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Research Article

An *In Vitro* Study on Effect of Lactic Acid and Ascorbic Acid on Etoposide-Induced Lipid Peroxidation

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Abstract

Lipid peroxidation, a free radical mediated process is involved in several injurious consequences like cardiomyopathy, neurodegenerative diseases, etc. Reactive oxygen species (ROS) is involved as a mediator of free radical related damage. Antioxidants have the capability to scavenge free radicals and protect tissues from ROS. Keeping in mind this fact the present in vitro study was carried out to evaluate the lipid peroxidation induction potential of etoposide, an antimalarial and its suppression with ascorbic acid and lactic acid using malondialdehyde (MDA) as laboratory marker. Liver homogenate was treated with drug as well as antioxidant and incubated for definite time period and MDA content was estimated in different samples. Result showed that etoposide could significantly induce lipid peroxidation and ascorbic acid and lactic acid could significantly inhibit etoposide-induced lipid peroxidation. It was further found from the study that antioxidant potential of lactic acid is more than that of ascorbic acid.

***Keywords*:** Lipid peroxidation, etoposide, malondialdehyde, lactic acid, ascorbic acid

Introduction

The oxidative deterioration of polyunsaturated lipids is known as lipid peroxidation, which is a free radical related complex process known to occur in both plants and animals. It causes alteration of the structure and function of cellular membrane.[1] The events which are involved in lipid peroxidation include formation and propagation of lipid radicals, the uptake of oxygen, rearrangement of double bonds in unsaturated lipids and the destruction of membrane lipids producing different breakdown products like ethers, alcohols, aldehydes and ketones.[2] Peroxides and lipid hydroperoxides which are generated during lipid peroxidation are decomposed to yield several cytotoxic end products, most of which are mainly aldehydes like malondialdehyde (MDA) and other aldehydes.[3] These aldehydes are found to be involved in some of the pathophysiological effects associated with oxidative stress in cells and tissues.[4] MDA is always produced when lipid hydroperoxides are metabolized in biological systems.[5] Identification and quantification of this compound give an indirect index of oxidative injury which results in lipid peroxidation. MDA is in many instances the most abundant aldehyde arising from lipid peroxidation.[6]

Etoposide is a semisynthetic derivative of epipodophyllotoxin usually used in combination with other anticancer compounds in the treatment of testicular, lung and other cancers as well as in Kaposi's sarcoma associated with AIDS. It is also used to treat leukemia either alone or in combination with other antitumor agents, or radiation therapy. In spite of its utility the drug shows remarkable toxicities[7] that might hinder its wide applications. In some studies it was found that etoposide has pro-oxidant effect also.[8] Toxicities of etoposide might have a link with its free radical generation capacity.[9]

Ascorbic acid is considered the most important water soluble antioxidant in human plasma[10-11] where it circulates at concentrations of 20-60 µmol/ml in unsupplemented individuals.[12-13] It acts as an antioxidant both in vitro and in vivo. It functions as a free radical scavenger for active and stable oxygen radicals. In all types of oxidative stress ascorbic acid successfully prevents detectable oxidative damage strongly, suggesting that it should be helpful in preventing degenerative and other diseases in which oxidative stress plays causative or exacerbating role.[14]

Lactic acid, an alpha hydroxy acid, also known as milk acid, is a chemical compound that plays a role in various biochemical processes. Lactic acid is miscible with water or ethanol, and is hygroscopic. In industry, lactic acid fermentation is performed by lactic acid bacteria. These bacteria can also grow in the mouth to produce tooth decay known as caries.[15-18] In medicine, lactate is one of the main components of Ringer Lactate Solution and Hartmann's Solution. Study showed that lactate ion has free radical scavenging and antioxidant activity.[19]

As part of our ongoing research work to explore drug-induced lipid peroxidation and inhibitory actions of different antioxidants on drug-induced lipid peroxidation,[20] our present study has been

designed to evaluate lipid peroxidation induction potential of etoposide in goat liver homogenate. MDA level is used as marker of lipid peroxidation and suppressive actions of two antioxidants like ascorbic acid and lactic acid were studied on etoposide-induced lipid peroxidation in an attempt to minimize etoposide-induced toxicities *in vivo*.

Materials and Methods

Materials

Goat (*Capra capra*) liver was used as the lipid source. The goat liver was selected because of its easy availability and close similarity to the human liver in its lipid profile.[21] 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 (I g/ml) using freshly prepared phosphate buffer (pH 7.4). For each antioxidant, the homogenate was divided into four equal parts as C (control), D (only drug treated), DA (drug plus antioxidant treated) and A (only antioxidant treated). Drug and antioxidants were added at their specific concentrations to respective samples. After drug and/or antioxidant treatment, the different portions of liver homogenate were shaken for I h and incubated below 20°C for up to 4h.

Estimation of MDA as an index of lipid peroxidation

MDA is a product of enzymatic polyunsaturated fatty acid (PUFA) peroxidation.[22] It is a low molecular weight end product of metabolically uncoupled PUFA oxidative degradation.[23] In the present study the extent of lipid peroxidation was estimated in terms of MDA content by thiobarbituric acid (TBA) reaction.[24] The estimation was done at 3h and 4h of incubation and repeated in five animal sets. In each case three samples of 2.5 ml of incubation mixture was treated with 2.5 ml of 10% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 30 min to precipitate protein. Then 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. Then the tubes were cooled to room temperature and the absorbance was measured at 530 nm against a TBA 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 absorbance of the mixture is directly proportional to concentration of MDA estimated from the standard curve.[24]

Results and Discussion

The results of the study are illustrated in Table 1 and Table 2 as well as in Fig. 1 and Fig. 2. Interpretation of the results is supported by Student's t-test. Incubation of the liver homogenates with etoposide caused an enhancement in MDA content with respect to control of corresponding time interval (Table 1-2; Fig. 1-2). These observations revealed the fact that the drug etoposide has lipid peroxidation induction potential. It was further found that enhancement MDA content due to etoposide was significantly suppressed when the liver homogenate was incubated with both the drug and antioxidants like ascorbic acid and lactic acid. This indicates that both ascorbic acid and lactic acid could significantly reduce the lipid peroxidation induction potential of etoposide due to their free radical scavenging capacity. When the liver homogenate was treated with only ascorbic acid / lactic acid, the MDA content was further decreased with respect to control and this indicates the antioxidant property of both ascorbic acid and lactic acid. It was further observed from the results that the antioxidant potential of lactic acid is greater than that of ascorbic acid. Thus lactic acid merit further study to establish its role as an effective and potential antioxidant.

Table 1. Effect of ascorbic acid (AA) on etoposide-induced lipid peroxidation: percent change in MDA content

Incubation Period	Animal set	Percent changes in MDA content		
		Samples		
		D	DA	A
3h	1	43.87	29.72	5.51
	2	9.80	-5.06	9.28
	3	56.22	26.93	-0.40*
	4	17.06	-4.29	-16.00
	5	23.33	12.24	-21.45
	Mean± SE	30.05 ± 8.66	11.90 ±7.39	-10.52±3.73

Incubation Period	Animal set	Percent changes in MDA content		
		Samples		
		D	DA	A
4h	1	21.53	-9.21	-48.75
	2	16.16	-47.62	-72.13
	3	55.25	15.80	-8.28
	4	10.14	-14.31	-27.30
	5	11.70	3.23	-32.72
	Mean± SE	22.95 ±8.31	-10.42±10.66	-37.83±10.73

*Percent changes with respect to controls of corresponding hours are shown. Reproducibility measured by 't' test and all the values are significant except marked with *. D, DA, A indicate etoposide-treated, etoposide & ascorbic acid-treated and ascorbic acid-treated respectively. Av = average of five animal sets; SE = standard error (df = 4); df = degrees of freedom.*

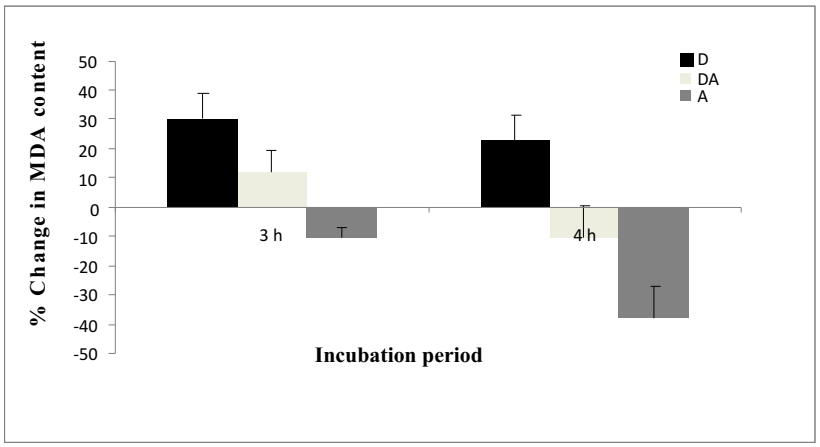

Fig. 1: Effect of ascorbic acid on etoposide-induced lipid peroxidation (D, DA and A indicate only etoposide-treated, etoposide and ascorbic acid-treated, and only ascorbic acid-treated samples, respectively)

Table 2. Effect of lactic acid (LA) on etoposide-induced lipid peroxidation: percent change in MDA content

Incubation Period	Animal set	Percent changes in MDA content		
		Samples		
		D	DA	A
3h	1	33.85	-39.12	-55.07
	2	39.74	-35.93	-49.46
	3	34.19	17.08	-2.13
	4	30.56	-14.82	-25.61
	5	41.49	-4.66	-15.38
	Mean± SE	35.96±2.02	-15.49±10.37	-29.53±10.03

Incubation Period	Animal set	Percent changes in MDA content		
		Samples		
		D	DA	A
4h	1	85.92	-28.14	-43.89
	2	52.99	-25.04	-42.10
	3	28.20	12.53	-40.11
	4	24.95	-18.98	-41.12
	5	43.71	-19.57	-28.71
	Mean± SE	47.15 ±10.95	-15.84±7.29	-39.18±2.69

Percent changes with respect to controls of corresponding hours are shown. Reproducibility measured by 't' test and all the values are significant. D, DA, A indicate etoposide-treated, etoposide & lactic acid-treated and lactic acid-treated respectively. Av = average of five animal sets; se = standard error (df = 4); df = degrees of freedom.

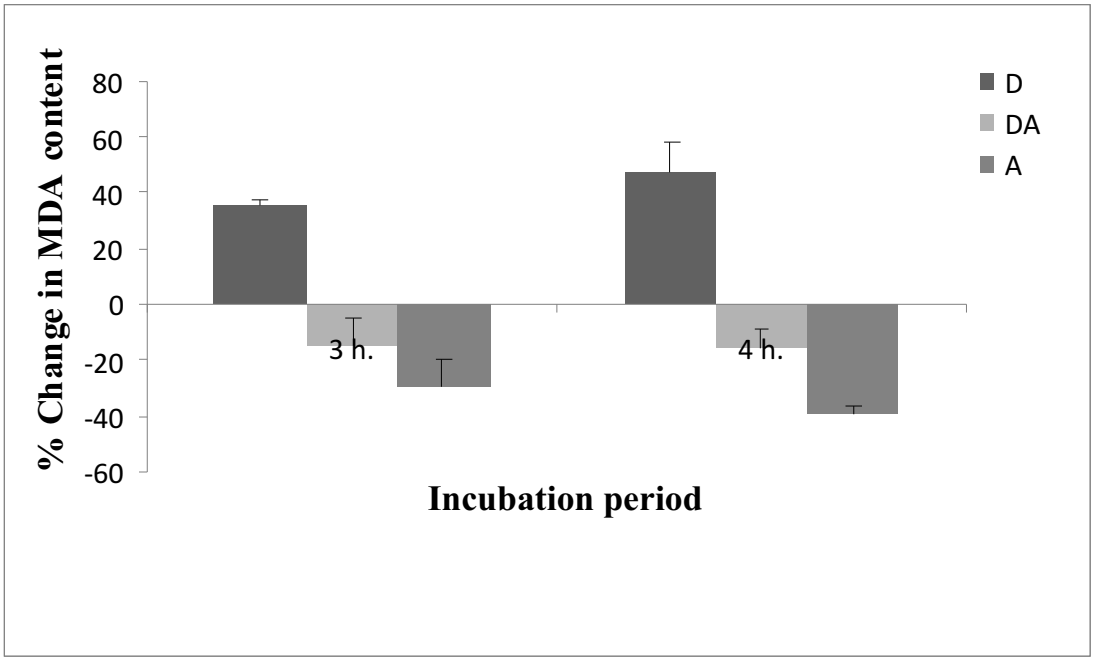

Fig. 2: Effect of lactic acid on etoposide-induced lipid peroxidation (D, DA and A indicate only etoposide-treated, etoposide and lactic acid-treated, and only lactic acid-treated samples, respectively).

Conclusion

One important reason behind the adverse reactions of etoposide might be its lipid peroxidation induction capacity that could be suppressed on co-administration of antioxidants like ascorbic acid and lactic acid. The antioxidant efficiency of lactic acid is found to be more than that of ascorbic acid. Thus lactic acid might be a future promising antioxidant. To draw any final conclusion a detail and extensive study using more parameters is required.

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