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Research Article

Role of Ascorbic Acid in Controlling Levofloxacin Induced Peroxidation of Lipid Using Malonaldehyde and Reduced Glutathione as Laboratory Markers, an *In Vitro* Study

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Abstract

A remarkable outcome of drug- lipid interaction is peroxidation of membrane lipid when the drug crosses the lipoidal membrane barrier to elicit biological response. These may lead to free radical mediated and reactive oxygen species (ROS) - linked adverse drug reactions that include aminoglycoside-induced-ototoxicity and nephrotoxicity, adriamycin and azidothymidine- induced cardiomyopathy, cisplatin-induced ototoxicity, etc. Antioxidants have the competence to minimize ROS associated adverse drug reactions. Considering the above phenomenon the present work was carried out to explore peroxidation potential of a fluoroquinolone antibacterial agent, levofloxacin and its regulation using ascorbic acid as antioxidant of choice. Goat liver homogenate that was used as lipid source was treated with the drug and the antioxidant alone and in combination. The study showed that levofloxacin has the potential to induce peroxidation of lipids that was suppressed by ascorbic acid.

Keywords: Peroxidation, adverse drug reaction, levofloxacin, ascorbic acid

Introduction

Before binding with its specific receptor to show its biological activity a drug has to cross many lipid barriers. Whenever a drug crosses a biomembrane a cascade of chemical reactions takes place and one important such reaction include its interaction with membrane lipids that may lead to peroxidation of the same. Lipid peroxidation is the oxidative decomposition of membrane lipid that leads to generation of reactive oxygten species (ROS) like lipid peroxides, hydroperoxides, etc that ultimately causes generation of toxic end products such as malonaldehyde (MDA) [1], hydroxy alkenals that include 4-hydroxy-2-nonenal (4-HNE) [2] and other damaging molecules. These treacherous compounds are found to be involved in many diseases and ailments like diabetes mellitus [3], aging [4], neurodegenerative disorders [5], gastric mucosal damage [6], asthma [7], atherosclerosis [8], retinopathy [9] and many others.

There are many ways by which lipid peroxidation could be counteracted. These include (i) destruction of free radicals that are already formed, (ii) supply of competitive substrate for unsaturated lipids in the membrane, and (iii) acceleration of the repair mechanism of damaged cell membrane. Different natural and synthetic antioxidants have the potential to suppress the lipid peroxidation process [10, 11]. Whenever there is diminished in vivo antioxidant status and uncontrolled generation of free radicals that are not checked by endogenous antioxidant defense, exogenous antioxidant supplement may be helpful to conquer free radical-induced oxidative damage. Levofloxacin is a second generation fluoroquinolone antibacterial agent that is used widely in the treatment of different infections caused by the pathogenic microorganisms. It is the active levo isomer of ofloxacin having improved activity against Streptococcus pneumonia and some other gram-positive and gram-negative bacteria. Anaerobes are moderately susceptible. 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 [12]. Despite its wide application, the drug can produce numerous side effects some of which are potentially hazardous [12-14]. It is reported that fluoroquinolones have the capability of inducing oxidative stress [15, 16]. Antioxidants play promising role in counteracting drug-induced oxidative stress. Many antioxidants are found to be effective in scavenging free radicals generated due to interaction of drugs with lipids. It is reported that ascorbic acid, the antioxidant vitamin, played a great role in minimizing drug-induced lipid peroxidation and tissue damage [17-19]. Another important antioxidant vitamin that is present naturally in our blood as well as in different food materials is alpha tocopherol (vitamin E) that also has good free radical scavenging property [20, 21]. Many study showed that remarkable antioxidant feature is present in lactic acid and lactic acid bacteria that is present in milk product like curd. Lactate ion itself is a free radical scavenger and can provide protection against free radical-induced damage [22, 23].

The present study has been designed to evaluate the peroxidation induction ability of the fluoroquinolone antibacterial levofloxacin and its possible suppression with ascorbic acid, in vitro. The laboratory markers that have been considered in the study to determine the extent of lipid peroxidation are MDA and reduced glutathione (GSH) as model molecules.

Materials and Methods

For in *vitro study* as lipid source different materials may be used. One of the important of them is goat (Capra capra) liver. The easy availability and close similarity to human liver in its lipid profile are the basis of selecting goat liver as lipid source [24]. 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 grinded to make a tissue homogenate (l g/ml) using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts as C (control), D (only drug treated), DAA (drug plus antioxidant treated) and AA (only antioxidant treated). Drug and antioxidant 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 done 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

The MDA content of the sample was determined by thiobarbituric acid (TBA) method [25]. 2.5 ml of the filtrate 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 concentration of MDA present in the sample was calculated from the standard curve [25].

Estimation of GSH

The estimation of GSH content was performed following Ellman's method [26]. 1 ml of the filtrate was taken in stoppered test tubes and it was treated with 0.4 ml of DTNB solution and then 5 ml of phosphate buffer was added and mixed. The absorbance of the resulting solution was measured at 412 nm against a blank containing 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 the control.

Results and Discussion

Results of the study are shown in Tables 1-2. The result is validated statistically by analysis of variance (ANOVA) study followed by multiple comparisons using a least significant difference procedure [27, 28].

Table 1: Effect of ascorbic acid on levofloxacin-induced lipid peroxidation (percent change in MDA content with respect to control)

Incubation period	Animal set	Percent changes in MDA content			Analysis of variance and multiple comparison
		Samples			
		D	DAA	AA	
4h	1	21.64	-37.00	-42.34	F1 = 267.50 (df 2,8)
	2	25.08	-25.70	-45.43	F2 = 6.47 (df 4,8)
	3	22.43	-26.29	-31.64	Pooled variance
	4	23.49	-23.90	-40.12	$(s^2)^*$ = 20.00
	5	32.17	-15.35	-21.74	Critical difference (p=0.05)#
	Av.	24.96	-25.64	-36.25	LSD = 6.16
	(± se)	(±1.89)	(± 3.45)	(± 4.29)	Ranked means** (D) (DAA) (AA)
6h	1	17.13	-54.40	-45.32	F1= 614.17 (df 2,8)
	2	19.29	-56.23	-39.13	F2= 7.88 (df 4,8)
	3	16.10	-58.73	-49.06	Pooled variance
	4	11.92	-70.23	-64.69	$(s^2)^*$ = 13.74
	5	14.88	-58.45	-51.68	Critical difference (p=0.05) #
	Av.	15.86	-59.60	-49.97	LSD = 5.11
	(± se)	(± 1.22)	(± 2.77)	(± 4.24)	Ranked means** (D) (DAA) (AA)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by't' test and the values are significant at P< 0.05. F1 and F2 correspond to variance ratio between samples and between animal sets respectively. D, DAA and AA indicate levofloxacin-treated, levofloxacin & ascorbic acid-treated and only ascorbic acid-treated samples respectively. Av. = average of five animal sets; se = standard error (df = 4); 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: Effect of ascorbic acid on levofloxacin-induced lipid peroxidation (percent change in GSH content with respect to control)

Incubation period	Animal set	Percent changes in MDA content			Analysis of variance and multiple comparison
		Samples			
		D	DAA	AA	
4h	1	-9.34	28.05	24.38	F1 = 135.56 (df 2,8)
	2	-17.48	19.92	22.36	F2 = 4.01 (df 4,8)
	3	-24.67	11.24	22.07	Pooled variance
	4	-15.05	11.94	13.69	$(s^2)^*$ = 16.73
	5	-17.36	21.92	24.67	Critical difference (p=0.05)#
	Av.	-16.78	18.61	21.43	LSD = 5.64
	(± se)	(± 2.46)	(±3.16)	(±2.00)	Ranked means** (D) (DAA, AA)
6h	1	-22.02	33.02	48.15	F1= 139.69 (df 2,8)
	2	-18.72	49.04	53.85	F2= 3.52 (df 4,8)
	3	-14.50	57.84	85.57	Pooled variance
	4	-18.45	36.53	63.93	$(s^2)^*$ = 71.45
	5	-22.90	58.37	77.13	Critical difference (p=0.05) #
	Av.	-19.31	46.96	65.72	LSD = 11.65
	(± se)	(±1.49)	(±5.27)	(±6.98)	Ranked means** (D), (DAA), (AA)

*Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by't' test and the values are significant at P< 0.05. F1 and F2 correspond to variance ratio between samples and between animal sets respectively. D, DAA and AA indicate levofloxacin-treated, levofloxacin & ascorbic acid-treated and only ascorbic acid-treated samples respectively. Av. = average of five animal sets; se = standard error (df = 4); 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.*

It is evident from Tables 1-2 that the drug levofloxacin could induce lipid peroxidation significantly since there is an elevation of MDA level and reduction in GSH level. Study shows that MDA is one of the remarkable end products of peroxidation [29] and is found to be involved in drug-induced toxicities [30-31]. Table 1 also shows that the elevated level of MDA was reduced in samples that are treated with both ascorbic acid and levofloxacin..

It was further found from the study that the drug levofloxacin caused a reduction in GSH content in drug-treated samples with respect to control which might be an outcome of levofloxacin-induced lipid peroxidation (Table 2). It is reported that GSH is an important part of the antioxidant defense mechanism [32] of the body. In case of samples that are treated with both levofloxacin and ascorbic acid the GSH level is elevated compared to only levofloxacin treated samples. The liver homogenate that was treated with only ascorbic acid shows an enhancement of GSH content with respect to control sample. This increased GSH level supports the fact that ascorbic acid has antiperoxidative as well as antioxidant potential.

Conclusion

The fluoroquinolone antibacterial levofloxacin that has wide applications as chemotherapeutic agent [12] could also induce peroxidation of lipids significantly. Such capability of the drug might have some link with its adverse reactions. Some studies showed that fluoroquinolones have the ability of inducing oxidative stress [15-16]. The study supports the antioxidant capacity of ascorbic acid. It is already found that ascorbic acid has free radical scavenging property [33, 34] that might be beneficial in minimizing drug-induced oxidative stress. Thus to improve the therapeutic outcome of a drug one important tool may be minimization of drug toxicity upon antioxidant co-administration. Future formulation design may consider combination of drug with antioxidant that will be helpful in minimizing drug-induced adverse reactions and hazards.

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Conflicts of interest

The authors declare that there is no competing interest regarding publication of this paper.

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