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Research Article

Effect of Lincomycin and Ascorbic Acid, Alone and in Combination, on Peroxidation Parameters of Rabbit Blood Plasma

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Abstract

Peroxidation of membrane lipids is an important phenomenon that happens when a drug, after administration, crosses biological lipoidal barrier to reach its site of action. Lipid peroxidation, that may lead to injurious consequences on cells and tissues, is considered to be one of the underlying facts behind the development of several life threatening diseases and disorders. Considering the above statement the *in vivo* study was conducted to evaluate the peroxidation induction potential of the lincosamide antibiotic lincomycin using rabbit as animal model. The study reveals that lincomycin has considerable lipid peroxidation induction potential that was indicated by its ability to raise plasma MDA (malondialdehyde) and HNE (4-hydroxy-2-nonenal) level and suppressing plasma GSH (reduced glutathione) and NO (nitric oxide) level. It was further found from the study that ascorbic acid, the well known and easily available antioxidant, has the ability to minimize lincomycin-induced lipid peroxidation.

Keywords: Lipid peroxidation, injurious consequences, lincomycin, ascorbic acid

Introduction

Extent of drug-induced peroxidation of plasma lipids can be estimated by measuring the peroxidation parameters of blood plasma like MDA, HNE, GSH, NO, etc content after drug administration. Lipid peroxidation, the oxidative deterioration of polyunsaturated lipids, is a highly destructive process that induces a wide variety of changes in cellular membranes both structurally and functionally [1]. It is an important cause of certain ailments like gastric ulcer, diabetes mellitus, neurodegenerative diseases [2], aging [3], atherosclerosis [4], etc. The consequences of drug-induced lipid peroxidation and reactive oxygen species (ROS) formation include indomethacin-induced gastric mucosal damage [5], doxorubicin-induced cardiac damage [6], azidothymidine-induced myopathy, and cisplatin-induced ototoxicity [7].

One important lincosamide class of antibiotics is lincomycin whose mode of action and activity spectrum is similar to that of erythromycin [8]. It inhibits most gram positive cocci that include streptococci, staphylococci, etc. A large number of staphylococci strains which are resistant to methicillin, ampicillin, tetracycline, streptomycin, chloramphenicol, and erythromycin were found to be sensitive to lincomycin [9]. Despite its wide utility, this antibiotic is found to be involved in the production of different adverse reactions, some of which may be life threatening [10-13]. The aim of this in vivo study is to find out the lipid peroxidation induction potential of lincomycin and its subsequent control on co-administration of ascorbic acid, a promising and easily available antioxidant that has free radical scavenging capability [14-15]. Application of antioxidants as adjuvant therapy may become a promising approach in reducing drug-induced, peroxidation-related hazards [16-18].

Materials and Methods

Lipid peroxidation induction potential of the antibiotic lincomycin was measured by estimating malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), reduced glutathione (GSH) and nitric oxide (NO) levels that are considered as laboratory markers of lipid peroxidation. New Zealand White rabbit (Oryctolagus cuniculus) was used as animal model. The reagents that are used in this study were of analytical grade. Institutional Animal Ethical Committee has approved the design of the study protocol.

Collection of blood

Rabbits were divided into different experimental groups such as control (C), drug-treated (D), drug co-administered with antioxidant (DA) and only antioxidant-treated (A). The antibiotic lincomycin was administered via intramuscular route at a dose of 25 mg/kg body weight [19] to animal groups marked as D and DA. Administration of ascorbic acid was done at a dose of 40 mg/kg body weight [20] to animal groups marked as DA and A. Collection of blood from marginal ear vein of animal was done after 4.5 and 24 h of drug and/or antioxidant administration. The collected blood was mixed well with equal volume of trichloroacetic acid (10% w/v) in centrifuge tubes marked accordingly and it was centrifuged at 4000 rpm for 30 min and then filtered. The protein free supernatant was used for estimation of different laboratory markers of lipid peroxidation for evaluating the effect of lincomycin and ascorbic acid on peroxidation parameters of rabbit blood plasma.

Estimation of MDA level

The supernatant was taken in test tube with stopper and equal volume of thiobarbituric acid (TBA) reagent was mixed and it was heated in a boiling water bath for 30 min. The absorbance of the pink coloured sample was measured at a wave length of about 530 nm against a blank. The concentration of MDA present in the sample was estimated from the standard curve prepared using tetraethoxy propane (TEP) and TBA (1:1) [21].

Estimation of HNE level

The supernatant was mixed with equal volume of 2, 4-dinitrophenylhydrazine (DNPH) solution (100 mg % in 0.5 M HCl) and incubated at room temperature for 1 h. After that, the mixture was extracted with hexane followed by removal of hexane by heating at a temperature not more than 700°C and then addition of methanol was done. The absorbance of the resultant solution was measured at 350 nm against methanol as blank. The concentration of HNE was determined from the standard curve [22].

Estimation of GSH level

The GSH content of the sample was measured by treating it with 5, 5/-dithiobis (2-nitrobenzoic acid) (DTNB) to give a colour complex

(Ellman's method) [23]. The supernatant was mixed with DTNB (1:3) solution (0.01% in phosphate buffer 0.1 M, pH 8) and the absorbance of the resultant mixture was measured at 412 nm against a blank prepared using the phosphate buffer and DTNB solution at the same ratio. Concentration of GSH present in the sample was calculated from the standard curve [23].

Estimation of NO level

NO content of the sample was determined by reacting the sample with Griess reagent [1:1 sulfanilamide (1% w/v in 3 M HCl) and 0.1% w/v N-(1-naphthyl) ethylenediamine dihydrochloride]. The pH of the mixture was adjusted to 6.7 by adding Na_2HPO_4. The absorbance of the resultant mixture was measured at a wave length of 540 nm. The concentration of NO present in the sample was calculated from the standard curve [24].

The percent changes in MDA, GSH, HNE, and NO content of different samples at different time interval were calculated with respect to the control.

Results and Discussion

Results of the study are presented in Tables 1-4. The validation of the results is done statistically by analysis of variance (ANOVA) study followed by multiple comparisons using a least significant difference procedure [25-26]. Table 1-4 shows that the drug lincomycin has significant lipid peroxidation induction potential that is involved behind the elevation of MDA and HNE level and reduction in GSH and NO level. Study shows that MDA and HNE are the end products of lipid peroxidation process [27] and are found to be involved in drug-induced toxicities [5-6]. The elevated level of MDA and HNE was found to be reduced upon co-administration of the antioxidant ascorbic acid (Table 1-2).

Table 1: Effect of ascorbic acid on lincomycin-induced lipid peroxidation: percent change in MDA content

Time period	Animal Set	% changes in MDA content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
4.5 h	1	9.42[a]	3.69[a]	-5.23[a]	F1 = 91.87 (df 2,8)
	2	8.16[a]	4.87[a]	-6.04[a]	F2 = 1.86 (df 4,8)
	3	7.92[a]	3.41[a]	-6.77[a]	Pooled variance
	4	13.69[a]	4.54[a]	-5.87[a]	(s^2)* = 4.08
	5	15.69[a]	7.56[a]	-6.84[a]	Critical difference (p=0.05)#
	Av (±se)	10.96 (±1.56)	4.71 (±10.79)	-6.15 (±0.29)	LSD = 2.78 Ranked means** (D) (DA) (A)
24 h	1	4.56[a]	1.76[c]	-1.33[a]	F1= 40.10 (df 2,8)
	2	3.58[a]	1.81[a]	-2.50[a]	F2= 1.13 (df 4,8)
	3	3.57[a]	0.22[a]	-1.87[a]	Pooled variance
	4	7.21[a]	4.15[a]	-3.94[a]	(s^2)* =1.58
	5	5.45[a]	1.59[d]	-1.95[b]	Critical difference (p=0.05)
	Av (±se)	4.88 (±0.68)	1.901 (±0.63)	-2.22 (±0.35)	LSD = 1.73 Ranked means** (D) (DA) (A)

Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05; bP<0.10, cP<0.12, dP<0.28, eP<0.42. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated 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 (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.

Table 2: Effect of ascorbic acid on lincomycin-induced lipid peroxidation: percent change in HNE content

Time period	Animal Set	% changes in HNE content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
4.5 h	1	14.01[a]	11.32[a]	-6.51[a]	F1 = 190.39 (df 2,8)
	2	13.71[a]	7.43[a]	-8.21[a]	F2 = 2.23 (df 4,8)
	3	11.50[a]	5.92[a]	-9.74[a]	Pooled variance
	4	14.13[a]	3.56[a]	-6.12[a]	(s^2)* = 3.12
	5	16.10[a]	8.03[a]	-6.43[a]	Critical difference (p=0.05)
	Av (±se)	13.98 (±0.73)	7.25 (±1.27)	-7.40 (±0.68)	LSD = 2.43 Ranked means** (D) (DA) (A)
24 h	1	6.82[a]	3.59[a]	-2.92[a]	F1= 40.09 (df 2,8)
	2	5.46[a]	2.26[a]	-1.59[a]	F2= 0.68 (df 4,8)
	3	4.40[a]	2.02[a]	-1.40[a]	Pooled variance
	4	2.98[a]	1.09[a]	-1.54[a]	(s^2)* = 1.80
	5	7.36[a]	2.71[b]	-3.36[a]	Critical difference (p=0.05)
	Av (±se)	5.40 (±0.79)	2.33 (±0.4)	-2.16 (±0.4)	LSD = 1.85 Ranked means** (D) (DA) (A)

Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05; bP<0.07. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated 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 (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.

Table 3: Effect of ascorbic acid on lincomycin-induced lipid peroxidation: percent change in GSH content

Time period	Animal Set	% changes in GSH content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
4.5 h	1	-7.98[a]	-3.29[a]	4.28[a]	F1 = 235.68 (df 2,8)
	2	-12.12[a]	-5.89[a]	5.20[a]	F2 = 2.44 (df 4,8)
	3	-10.11[a]	-3.62[a]	5.95[a]	Pooled variance
	4	-10.68[a]	-5.30[a]	3.58[a]	(s^2)* = 1.19
	5	-8.18[a]	-4.63[a]	5.84[a]	Critical difference (p=0.05)
	Av (±se)	-9.81 (±0.75)	-4.55 (±0.49)	4.97 (±0.45)	LSD = 1.50 Ranked means** (D) (DA) (A)

	1	-3.20[a]	-1.81[c]	2.29[a]	F1 = 105.43 (df 2,8)
	2	-5.79[a]	-3.09[b]	1.77[a]	F2 = 2.69 (df 4,8)
24 h	3	-4.96[a]	-2.77[a]	2.79[a]	Pooled variance
	4	-4.93[a]	-1.88[a]	2.02[a]	(s^2)* = 0.52
	5	-2.77[a]	-1.12[a]	1.98[a]	Critical difference (p=0.05)
	Av	4.33	1.901	2.17	LSD = 0.99
	(±se)	(±0.57)	(±0.63)	(±0.17)	Ranked means** (D) (DA) (A)

Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.06. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated 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 (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.

It was further found from the study that the drug lincomycin caused reduction in GSH and NO contents in drug-treated group with respect to control which might be due to lipid peroxidation induction potential of the drug (Table 3-4). GSH and NO are involved in the antioxidant defense mechanism [28-29] of the body. In case of animals received lincomycin co-administered with ascorbic acid, the GSH and NO levels are elevated in comparison to the animal group that received lincomycin only. The animal group that received only ascorbic acid shows elevated levels of these parameters compare to the control group. This increased GSH and NO level (in respective animal groups) reveals the antiperoxidative as well as antioxidant power of ascorbic acid [16-18].

Table 4. Effect of ascorbic acid on lincomycin induced lipid peroxidation: percent change in NO content

Time period	Animal Set	% changes in NO content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
	1	-13.33[a]	-7.39[a]	14.58[a]	F1 = 118.74 (df 2,8)
	2	-16.99[a]	-8.49[a]	13.54[a]	F2 = 0.46 (df 4,8)
4.5 h	3	-19.28[a]	-7.78[a]	13.36[a]	Pooled variance
	4	-11.83[a]	-5.30[a]	10.18[a]	(s^2)* = 10.24
	5	-17.42[a]	-8.27[a]	20.47[a]	Critical difference (p=0.05)
	Av	-15.77	-7.45	14.43	LSD = 4.41
	(±se)	(±1.37)	(±0.56)	(±1.68)	Ranked means** (D) (DA) (A)
	1	-5.83[a]	-3.85[b]	5.52[a]	F1= 49.61 (df 2,8)
	2	-8.10[a]	-3.05[a]	3.98[a]	F2= 0.24 (df 4,8)
24 h	3	-9.64[a]	-5.24[a]	8.29[a]	Pooled variance
	4	-5.30[a]	-2.34[a]	3.56[a]	(s^2)* = 4.00
	5	-8.77[a]	-2.61[a]	3.26[b]	Critical difference (p=0.05)
	Av	-7.43	-3.42	4.02	LSD = 2.76
	(±se)	(±0.81)	(±0.52)	(±0.92)	Ranked means** (D) (DA) (A)

Percent changes with respect to control of corresponding hours are shown. Reproducibility measured by 't' test and the values are significant at aP< 0.05, bP< 0.08. F1 and F2 correspond to variance ratio between samples and between animals respectively. D, DA and A indicate lincomycin-treated, lincomycin & ascorbic acid-treated and ascorbic acid-treated 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 (Ref. 197 and 198). **Two means not included within same parenthesis are statistically significantly different at P <0.05.

Conclusion

The antibiotic lincomycin that has wide applications in the treatment of different bacterial infections [8-9] also has to some extent peroxidation induction potential that might have a connection with the adverse drug reactions of this drug. It was already reported that another lincosamide antibiotic, clindamycin has peroxidation induction capability [30]. Study also showed that clindamycin-induced neurotoxicity, colon related toxicity, and pseudomembranous enterocolitis are oxidative stress associated and that may have a link with its peroxidation induction ability [31]. The free radical scavenger ascorbic acid [14-15] has the capacity to control these toxicities effectively upon co-administration. Antioxidant co-therapy may be an effective tool during future formulation design with an aim of minimizing adverse drug reactions and drug-induced toxicities. Obviously, this approach will improve patient compliance and will enhance therapeutic index of the drug concerned.

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Conflict of interest

The author declares no conflict of interest.

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