Research Article

Protective role of α-tocopherol on gemcitabine-induced lipid peroxidation

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Received: 9 November 2018 Revised: 7 January 2019 Accepted: 29 January 2019

Abstract

Objective: The work was aimed to explore protective role of α-tocopherol on gemcitabine-induced lipid peroxidation. **Material and methods:** The goat liver tissue homogenate was used a source of lipid. The work was carried out *in vitro*. Estimation of malondialdehyde and reduced glutathione were considered as marker of lipid peroxidation. They were estimated as per the standard procedure with little modification **Results and conclusion:** The results showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent by increasing the malondialdehyde content / reducing the reduced glutathione content in the liver tissue homogenate. But α-tocopherol reduces the malondialdehyde content / increase the reduced glutathione content. These indicate the potential of α-tocopherol to suppress the gemcitabine-induced lipid peroxidation.

***Keywords**:* Gemcitabine, α-tocopherol, lipid peroxidation, malondialdehyde, reduced glutathione

Introduction

Alpha-tocopherol is one of widely used vitamin applied as antioxidant. One such work reported that use of vitamin E protects the acrosome from oxidative damage, as well as decreases intracellular oxidative activity (Adani et al., 2018). Another study showed that alpha-tocopherol along with omega-3 fatty acid has beneficial role on malnutrition-inflammation score (Asemiet al., 2016). Lipid peroxidation is a free radical related process that may occur in the biological system under enzymatic control or non-enzymatically (Gutteridge and Halliwell, 2000; Stohs, 1995; Romeroet al., 1998). The cytotoxic end products of lipid peroxidation are mainly aldehydes as exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc. Gemcitabine is a newer anticancer drug and mainly used in breast cancer and ovarian 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 (Zhang et al., 2017). In one work, it is reported that gemcitabine has improved efficacy when used along with vitamin E (Abu-Fayyad et al., 2017). In view of the above findings, the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of α-tocopherol on gemcitabine-induced lipid peroxidation.

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Materials and methods

Materials

Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi. 1,1,3,3, tetraethoxypropane and reduced glutathione were from Sigma chemicals Co. St. Louis, MO, USA. 5, 5' dithiobis (2-nitrobenzoic acid) was from SRL Pvt. LTd., Mumbai. Morin was from CDH Pvt. Ltd., New Delhi. 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 (Hilditch and Williams, 1964). 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)

DOI: https://doi.org/10.31024/ajpp.2019.5.3.20

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 α-tocopherol at a concentration of 0.1666 mg/g homogenate (DA) and the fourth portion was treated only with α-tocopherol 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 thiobarbuturic acid (TBA) method (Ohkawaet al., 1979). 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 absorbance was measured at 530 nm against TBA as blank. By plotting absorbance 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 (George, 1959). 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 absorbance of the solutions was 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 absorbance of each solution was noted at 412 nm against a blank containing 9.60 ml phosphate buffer and 0.04 ml DTNB solution. By plotting absorbance 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 (Snedecor and Cochran, 1967; Bolton, 2000) were also performed on the percent changes data of various groups such as gemcitabine-treated (D), gemcitabine and α-tocopherol (DA) and only α-tocopherol-treated (A) with respect to control group of corresponding time.

Results and discussion

The percent changes in MDA and GSH content of different samples at two hours of incubation were calculated with respect to the control of the corresponding time of incubation and was considered as indicator of the extent of lipid peroxidation.

From table 1 it was evident that tissue homogenates treated with gemcitabine showed an increase in MDA (22.33 %) content in samples with respect to control at two hours of incubation to a significant extent. The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. But the MDA (-7.75 %) content were significantly reduced in comparison to gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with α-tocopherol. Again the tissue homogenates were treated only with the α-tocopherol then the MDA (-3.47%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging property of the α-tocopherol.

Tissue homogenates treated with gemcitabine showed a

Table 1. Effect of α-tocopherolon 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	21.34[a]	-7.12[a]	-4.10[a]	F1=395.94 [df=(2,4)]
	AL2	24.82[a]	-8.20[b]	-3.23[a]	F2=0.65 [df=(2, 4)]
	AL3	20.84[a]	-7.94[a]	-3.08[a]	Pooled variance
	AV. (±	22.33	-7.75	-3.47	$(S^2)^*$ =2.007
	S.E.)	(±1.25)	(±0.32)	(±0.32)	Critical difference,(p=0.05)[#]
					LSD =2.66
					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=6.94 [df=(2,4)], F2=6.94 [df=(2, 4)], F1 and F2 corresponding to variance ratio between groups and within groups respectively. D, DA, A indicate gemcitabine-treated, gemcitabine andα-tocopherol-treated, α-tocopherol-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(Bolton, 2000) **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

Table 2. Effect of α-tocopherol 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	-2.32[b]	7.28[b]	4.12[b]	F1=463.15 [df=(2,4)]
	AL2	-3.17[b]	8.10[a]	3.88[a]	F2=0.033 [df=(2, 4)]
	AL3	-2.88[a]	7.76[a]	4.10[a]	Pooled variance
	AV.	-2.79	7.71	4.03	$(S^2)^*$ =0.1839
	(± S.E.)	(±0.24)	(±0.24)	(±0.07)	Critical difference,(p=0.05)[#]
					LSD =0.81
					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=6.94 [df=(2,4)], F2=6.94 [df=(2, 4)], F1 and F2 corresponding to variance ratio between groups and within groups respectively. D, DA, A indicate gemcitabine-treated, gemcitabine andα-tocopherol -treated, α-tocopherol -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 (Bolton, 2000) **Two means not included within same parenthesis are statistically significantly different at p=0.05 level

decrease in GSH (-2.79%) content in samples with respect to control to a significant extent (**Table 2**). The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. But the GSH content was significantly increased (7.71%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with α-tocopherol. Again the tissue homogenates was treated only with α-tocopherol then the GSH level was increased (4.03%) in comparison to the control and the gemcitabine treated group. This increase may be explained by the free radical scavenging property of α-tocopherol.

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 gemcitabine-treated, gemcitabine and morin-treated and only morin-treated. But within a particular group, differences (F2) are insignificant which shows that there is no statistical difference in animals in a particular group (Tables 1 & 2). The Table 1and 2 also indicated that for MDA/ GSH content, gemcitabine-treated group, gemcitabine and α-tocopherol-treated and only α-tocopherol-treated groups are statistically significantly different from each other.

Conclusion

The results showed that gemcitabine has lipid peroxidation induction potential which may be related to its toxic potential. The results also suggest the antiperoxidative effects of alpha-tocopherol and demonstrate its potential to reduce gemcitabine induced toxic effects.

Conflict of Interest

The author declares no conflicts of interest

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