Detection of oxidative stress induced by ciprofloxacin in imatuer rat

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ABSTRACT

Fluoroquinolones are a group of antibiotics broadly used because of their wide spectrum activity against both Gram-negative and Gram positive bacteria. It is well tolerated in patients but their uses have been associated with numerous adverse effects, including adverse central nervous system (CNS) effects and juvenile joint toxicity due to oxidative stress.

This study designed to detect the oxidative stress caused by administration of ciprofloxacin alone and in concert with glutathione. Ciprofloxacin (400 mg/kg body weight) administered to rats alone or with glutathione (100 mg/kg) body weight for7 days injection via intra peritoneal route to detect effects on glutathione peroxidase and glutathione reductase as oxidative stress enzymes markers. The data obtained demonstrated presence of the two enzymes which supplies further evidence that fluoroquinolones at therapeutic doses promote oxidative stress and the crucial role of glutathione in ptotection against ciprofloxacin side effects on bone.

***Keywords**: Ciprofloxacin, Oxidative stress, side effects, antioxidants.*

1-INTRODUCTION

Ciprofloxacin is a second generation fluoroquinolone with a broad spectrum of antibacterial activity. It has a respectable bioavailability after oral administration, moral to excellent tissue penetration and comparatively safe **(Papich, 1998)**. It is very active against wide type of pathogenic bacteria including some gram-positive and most grain-negative organism **(Hooper and Wolfson, 1985)**.It is used in a diversity of human clinical infections **(Sub and Lorber, 1995)**.Ciprofloxacin exerts its action by stopping bacterial DNA synthesis through inhibition of bacterial topoisomerase ii (DNA gyrase) plus topoisomerase iv**.(Hooper, 2000)**.

Ciprofloxacin is generally well tolerated and persistsone of the safest of all antibiotics with remarkably limited reactions **(Ball, 1986)**. These reactions involve central nervous system, gastrointestinal tract and hematological system **(Petri, 2001)**.

Regardless of its safe outline there are reported cases of Ciprofloxacin induced Tendinopathy, chrondrotoxicity and tendon rupture in human and animals **(Channaet al., 2008)**. It was reported that

Ciprofloxacin less ended thickness of the articular cartilage of the femoral condyle, inhibit proliferation of cultivated chondrocytes and secretion of soluble proteoglycans in a concentration and time dependant mode **(Li et al., 2004).** Ciprofloxacin induced damage of the articular and epiphysical growth plate cartilage of knee joint, tendon rupture and tendinopathy **(Halawa, 2010; Kim, 2010)**.

Previous studies attached cartilage damage in growing animal to oxidative stress, lipid peroxidation and DNA oxidative damage of collagen and the chondrocytes **(Simoninet al., 1999; Li et al., 2004).**Other authors' referred to Ciprofloxacin induced tendinopathy to their inhibitory effects on DNA, proteoglycan and collagen synthesis**(Maslankaet al., 2004).** As of July 2008, the United State Food and Drug administration assigned that Ciprofloxacin product should have a black-box warning indicating an increased threat in adverse events incorporating tendon rupture **(Kim,2010).**

Antioxidant systems block the uncontrolled formation of free radicals, and inhibit ROS and its reaction with biological structures. Increases in ROS, such as those that may arise during periods of oxidative stress, can be reduced by regulatory molecules of the cell redox state, which initiatea homeostatic response to prevent cell injury. Antioxidant molecules, for instance reduced glutathione, act against numerous oxidant compounds, such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH_) ,superoxide anion (O2 _) and reactive species of carbon. Glutathione can additionally be oxidized spontaneously in the presence of ROS and consequently neutralize them by its antioxidant ability. Furthermore, glutathione protects cells from the effects of the free radicals generated throughout metabolism **(Manfredini et al., 2005; Cexiong et al., 2009).**

2-MATERIALS AND METHODS

Experimental design

Thirty two weeks old male Swiss Albino rat weighing 75-85 grams each was used in this investigation. Rat were maintained in an animal facility under standard laboratory conditions for 2 weeks prior to experiments. The rat were housed at 23-25 C and in daily dark/light cycle. Rat were caged (8 per cage) in fully ventilated cages and were provided with water and standard chow ad libitum. All experiments were carried out in accordance with protocols approved by the local experimental animal's ethics committee. rats were divided into four groups (8rat/group):

Group1: the Control group injected with distilled water.
Group2: ciprofloxacin group(cipro) injected with 400 mg\kg ciprofloxacin.
Group 3: glutathione group (G) injected with 100 mg\kg *glutathione*.
Group 4: ciprofloxacin +glutathione group(G+cipro) injected with 400 mg\kg ciprofloxacin and 100 mg\kg ***glutathione*** all doses were given via the intra-peritoneal route for 7 days.All rats were scarified 24 hours from the last injection and bone tissues were prepared for PCR studies.

PCR expermint

1- RNA was isolated from tissue and the purity was determined by measuring its absorbance at 260 nm(A260)it should be greater than 0.15to ensure significance .(Wang et al.,2000)
2- For formation of cDNA, samples were prepared in a final volume of 20ul containing RT buffer, 5.5 mM MgCl2, 500 mM each dNTP, 2.5 mM random hexamers, 0.4 U/mL RNase inhibitor, 1.25ul MultiScribe reverse transcriptase (PE Applied Biosystems), and 10ul of total RNA.
3- Then the samples were incubated at 25°C for 10 minutes and at 48°C for 30 minutes. Heating to 95°C for 5 minutes inactivated the reverse transcriptase on 2720 thermal cycler singapore.

4-CDNA ampilified for detection of glutathione peroxidase and glutathione reductase (Quantitative SYBR Green PCR kit with ready made quantitec primer Assay, Qiagen, Applied Biosystwm,USA2012).Appropriate volumes were dispensed into PCRvessels.

5-Thereal timecycler was programmed according to standard conditions for each detected gene and melting curve analysis of the PCR product was performed to verify their specifity and identity by using 7500 soft ware version 2.0.1 and melting curve cycling program is 95 c for 15 s 60 c for 1 min florescent data collection 95c for 30s ,60 for 15 s.

3-RESULTS AND DISCUSSION

Table 1.pcr results

Pcr results	control	cipro	G	G+cipro
GPX	1.33	9.7	3.55	1.9
GSR	1.4	18.5	4.1	1.7

These results indicate that Quinolones enhance the over expression of oxidative stress genes glutathione peroxidase (gpx) and glutathione reductase (gsr) as Fluoroquinolones are known to induce the formation of singlet oxygen and superoxide anion, which are responsible for the side effect of the fluoroquinolones. A number of diverse cellular processes that lead to cell death are also mediated through ROS **(Kohanski et al., 2010)**

And these results come in harmony with **(lee. Et al,.2015)** as they stated that

Oxidative stress has been proposed as an underlying mechanism of many diseases such as cancer, atherosclerosis, rheumatoid arthritis and osteoporosis **(lee. Et al,.2015).** Previous studies that evaluated the effects of quinolones on juvenile mammals showed that these substances can have negative effects on the musculoskeletal system Moreover, some studies have reported that the negative effects of quinolones on cells and tissues of the musculoskeletal system as a result of oxidative stress. **(Pouzaud et al,. 2004;Li et al ,.2010)**.

Despite its safe profile there are reported cases of Ciprofloxacin induced chrondrotoxicity, Tendinopathy and tendon rupture in animals and humans **(Channaet al., 2008).** It was reported that Ciprofloxacin decreased thickness of the articularcartilage of the femoral condyle, inhibit proliferation of cultivated chondrocytes and secretion of soluble proteoglycans in a concentration and time dependant manner **(Li et al., 2004)**. Ciprofloxacin induce damage of the articular and epiphysical growth plate cartilage of knee joint, tendinopathy and tendon rupture **(Halawa, 2010; Kim, 2010).**

The pathogenesis of Ciprofloxacin inducedchrondrotoxicity, tendinopathy and tendon rupture is amultifactoral event **(Halawa, 2010).** Previousstudies attributed cartilage damage in growing animalsto oxidative stress, lipid

peroxidation and DNA oxidative damage of the chondrocytes and collagen **(Simoninet al., 1999; Li et al., 2004)**.

Recommended,as the result was promising it will require more additional studies of biochemical markers of oxidative stress and more higher doses of ciprofloxacine and different doses of antioxidants with longer duration to highlight the antioxidant role .

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