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Evaluation of Ascorbic Acid as an Inhibitor of Lipid Peroxidation Induced by Cefotaxime sodium and Metoprolol

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Ascorbic acid (AA) has been evaluated at three different concentrations equivalent to human doses of 100, 250 and 500 mg as an inhibitor of lipid peroxidation (LP) induced by two drugs, cefotaxime sodium (CS) and metoprolol (M) using goat whole blood as the lipid source and malonaldehyde (MA) content as the measure of LP. AA was found to significantly suppress *in vitro* lipid peroxidation caused by CS and M at all three dose levels. Paradoxically, AA *per se* increased MA content when used without drug. These findings are discussed in an attempt to explore possible potential of AA to reduce toxic effects of the drugs considering LP as a toxicity mediating process.

In the course of intervening events between drug administration and biological response[1], drugs may bring about varied degrees of changes in lipid pattern of biomembranes depending on the partition coefficient parameter[2]. The changes include (a) changes in the composition of fatty acids (FA) which control membrane fluidity[3] and are precursors of eicosanoids, important pathophysiological modulators; (b) binding with phospholipids (PL) of biomembranes; (c) lipid peroxidation leading to generation of toxic peroxides and hydroperoxides which have been related to the pathogenesis of several cardiovascular, pulmonary and hepatic diseases[4].

To explore drug lipid interaction, preliminary *in vitro* studies were initiated with several groups of drugs. Changes in composition of FA and binding with PL have been related to the therapeutic effects, and lipid peroxidation to the toxic effects of the drugs[5-11]. The present investigation deals with LP induced by two drugs, cefotaxime sodium and metoprolol, and its suppression with ascorbic acid.

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MATERIALS AND METHODS

Whole blood collected from goat (*Capra capra*) and chemicals of analytical grade were used for the present study. The samples of cefotaxime sodium and metoprolol tartrate were provided by Roussel India Ltd., Bombay and Concept Pharmaceuticals Ltd., Aurangabad respectively.

Goat blood was collected in a sterile vessel containing anticoagulant solution (sodium citrate in saline) and freed of foreign materials like dust, and hair by filtration through sterile cloth. The collected blood was preserved under nitrogen atmosphere in a refrigerator for further work.

The preserved blood was divided into different portions for treatment with drug and/or ascorbic acid. The blood samples were stirred below 20° for one hour with drug (CS or M) and/or AA (without drug and AA in case of control) and incubated at 15° for 5 days. The drug solutions (in saline) were used in concentrations of 40 mg% and 0.2 mg% in cases of CS and M respectively. AA was used at three equivalent human dose levels of 100 mg, 250 mg and 500 mg, i.e., the effective concentrations were 2 mg%, 5 mg% and 10 mg% respectively.

Table 1: Effects of Ascorbic acid on cefotaxime (CS)-and Metoprolol (M)- Induced Lipid Peroxidation

	Relative percent changes in MA content with respect to control (0) day (Mean ± s.e.) Days of incubation			F ratio (Df) Between	
	1	3	5	days	samples
CS	80.78 ± 18.84	152.11 ± 26.13	262.25 ± 42.79	30.84#	
CSA100	36.73 ± 3.25	46.01 ± 7.47	111.52 ± 19.98	(2,6)	
CSA250	40.29 ± 12.16	100.24 ± 38.74	232.95 ± 48.34		5.72#
CSA500	30.72 ± 4.20	82.47 ± 13.24	227.81 ± 52.33		(3,6)
M	120.35 ± 23.06	160.59 ± 30.08	204.19 ± 34.62	0.84	
MA100	18.05 ± 8.35	21.18 ± 11.99	24.50 ± 8.94	(2,6)	
MA250	21.46 ± 6.78	22.10 ± 7.22	29.62 ± 6.13		26.90#
MA500	100.00 ± 0.00	86.59 ± 3.20	81.74 ± 5.61		(3,6)
A100	4.48 ± 2.30	10.51 ± 1.59	19.56 ± 3.49	25.67#	
A250	9.39 ± 2.85	12.99 ± 4.29	21.75 ± 3.75	(2,4)	
A500	6.12 ± 3.53	18.85 ± 9.80	28.28 ± 11.30		3.64 (2,4)

Number of animal sets (n) =3

#significant at 5% level

Keys :

M, CS-Blood samples treated with M or CS

MA100/MA250/MA500, CSA100/CSA250CSA500-Blood samples treated with AA (2/5/10 mg%) and M or CS

A100/A250/A500-Blood samples treated with AA (2/5/10 mg%)

The extent of LP was estimated in terms of malonaldehyde (MA) content on day 0 for control sample and on 1st, 3rd and 5th days of incubation for all samples following the method of Tarladgis *et. al.*[12,13] after suitable modification as described here briefly. The method involves precipitation of the protein part of blood by treatment with 10% trichloroacetic acid solution and centrifugation at 3000 rpm for 30 minutes followed by filtration of the supernatant and treatment of the filtrate with 0.002 molar thiobarbituric acid (TBA) solution and boiling of the mixture for half an hour. Absorbance of the resultant solution was measured at 530 nm against a TBA blank (prepared from equal volumes of TBA solution and distilled water) using EC digital spectrocolorimeter GS5700A. MA content was calculated from the regression equation of TBA-MA standard curve prepared using tetraethoxypropane and TBA following the method of Tarladgis *et. al*[13].

RESULTS AND DISCUSSION

Control blood samples did not show significant changes in malonaldehyde content in different days of incubation. Drug-treated blood samples (CS or M) showed significant increase in the MA content with respect to control (0 day) in a time dependent manner (Table 1). The increase in MA (which is a LP breakdown product) content due to presence of drug (CS or M) is possibly due to enhanced lipid peroxidation. Lipid peroxidation, being a cell injury mechanism generating reactive oxygen species (ROS) which play important role in different disease condition[14,15],

Table - 2 : Relative Percent reduction in cefotaxime -and metoprolol-induced lipid peroxidation due to presence of AA

A. Reduction in Cefotaxime induced peroxidation (measured as MA)

Days of incubation	A100	A250	A500
1	54.53	50.12	61.97
	t 2.30 (c)	t 1.81 (d)	t 2.59 (c)
3	69.75	34.10	45.78
	t 3.91 (a)	t 1.12 (e)	t 2.38 (c)
5	57.47	11.17	13.13
	t 3.19 (b)	t 0.45 (e)	t 0.52 (e)

ANOVA : F ratio±between doses, 3.32; between days, 3.40

B.Reduction in Metoprolol induced peroxidation (measured as MA)

Days of incubation	A100	A250	A500
1	85.00	82.17	16.91
	t 4.17 (a)	t 4.11 (a)	t 0.88 (e)
3	86.81	86.24	46.08
	t 4.32 (a)	t 4.48 (a)	t 2.45 (e)
5	88.00	85.49	59.97
	t 5.03 (a)	t 4. 97 (a)	t 3.49 (b)

ANOVA : F ratio±between doses, 14.52#; between days, 1.5##,
Confidence limits a : > 99% b: 97.5-99%, C : 95-97.5%, d : 90-95%, e : <90% (One way t tabulation, df = 4), + Degress of freedom of F values (2, 4), # Significant (5% level)

Keys : A100/A250/A500 -AA at doses 2/5/10 mg %

may be related to the toxic effects of the drugs. It will not be out of context to mention here that recently doxorubicin-induced cardiotoxicity has been reported to be caused by a free radical mediated process[16].

In cases of blood samples treated with either of the drug (CS or M) and AA, the increase in MA content was significantly reduced than that of drug-treated (CS or M) blood samples (Table 1). Table 2 depicts percent reduction in MA increase in drug induced lipid peroxidation due to presence of AA supported by statistical analyses like 't' test and ANOVA. Such reduction is possibly due to suppressive action of AA on Drug-induced lipid peroxidation. Thus it may be presumably hypothesized that owing to inhibitory action on LP, AA might have the potential of reducing toxic effects of the drugs (Which are mediated through drug-induced LP) and thus increasing therapeutic indices of the drugs. Such presumption is supported by reported uses of antioxidants for treatment of different disease conditions such as inflammation, atherosclerosis and traumatic brain injury[17]. The biological antioxidant property of AA is well established [18] and its protective role against oxidative damage is evident from the report that low plasma concentrations of AA and α - tocopherol (i.e., reduced antioxidant defence) may be associated with higher incidence of myocardial infarction[17]. Thus, in addition

to significant contribution to the treatment of physiological oxidative damage, ascorbic acid may have a role in reducing iatrogenic disorders also.

It is interesting to note that blood samples treated with AA only (i.e., without drug) showed some increase in MA content in comparison to the control (0 day) (Table 1). Such increase may be explained on the basis of the fact that AA can reduce Fe^{3+} to Fe^{2+} through Fenton's reaction and Fe^{2+} increases concentration of ROS having potential of covalent bond breaking in biomolecules[19]. The double role (antioxidant, and prooxidant in presence of metal ions, particularly, iron and copper) of AA is already reported[20]. Thus it will be appropriate to mention that for obtaining beneficial antioxidant effects of AA, iron level in blood should be monitored[21].

In conclusion, AA appears to possess the potential to inhibit generation of peroxidation breakdown products responsible for expression of various toxic effects of drugs and this may be exploited during future formulation design to enhance the therapeutic indices of drugs. Further work is required to be done to confirm this hypothesis.

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