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Research Article

An *In Vitro* Study on Effect of Lactic Acid on Levofloxacin Induced Peroxidation of Lipids

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Abstract

Antioxidants play vital role in protecting body from free radical mediated attacks. One possibility of free radical production in body may be drug-induced lipid peroxidation in individuals under drug therapy. The present study was carried out to explore the protective role of lactic acid on lipid peroxidation induced by levofloxacin, a fluoroquinolone antibacterial, using goat liver homogenate as lipid source. The study reveals the fact that lactic acid has enough potential to suppress levofloxacin induced lipid peroxidation.

Keywords: Antioxidant, lipid peroxidation, levofloxacin, lactic acid

Introduction

Lipids are susceptible to oxidation and lipid peroxidation products are potential biomarkers for oxidative stress status *in vivo* and its related diseases [1]. Reactive oxygen species (ROS) like lipid peroxides, hydroperoxides etc. cause generation of toxic end products such as malonaldehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) and other damaging molecules [2, 3]. Many serious consequences like cancer, cardiovascular diseases, neurodegenerative disorders, etc. may result due to generation of lipid peroxidation end products.

Effective control over lipid peroxidation could be achieved in many ways such as destruction of generated free radicals, supply of competitive substrate for unsaturated lipids in the membrane, acceleration of the repair mechanism of damaged cell membrane etc. Antioxidants of both natural and synthetic origin have the capability to suppress lipid peroxidation. Situations may arise when, there is diminished *in vivo* antioxidant status and uncontrolled generation of free radicals that are not checked by endogenous antioxidant defense. In such cases, exogenous antioxidant supplement may be helpful to heal ROS mediated injury to the tissue [4,5,6].

Levofloxacin, a second generation fluoroquinolone antibacterial, is used widely in the treatment of infections caused by different pathogenic microorganisms. It is actually the levo isomer of ofloxacin having greater effectiveness against *Streptococcus pneumoniae* and some other gram-positive and gram-negative bacteria. It has wide application in the treatment of pneumonia, chronic bronchitis, sinusitis, pyelonephritis, prostatitis and other urinary tract infections as well as skin and soft tissue infections [7]. Despite its wide application, the drug can produce numerous side effects, some of which are potentially hazardous[8,9]. In some studies, it is already found that f luoroquinolones are potentially damaging molecules due to their ability to induce oxidative stress [10,11].

Steps can be taken against drug-induced free radical generation and free radical mediated tissue damage. Most important of them is considering antioxidants' role in counteracting drug-induced oxidative stress since they have enough potential to scavenge free radicals, generated during interaction of drugs with lipids. Different compounds, which are reported to have established antioxidant activity, include ascorbic acid [12,13], alpha tocopherol [14,15], beta carotene [16,17] etc. Many studies revealed that promising antioxidant property is present in lactic acid and lactic acid bacteria, found in milk product like curd. Free radical scavenging property is present in lactate ion which can provide protection against free radical-induced damage [18,19]. The present *in vitro* study has been designed in an attempt to evaluate the protective role of lactic acid on levofloxacin induced lipid peroxidation considering MDA and reduced glutathione (GSH) as laboratory markers to determine the extent of lipid peroxidation.

Materials and Methods:

In this study goat (Capra capra) liver has been used as lipid source due to its easy availability and close similarity to human liver in its lipid profile [20]. Goat liver was collected in a sterile vessel containing phosphate buffer (pH 7.4) solution. The buffer solution was drained completely and the liver was immediately ground to make a tissue homogenate (I g/ml), using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts as C (control), D (only levofloxacin treated), DLA (levofloxacin plus lactic acid treated) and LA (only lactic acid treated). Levofloxacin and lactic acid were added at their respective concentrations to corresponding samples. After drug and/or antioxidant treatment, the different portions of liver homogenate were shaken for l h and incubated below 200 C for up to 6 h. The estimation of MDA and GSH content was made at 4 h and 6 h of incubation and it was repeated in five animal sets. In each case three samples of 3.5 ml of incubation mixture was treated with 3.5 ml of 10% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 30 min to precipitate protein. Then it was filtered and the protein free filtrate was used to determine MDA and GSH content of the samples.

Estimation of MDA in liver homogenate

Estimation of MDA content of the sample was done by following Thiobarbituric acid (TBA) method [21]. 2.5 ml of the filtrate was taken in clean test tube and it was treated with 0.002 (M) TBA solution (5.0 ml) and the volume was made up to 10.0 ml with distilled water. The mixture was heated for 30 min in boiling water bath and cooled to room temperature. The absorbance was measured at 530 nm against a blank prepared from 5.0 ml of TBA solution and 5.0 ml of distilled water using Thermo Scientific (Genesis 10 UV Scanning) spectrophotometer. The content of MDA present in the sample was calculated from the standard curve [21].

Estimation of GSH in liver homogenate

GSH content estimation of the sample was performed following Ellman's method [22]. 1 ml of filtrate was treated with 0.4 ml of DTNB solution in stoppered test tube and then 5 ml of phosphate buffer was added and mixed. The absorbance of the resulting solution was measured at a wave length of about 412 nm against a blank prepared using 6 ml of buffer solution and 0.4 ml of DTNB solution. The concentration of GSH in the sample was calculated from the standard curve.

The percent changes in MDA and GSH content of different samples at different time interval were calculated with respect to control.

Results and Discussion:

Results of the study have been presented in figures 1 and 2. Statistical validation of the result has been done by analysis of variance (ANOVA) study followed by multiple comparisons using a least significant difference (LSD) procedure [23, 24]. Figures 1 and 2 reveal the fact that the drug levofloxacin could induce lipid peroxidation to a significant extent since there is an elevation of MDA level and reduction in GSH level with respect to control in drug treated group. It is found from the study that MDA is one of the remarkable end products of lipid peroxidation [25] and it plays enormous role in the development of drug-induced toxicities [26, 27]. Figure 1 also gives the evidence of reduction in MDA level in samples treated with both lactic acid and levofloxacin.

Study also discloses the ability of levofloxacin to reduce GSH content in drug-treated samples with respect to control which is a clear indication of levofloxacin-induced lipid peroxidation (Figure 2). There are many evidences behind the fact that GSH is an essential component of the antioxidant defense system of the body [28-30]. Samples that are treated with both levofloxacin and lactic acid the GSH level is elevated compared to only levofloxacin treated samples. In case of only lactic acid treated samples there is an enhancement of GSH level with respect to control value. This enhancement in GSH content supports the fact that lactic acid has antiperoxidative as well as antioxidant capability.

Tables 1 and 2 show the statistical validation of the results. For comparing means of more than two samples, multiple comparison analysis along with analysis of variance was performed on the percent changes data with respect to control of corresponding hours. Both tables indicate that there are significant differences between various groups (F1) such as levofloxacin-treated, levofloxacin and lactic acid-treated and only lactic acid treated. But within a particular group, the differences (F2) are insignificant that means there is no statistical difference between samples in a particular group (Table 1 and 2).

Table 1: ANOVA and multiple comparison for changes in MDA content

Name of the antioxidant	Incubation Period (hr.)	Analysis of variance and multiple comparison
Lactic acid	4	F1=229.69 (df 2,8); F2=1.59 (df 4,8); Pooled variance (s^2)* = 25.29; Critical difference (p=0.05)# LSD=6.93; Ranked means** (D) (DLA) (LA)
	6	F1=793.29 (df 2,8); F2=4.12 (df 4,8); Pooled variance (s^2)* = 13.81; Critical difference (p=0.05)# LSD=5.12; Ranked means** (D) (DLA) (LA)

F1 and F2 correspond to variance ratio between samples and between animal sets respectively. D, DLA and LA indicate levofloxacin-treated, levofloxacin & lactic acid-treated and only lactic acid-treated samples respectively. df = degrees of freedom. *Error mean square. #Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at $P < 0.05$.

Table 2: ANOVA and multiple comparison for changes in GSH content

Name of the antioxidant	Incubation Period (hr.)	Analysis of variance and multiple comparison
Lactic acid	4	F1=24.10 (df 2,8); F2=1.53 (df 4,8); Pooled variance (s^2)* = 56.80; Critical difference (p=0.05)# LSD=10.38; Ranked means** (D) (DLA, LA)
	6	F1=106.93 (df 2,8); F2=3.61 (df 4,8); Pooled variance (s^2)* = 69.65; Critical difference (p=0.05)# LSD=11.50; Ranked means** (D) (DLA) (LA)

F1 and F2 correspond to variance ratio between samples and between animal sets respectively. D, DLA and LA indicate levofloxacin-treated, levofloxacin & lactic acid-treated and only lactic acid-treated samples respectively. df = degrees of freedom. *Error mean square. #Critical difference according to least significant difference (LSD) procedure. **Two means not included within same parenthesis are statistically significantly different at $P < 0.05$.

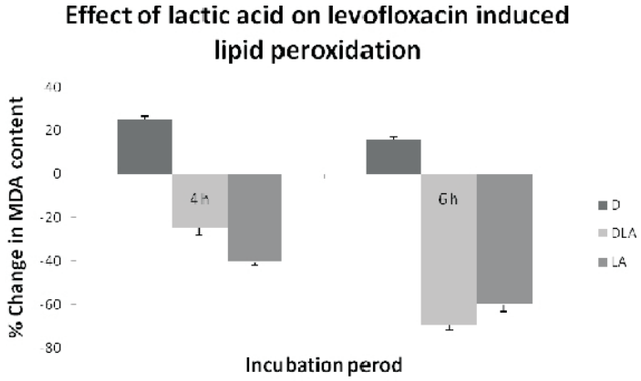

Figure 1: Effect of lactic acid on levofloxacin-induced lipid peroxidation (percent change in MDA content with respect to control, n=5); D, DLA and LA indicate levofloxacin-treated, levofloxacin & lactic acid-treated and only lactic acid-treated samples respectively

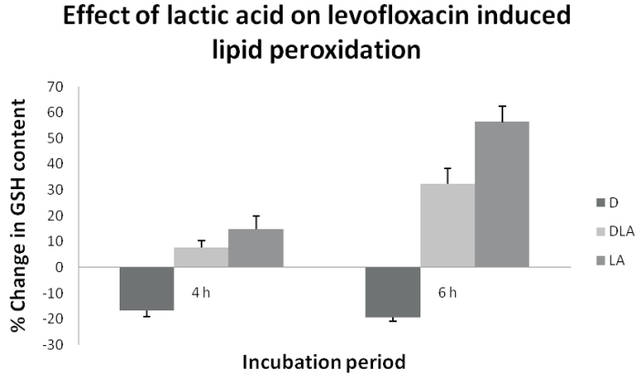

Figure 2: Effect of lactic acid on levofloxacin-induced lipid peroxidation (percent change in GSH content with respect to control, n=5); D, DLA and LA indicate levofloxacin-treated, levofloxacin & lactic acid-treated and only lactic acid-treated samples respectively

Conclusion

The findings of the study reveal that levofloxacin has significant peroxidation induction potential that might have a link with its toxicities or adverse drug reactions. The results also suggest the peroxidation inhibition capacity of lactic acid and demonstrate its possible role in suppressing levofloxacin induced adverse drug reactions. Thus maximum benefit from fluoroquinolone therapy may be achieved by antioxidant co-administration either in the form of formulation or as food supplements.

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Conflict of Interest

The author declares no conflict of interest.

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