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Research Article

ROLE OF APIGENIN ON GEMCITABINE-INDUCED LIPID PEROXIDATION

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ABSTRACT

The present study explores the role of apigenin on gemcitabine-induced lipid peroxidation. The work was carried out *in vitro* and goat liver was used as model lipid source. Estimation of malondialdehyde and reduced glutathione of the tissue content were used as marker for the model. The results showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent and it was also found that apigenin has the ability to suppress the gemcitabine-induced toxicity.

KEYWORDS: Gemcitabine, lipid peroxidation, apigenin, malondialdehyde, reduced glutathione

INTRODUCTION

Free radical generated in the body due to oxidative stress is responsible for several diseases. One such free radical is reactive oxygen species. It is formed from molecular oxygen by partial reduction. Free radicals attack the lipid and are responsible for lipid peroxidation. Lipid peroxidation is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically[1-3]. The cytotoxic end products of lipid peroxidation are mainly aldehydes as exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc[4].

Gemcitabine belongs to antimetabolites. Chemically it is a pyrimidine nucleoside prodrug. It is used mainly in breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer and bladder cancer. But along with its use the compound also produces several side effects such as pale skin, easy bruising or bleeding, numbness or tingly feeling, weakness, nausea, vomiting, upset stomach, diarrhea, constipation, headache, skin rash, drowsiness, hair loss etc[5].

Apigenin, a common dietary flavonoid abundantly present in fruits and vegetables. One study shows that apigenin inhibits the growth of androgen-responsive human prostate carcinoma LNCaP cells[6]. Another work shows the role of apigenin in lipopolysaccharide-Induced Inflammatory in Acute Lung Injury by Suppressing COX-2 and NF-kB Pathway[7].

In view of the above findings and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation[8-9] the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of apigenin on gemcitabine-induced lipid peroxidation.

MATERIALS AND METHODS

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi. Apigenin and 1,1,3,3, tetraethoxypropane were from Sigma chemicals Co. St. Louis, MO, USA. 5, 5' dithiobis (2-nitrobenzoic acid) was from SRL Pvt. Ltd., Mumbai. Pure sample of gemcitabine used in present study was obtained from Parchem, New Rochelle, New York, USA. All other reagents were of analytical grade. Goat liver was used as the lipid source.

Preparation of tissue homogenate

Goat liver was collected from Silchar Municipal Corporation approved outlet. Goat liver was selected because of its easy availability and close similarity with human liver in its lipid profile[10]. 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 (C) while a second portion was treated with the gemcitabine (D) at a concentration of 1.2mg/g tissue homogenate. The third portion was treated with both gemcitabine at a concentration 1.2mg/g tissue homogenate and apigenin at a concentration of 0.1666 mg / g homogenate (DA) and the fourth portion was treated only with apigenin at a concentration of 0.1666 mg / g tissue homogenate (A). After gemcitabine and /or morin treatment, the liver tissue homogenate samples were shaken for two hours and the malondialdehyde and reduced glutathione content of various portions were determined.

Estimation of malondialdehyde (MDA) level from tissue homogenate

The extent of lipid peroxidation was measured in terms of malondialdehyde (MDA) content using thiobarbituric acid (TBA) method[11]. The estimation was done at two hours of incubation and repeated in three animal sets. In each case three samples of 2.5 ml of incubation mixture were treated with 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) and centrifuged at room temperature

at 3000 rpm for 30 minutes to precipitate protein. Then 2.5 ml of the supernatant was treated with 5 ml of 0.002 (M) TBA solutions and then volume was made up to 10 ml with distilled water. The mixture was heated on a boiling water bath for 30 minutes. Then tubes were cooled to a room temperature and the absorbance was measured at 530 nm against a TBA blank (prepared from 5 ml of TBA solution and 5 ml of distilled water). The concentrations of MDA were determined from standard curve, which was constructed as follows. Different aliquots from standard 1, 1, 3, 3-tetrahydroxypropane (TEP) solution were taken in graduated stoppered test tubes and volume of each solution was made up to 5 ml. To each solution, 5 ml of TBA solution was added and the mixture was heated in a steam bath for 30 minutes. The solutions were cooled to a room temperature and their absorbances were measured at 530 nm against TBA as blank. By plotting absorbances against concentrations a straight line passing through the origin of grid was obtained. The best-fit equation is A=0.006776 M + 0.003467, where M= nanomoles of MDA, A= absorbance, r = 0.996, SEE= 0.0037, F=1068.76 (df=1,8).

Estimation of reduced glutathione (GSH) level from tissue homogenate

The extent of lipid peroxidation was measured in terms of reduced glutathione level by Ellman's method[12]. 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 2000 g for 10 minutes. After that 1 ml of the filtrate was mixed with 5 ml of 0.1M phosphate buffer (pH 8.0) and 0.4 ml of 5, 5'-dithiobis (2-nitrobenzoic acid in 0.01% in phosphate buffer pH 8.0) (DTNB) was added to it. The absorbances of the solutions were measured at 412 nm against blank (prepared from 6.0 ml of phosphate buffer and 0.4 ml of DTNB) (0.01% in phosphate buffer). The concentrations of reduced glutathione were determined from standard curve, which was constructed as follows. Different aliquots from standard reduced glutathione solution were taken in 10.00 ml volumetric flask. To each solution 0.04 ml of DTNB solution was added and volume was adjusted up to the mark with phosphate buffer. The absorbances of each solution were noted at 412 nm against a blank containing 9.60 ml phosphate buffer and 0.04 ml DTNB solution. By plotting absorbances against concentrations a straight line passing through the origin was obtained. The best-fit equation is A=0.001536 M - 0.00695, where M= nanomoles of GSH, A= absorbance, r = 0.995, SEE= 0.0067, F=1638.83 (df=1,8).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure[13-14] were also performed on the percent changes data of various groups such as gemcitabine-treated (D), gemcitabine and apigenin (DA) and only apigenin-treated (A) with respect to control group of corresponding time.

Table 1: Effect of apigenin on gemcitabine induced lipid peroxidation: Changes in MDA profile

Hours of incubation	Animal sets	% Changes in MDA content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
2	AL1	14.24^{b}	-5.26^{a}	-2.32^{b}	F1=192.77 [df= (2,4)]
	AL2	19.07^{c}	-4.38^{a}	-3.11^{a}	F2=0.90 [df= (2, 4)]
	AL3	17.18^{b}	-5.75^{c}	-2.82^{c}	Pooled variance $(S^2)^{*}$ =2.259
	AV.	16.83	-5.13	-2.75	Critical difference, (p=0.05) #
	(± S.E.)	(±1.40)	(±0.40)	(±0.23)	LSD =2.825
					Ranked means**
					(D) (DA, A)

% Changes with respect to controls of corresponding hours are shown: a>99%; b=97.5-99%; c=95-97.5%; d=90-95%; e=80-90%; f=70-80%; g=60-70%; h<60%; Theoretical values of F: p=0.05 level F1=4.46 [df=(2,4)], F2=3.84 [df=(2,4)]; P=0.01 level F1=8.65 [df=(2,4)], F2=7.01 [df=(2, 4)]. F1 and F2 corresponding to variance ratio between groups and within groups respectively.
D, DA, A indicate gemcitabine-treated, gemcitabine and apigenin-treated, apigenin-treated respectively. AV.= Averages of three animal sets; S.E.= Standard Error (df=2); df= degree of freedom; * Error mean square, # Critical difference according to least significant procedure[14] ** Two means not included within same parenthesis are statistically significantly different at p=0.05 level

Table 2: Effect of apigenin on gemcitabine induced lipid peroxidation: Changes in GSH profile

Hours of incubation	Animal sets	% Changes in GSH content			Analysis of variance & multiple comparison
		Samples			
		D	DA	A	
2	AL1	-20.34^{a}	2.34^{b}	1.02^{a}	F1=1156.29 [df= (2,4)]
	AL2	-18.12^{c}	3.17^{a}	0.98^{b}	F2=1.92 [df= (2, 4)]
	AL3	-19.62^{c}	3.29^{b}	1.12^{c}	Pooled variance $(S^2)^{*}$ =0.396
	AV.	-19.36	2.93	1.04	Critical difference, (p=0.05) #
	(± S.E.)	(±0.65)	(±0.29)	(±0.04)	LSD =1.18
					Ranked means**
					(D) (DA) (A)

% Changes with respect to controls of corresponding hours are shown: a>99%; b=97.5-99%; c=95-97.5%; d=90-95%; e=80-90%; f=70-80%; g=60-70%; h<60%; Theoretical values of F: p=0.05 level F1=4.46 [df=(2,4)], F2=3.84 [df=(2,4)]; P=0.01 level F1=8.65 [df=(2,4)], F2=7.01 [df=(2, 4)]. F1 and F2 corresponding to variance ratio between groups and within groups respectively.
D, DA, A indicate gemcitabine-treated, gemcitabine and apigenin-treated, apigenin-treated respectively. AV.= Averages of three animal sets; S.E.= Standard Error (df=2); df= degree of freedom; * Error mean square, # Critical difference according to least significant procedure[14] ** Two means not included within same parenthesis are statistically significantly different at p=0.05 level

RESULTS AND DISCUSSION

The markers used for measuring the lipid peroxidation are percent changes in MDA and GSH content with respect to corresponding control.

Treatment of liver tissue homogenate with gemcitabine indicated an increase in MDA level (16.83%) with respect to control. The finding showed the lipid peroxidation induction potential of gemcitabine. Liver tissue homogenate when treated both with gemcitabine and apigenin the MDA level (-5.13%) was reduced in respect to both control and only gemcitabine treated group. Again, the tissue homogenates were treated only with apigenin then the MDA (-2.75%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging activity of apigenin (**Table 1**).

In case of GSH estimation, it is observed that there was decrease in GSH content (-19.36%) when liver tissue homogenates were treated with gemcitabine. This indicated the lipid peroxidation induction potential of the drug. However, GSH content was increased when the samples were treated both gemcitabine and apigenin (2.93%). But the GSH content was significantly increased (2.93%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with apigenin. When the samples were treated only with apigenin, GSH level was also increased (1.04%). This increase may be explained by the free radical scavenging property of apigenin (**Table 2**).

To observe the statistical significance between various groups (F1) and within a particular group (F2), ANOVA and multiple analyses were carried out. In case of MDA content gemcitabine-treated group is statistically different from gemcitabine and apigenin-treated and only apigenin-treated groups. But there is no difference between gemcitabine and apigenin-treated and only apigenin-treated groups (**Table 1**). However, for GSH content all three groups i.e. gemcitabine –treated, gemcitabine and apigenin-treated and only apigenin-treated groups are statistically significantly different from each other (**Table 2**).

CONCLUSION

The results showed the lipid peroxidation capacity of gemcitabine and demonstrate the antiperoxidative property of apigenin on drug induced lipid peroxidation. However, a detailed study has to be carried out to advance the hypothesis.

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