro collagen degradation in chondrocytes by inhibiting advanced lipoxidation reaction and protein oxidation, Arthritis research, Therapy, 2007, 9:R76(doi:10.1186/ar 2274).
 24. Jacksony C G, A. H. Plaasz, J. D. Sandyz, C. Huax, S. Kim-Rolandsx, J. G. Barnhillk ,C. L. Harrisk and D. O. Cleggy. The human pharmacokinetics of oral ingestion of glucosamine and chondroitin sulfate taken separately or in combination, Osteoarthritis and Cartilage 18, 2010, 297e302.
 25. Stahlmann Ralf, Rstar Christian, Shakibaei Mehdi, Vormann Rgen, Nther Theodor, Merker Hans. Magnesium Deficiency Induces Joint Cartilage Lesions in Juvenile Rats Which Are Identical to Quinolone-Induced Arthropathy, ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Vol. 39, No.9, 1995, p. 2013–2018.

