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Original Research

Evaluation of protective role of nifedipine on lipid peroxidation using reduced glutathione as model marker

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Abstract

This study was designed with an aim to explore any possible relationship existing between nifedipine-lipid interaction (lipid peroxidation) using reduced glutathione as model marker. Also attempts have been made to explore the effects of various antioxidants like ascorbic acid, butylated hydroxytoluene and naringin on this interaction. The study was performed *in vitro* using goat liver as lipid source. The study reveals that the nifedipine could significantly arrest the lipid peroxidation process. The results also show that along with antioxidants the antiperoxidative action of nifedipine is enhanced.

INTRODUCTION

Lipid peroxidation leads to oxidative damage in cell membranes, lipoproteins, and other lipid-containing structures [1]. It is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically [2-4]. The latter form is associated mostly with cellular damage as a result of oxidative stress [5]. Free radicals are constantly being generated in the body through various mechanisms and also being removed by endogenous antioxidant defense mechanism that acts by scavenging free radicals, decomposing peroxides and/or binding with pro-oxidant metal ion. Free radical mediated oxidative stress results usually from deficient natural antioxidant defense. Oxidative stress in lipid peroxidation is responsible for initiating and developing many condition and diseases of modern time like diabetes, liver cirrhosis, nephrotoxicity, aging *etc* [6-7]. In case of reduced or impaired defense mechanism and excess generation of free radicals that are not counterbalanced by endogenous antioxidant defense, exogenously administered antioxidants have been proven useful to overcome oxidative damage.

Nifedipine, an antihypertensive drug acts as an L-type voltage-gated Ca^{2+} channel blocker. But recent study shows that nifedipine treatment can reduce ischemic lesion volume after focal cerebral ischemia, possibly because of the decrease in oxidative stress with an increase in antioxidant activities within the ischemic area [8]. Another study indicates that nifedipine is beneficial as a protective agent against nephrotoxicity and oxidative stress induced by cyclosporine [9]. It was observed that melatonin and nifedipine has beneficial role on various antioxidant enzymes and different energy fuels in the blood and brain of global ischemic rats [10]. Another study shows that nifedipine has a remarkable chain breaking effect on the plasma oxidative damage induced by copper [11]. It was also observed that ascorbic acid, cimetidine and nifedipine have protective effect on diethyldithiocarbamate-induced hepatic toxicity in albino rats [12].

In view of the above findings the present experimental study is designed with an aim to explore any possible relationship existing between nifedipine-lipid interaction (lipid peroxidation) using reduced glutathione (GSH) as model marker. Attempts have also been made to explore the effects of various antioxidants like ascorbic acid, butylated hydroxytoluene and naringin on this interaction.

MATERIALS AND METHODS

Pure samples of nifedipine used in present study was provided by Abbott Health Care Pvt. Ltd. (Baddi, India); ascorbic acid and trichloroacetic acid (TCA) were purchased from CDH Bioscienses (New Delhi, India); GSH was from Sigma Chemicals (St. Louis, MO, USA); 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was from SRL Diagnostics (Mumbai, India); butylated hydroxytoluene and naringin were from Merck (Mumbai, India); all other reagents were of analytical grade and locally purchased.

Preparation of tissue homogenate

Goat liver was collected from Durgapur Municipal Corporation (DMC) approved outlet. Goat liver was selected because of its easy availability and close similarity with human liver in its lipid profile [13]. Goat liver perfused with normal saline through hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were then transferred in a sterile vessel containing phosphate buffer (pH 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately grinded to make a tissue homogenate (1 g/ml) using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts, which were then treated differently as mentioned below.

One portion of the homogenate was kept as control while a second portion was treated with the nifedipine at a concentration of 0.00336 mg/g tissue homogenate. The third portion was treated with both nifedipine at a concentration 0.00336 mg/g tissue homogenate and ascorbic acid/butylated hydroxytoluene naringin at a concentration of 0.03328 mg/g tissue homogenate and the fourth portion was treated only with ascorbic acid/butylated hydroxytoluene/naringin at a concentration of 0.03328 mg/g tissue homogenate. After nifedipine and/or ascorbic acid/butylated hydroxytoluene/naringin treatment, the liver tissue homogenate samples were shaken for two hours.

Estimation of reduced glutathione level from tissue homogenate

Reduced glutathione (GSH) was measured in accordance with Ellman's method [14]. The estimation was done at two hours of incubation and repeated in three animal sets. In each case three samples of 1 ml of incubation mixture were treated with 1 ml of 5% (w/v) TCA in 1 mM EDTA centrifuged at 2000g for 10 min. After that 1 ml of the filtrate was mixed with 5 ml of 0.1 M phosphate buffer (pH 8.0) and 0.4 ml of DTNB (0.01% in phosphate buffer, pH 8.0) was added to it. The absorbances of the solutions were measured at 412 nm against blank (prepared from 6 ml of phosphate buffer and 0.4 ml of DTNB). The concentrations of GSH were determined from standard curve, which was constructed as follows. Different aliquots of standard GSH stock solution were taken in 10 ml volumetric flasks. To each solution 0.4 ml of DTNB solution was added and volume was adjusted up to the mark with phosphate buffer (pH 8.0). The absorbance of each solution was measured at 412 nm against a blank containing 9.6 ml of phosphate buffer (pH 8.0) and 0.4 ml DTNB solution. By plotting absorbances against concentration a straight line passing through the origin of grid was obtained.

Statistical analysis

Interpretation of the results is supported by student t-test. Analysis of variance (ANOVA) and multiple comparison analysis using least significant different (LSD) procedure [15, 16] were also performed on the percent changes data of various groups such as nifedipine-treated, nifedipine and ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated with respect to control group of corresponding time.

RESULTS

From Tables 1, 2 and 3 it was evident that incubation of liver tissue homogenates with nifedipine showed an increase in GSH content with respect to control to a significant extent. The increase in GSH content was associated with a decrease in lipid peroxidation. Hence, it may be inferred that the drug could significantly arrest the lipid peroxidation process. When the tissue homogenates were treated both with nifedipine and ascorbic acid/butylated hydroxytoluene/naringin then also the GSH levels increased in comparison to control group of corresponding hours. Again, when the tissue homogenates were treated with ascorbic acid/butylated hydroxytoluene/naringin alone the GSH content was also increased in comparison to the control samples.

From the results it was also observed that when nifedipine was used in combination with butylated hydroxytoluene/naringin, the GSH contents were higher than only nifedipine treated group. But when ascorbic acid was used along with nifedipine then the GSH content is slightly lower than only nifedipine treated group.

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Table 1. Effects of ascorbic acid on nifedipine-lipid interaction (lipid peroxidation): % changes in GSH content

Hours of incubation	Animal sets	Percent changes in GSH content Samples			ANOVA & Multiple comparison
		D	DA	A	
2 h	AL1	15.31[a]	43.17[a]	64.05[b]	F1 = 4.76 [df = (2, 4)] F2 = 2.37 [df = (2, 4)] Pooled variance (S^2)* = 415.98 Critical difference (p = 0.05)# LSD = 38.4 Ranked means** (D, DA, A)
	AL2	12.59[b]	9.94[c]	15.24[a]	
	AL3	59.06[c]	31.65[a]	100.89[c]	
	AV (± SEM)	28.99 (± 15.1)	28.25 (± 9.74)	60.06 (± 24.8)	

Percent changes with respect to controls of corresponding hours were shown as [a]>99%, [b]97.5-99%, [c]95-97.5%; theoretical values of F: p = 0.1 level are F1 = 4.32 [df = (2, 4)], F2 = 4.32 [df = (2,4)]; p = 0.05 level F1 = 6.94 [df = (2, 4)], F2 = 6.94 [df = (2, 4)]. F1 and F2 correspond to variance ratio between groups and within groups, respectively; D, DA, and A indicate nifedipine-treated, nifedipine & ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated groups, respectively; AV= averages of three animal sets; SEM = standard error of the mean (df = 2); df = degree of freedom.; *error mean square; #critical difference according to least significant procedure; **two means not included within same parenthesis are statistically significantly different at p = 0.05 level.

Table 2. Effects of butylated hydroxytoluene on nifedipine-lipid interaction (lipid peroxidation): % changes in GSH content

Hours of incubation	Animal sets	Percent changes in GSH content Samples			ANOVA & Multiple comparison
		D	DA	A	
2 hrs	AL1	3.24[b]	9.3[a]	16.32[a]	F1 = 12.5 [df = (2, 4)] F2 = 3.73 [df = (2, 4)] Pooled variance (S^2)* = 106.01 Critical difference (p = 0.05)# LSD=19.38 Ranked means** (D, DA, A)
	AL2	31.3[c]	44.8[c]	76.1[b]	
	AL3	30.8[b]	40.9[b]	41.6[c]	
	AV (± SEM)	21.78 (± 9.27)	31.67 (± 11.23)	44.67 (± 17.32)	

Percent changes with respect to controls of corresponding hours were shown as [a]>99%, [b]97.5-99%, [c]95-97.5%; theoretical values of F: p = 0.1 level are F1 = 4.32 [df = (2, 4)], F2 = 4.32 [df = (2,4)]; p = 0.05 level F1 = 6.94 [df = (2, 4)], F2 = 6.94 [df = (2, 4)]. F1 and F2 correspond to variance ratio between groups and within groups, respectively; D, DA, and A indicate nifedipine-treated, nifedipine & ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated groups, respectively; AV= averages of three animal sets; SEM = standard error of the mean (df = 2); df = degree of freedom.; *error mean square; #critical difference according to least significant procedure; **two means not included within same parenthesis are statistically significantly different at p = 0.05 level.

Table 3. Effects of naringin on nifedipine-lipid interaction (lipid peroxidation): % changes in GSH content

Hours of incubation	Animal sets	Percent changes in GSH content Samples			ANOVA & Multiple comparison
		D	DA	A	
2 hrs	AL1	6.95[b]	7.91[d]	8.48[b]	F1 = 15.3 [df = (2, 4)] F2 = 2.55 [df = (2, 4)] Pooled variance (S^2)* = 20.35 Critical difference (p = 0.05)# LSD = 8.49 Ranked means** (D, DA, A)
	AL2	7.22[a]	5.04[b]	14.7[c]	
	AL3	16.99[b]	28.31[b]	32.8[a]	
	AV (± SEM)	10.39 (± 3.3)	13.75 (± 7.37)	18.66 (± 7.29)	

Percent changes with respect to controls of corresponding hours were shown as [a]>99%, [b]97.5-99%, [c]95-97.5%, [d]90-95%; theoretical values of F: p = 0.1 level are F1 = 4.32 [df = (2, 4)], F2 = 4.32 [df = (2,4)]; p = 0.05 level F1 = 6.94 [df = (2, 4)], F2 = 6.94 [df = (2, 4)]. F1 and F2 correspond to variance ratio between groups and within groups, respectively; D, DA, and A indicate nifedipine-treated, nifedipine & ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated groups, respectively; AV= averages of three animal sets; SEM = standard error of the mean (df = 2); df = degree of freedom.; *error mean square; #critical difference according to least significant procedure; **two means not included within same parenthesis are statistically significantly different at p = 0.05 level.

To compare 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. It is seen that there is significant differences among various groups (F1) such as nifedipine-treated, nifedipine and ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated. But within a particular group differences are insignificant (Tables 1-3). The Tables also indicate that there are no statistically significantly differences among the nifedipine-treated, nifedipine and ascorbic acid/butylated hydroxytoluene/naringin-treated and only ascorbic acid/butylated hydroxytoluene/naringin-treated groups.

DISCUSSION

From the results of the present study, it is evident that nifedipine could increase GSH content of liver homogenates with respect to the corresponding hours of controls to a significant extent after incubation for varying period of times. So, it may be concluded that the drug could significantly arrest the lipid peroxidation process.

Glutathione is a small protein composed of three amino acids, such as, cysteine, glutamic acid and glycine. It is an important antioxidant and plays a very important role in the defense mechanism for tissue against the reactive oxygen species [17]. The increase of GSH is associated with decrease in lipid peroxidation. The increase in GSH level may be a consequence of decreased utilization of this compound by the antioxidant enzymes glutathione peroxidase and glutathione-S-transferase. A very fast reaction occurred between GSH and hydroxyl radical (•OH) and gives thiol radicals. This radical can also be formed when GSH is oxidized by peroxidase, or by O_2 in the presence of transition metal ions. This thiol radical, also less reactive than •OH in conjugation with superoxide dismutase (SOD) and GSH, converts superoxide anions into hydrogen peroxide. Glutathione peroxidase converts hydrogen peroxide into water [18]. As a result of the second conversion, GSH is oxidized to glutathione disulphide (GSSG). In this way, GSH acts as a cofactor in the removal of toxic radicals from the body. During oxidative stress GSH level declines and GSSG level increases. So, there is a direct correlation between increase in lipid peroxidation and decrease in GSH level [19-20].

It was reported earlier that nifedipine increases endothelial nitric oxide release and thereby gives protective action against cyclo-sporine induced nephrotoxicity [9]. Another study shows that photodegraded product of nifedipine has beneficial role on oxidative stress [21]. The results also show that along with antioxidants the antiperoxidative action of nifedipine is enhanced. In summary, the above exploratory studies reveal that nifedipine has the capability to suppress lipid peroxidation occurring through free radical mechanism.

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