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

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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 norm, 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 value of maximum extension of right knee was assayed, the knee joint tissue sections were examined histopathologically, and serum lipid peroxidation (malondialdehide) was measured by flourimetric technique. The group treated with Pefloxacin showed more limited knee extension values and high serum levels of malondialdehide and more degenerative changes in the cartilage. There were significant improvement in the knee extension values and low serum levels of malondialdehide 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 malondialdehidein the groups of Vitamin E were less than the rest groups in reducing oxidative stress induced by pefloxacin in immature cartilage.

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

INTRODUCTION

Osteoarthritis is a chronic non-inflammatory disease which considered the most common joint disease in the world 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 lesions, this lesions may develop to arthritis in elderly. Quinolones are antibacterial drugs are used against a broad spectrum of bacteria, 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 arthritis. The chondrocyte is the first position which is attacked by quinolone which interact with the component of the extracellular matrix and damage it according to several theories: Chelating magnesium ions 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), increases the synthesis of fibronectin fragments which induce MMP (matrix metalloproteinase) enzymes, caspase 8, and as a result inducing apoptosis. 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 apoptosis. 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. Finally quinolones may affect directly mitochondria by causing diminishing of mitochondria membrane potential. 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 cartilage, especially in osteoarthritis where the microscopic shows that patients treated with GA have healthy cartilage in comparing with placebo, GA has anti-inflammatory and anti oxidant effects. 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 erosion. CS protects cartilage by enriching it with the normal substances which cartilage needs for repairing. CS suppresses catabolic enzymes and increases the concentration of hyaluraron in cartilage which increases concentration of the fluid in ECM. CS has anti-inflammatory effect too by suppression the production of IL-1b, and has anti-oxidative effect. 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.
Animals: Healthy male juvenile Wistar rats of same age group (24±3Days), obtained from Leein 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±1°C, with 50%±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 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 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.

Statistical Analysis: Data are presented as mean± 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 ±6.48degrees) 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).
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<0.001) (figure 2).

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).

In our study we approved the toxic effect of pefloxacine 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 radicals\(^4\), which may reach the level that can damage DNA\(^4\) this damage activate P53 which in turn activate Bax\(^2\). 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\(^1\), CS\(^1\) and CS\(^1\). And for their antioxidant 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\(^15\),\(^2\). 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 nucleus\(^12\). 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.\(^2\) 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-\(\alpha\)\(^1\) 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 pefloxacine and GA was better than CS, and the combination was less effective.

REFERENCES


