Research Article

Evaluation for protective role of quercetin on gemcitabine-induced lipid peroxidation using common laboratory markers

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Received: 3 August 2018 Revised: 19 August 2018 Accepted: 10 September 2018

Abstract

Objective: The work was designed is to explore the protective role of quercetin on gemcitabine-induced lipid peroxidation. **Material and methods**: The *in vitro* study was carried out using goat liver as model lipid source. Two common laboratory markers such as malondialdehyde and reduced glutathione were used for the model. **Results and conclusion**: The results showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent and it was also found that quercetin has the ability to suppress the gemcitabine-induced toxicity.

***Keywords*:** Gemcitabine, quercetin, lipid peroxidation, malondialdehyde, reduced glutathione

Introduction

The flavonoids has potential role as antioxidant. Quercetin is one of the flavonoids showed antioxidant activity and gives protection against the oxidative stress. In one the recent studies it was observed that quercetin prevents docetaxel induced testicular damage in rats (Altintaset al., 2015). Gemcitabine is a pyrimidine nucleoside pro-drug and has wide application in cancer therapy particularly breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer and bladder cancer etc (Zhang et al., 2017). Free radical played vital role in lipid peroxidation and it may occur in the biological system under enzymatic control or non-enzymatically (Gutteridge and Halliwell, 2000; Stohs, 1995; Romero et al., 1998). Malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc are the cytotoxic end products of lipid peroxidation. Resveratrol and capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model (Vendrely et al., 2017). In another study, it was observed that gemcitabine showed 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 quercetin on gemcitabine-induced lipid peroxidation.

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Materials and Methods

Materials and reagents

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. Quercetin was procured from Himedia Bioscience, 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 (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) using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts, which were

DOI: https://doi.org/10.31024/ajpp.2018.4.6.18

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 quercetin at a concentration of 0.1666 mg / g homogenate (DA) and the fourth portion was treated only with quercetin at a concentration of 0.1666 mg / g tissue homogenate (A). After gemcitabine and /or quercetin 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 (Ohkawa et al., 1979). The estimation was done at two h 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 m 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 m. 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 m. 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 (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 m. 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 (Snedecor and Cochran, 1967; Bolton, 2000) were also performed on the percent changes data of various groups such as gemcitabine-treated (D), gemcitabine and quercetin (DA) and only quercetin -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.

Results showed in table 1 were evident that tissue homogenates treated with gemcitabine showed an increase in MDA (29.46 %) 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. MDA is a highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism (Yahya et al., 1996). But the MDA (-16.67 %) content were significantly reduced in comparison to gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with quercetin. Again the tissue homogenates were treated only with the quercetin then the MDA (-15.19%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging property of the quercetin. So the decrease in

Table 1. Effect of Quercetin 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	23.88[a]	-12.01[b]	-8.64[a]	F1=16.27 [df=(2,4)]
	AL2	45.07[a]	-20.65[a]	-24.17[b]	F2=0.14 [df=(2, 4)]
	AL3	19.43[a]	-17.36[a]	-12.78[a]	Pooled variance
	AV. (± S.E.)	29.46 (±7.91)	-16.67 (±2.51)	-15.19 (±4.64)	$(S^2)^*$ =126.71
					Critical difference,(p=0.05)[#]
					LSD =12.23
					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 and quercetin-treated, quercetin-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 Quercetin 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	-12.52[a]	8.29[a]	6.64[b]	F1=283.84 [df=(2,4)]
	AL2	-13.85[b]	9.42[b]	5.54[a]	F2=0.28[df=(2, 4)]
	AL3	-11.06[a]	7.63[b]	6.72[a]	Pooled variance
	AV. (± S.E.)	-12.47 (±0.80)	8.45 (±0.52)	6.30 (±0.38)	$(S^2)^*$ =1.4
					Critical difference,(p=0.05)[#]
					LSD =2.22
					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 and quercetin-treated, quercetin-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

MDA content of samples, when treated with gemcitabine and quercetin implies the free radical scavenging property of quercetin.

It was also evident from table 2 that tissue homogenates treated with gemcitabine showed a decrease in GSH (-12.47%) content in samples with respect to control to a significant extent. The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. Glutathione is a small protein composed of three amino acid, such as cysteine, glutamic acid and glycine (Benet and

Schewartz, 1996). It is an important antioxidant and plays a very important role in the defense mechanism for tissue against the reactive oxygen species (Wilkinson, 2001). But the GSH content was significantly increased (8.45%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with quercetin. Again the tissue homogenates was treated only with quercetin then the GSH level was increased (6.3%) in comparison to the control and the gemcitabine treated group. This increase may be explained by the free radical scavenging property of the quercetin.

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 quercetin -treated and only quercetin -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). It was also observed that for both MDA and GSH content gemcitabine-treated group is statistically different from gemcitabine and quercetin-treated and only quercetin -treated groups. But there is no difference between gemcitabine and quercetin-treated and only quercetin -treated groups.

Conclusions

The findings of the work showed the lipid peroxidation induction potential of gemcitabine, which may be related to its toxic potential. The results also suggest the antiperoxidative effects of quercetin and demonstrate its potential to reduce gemcitabine induced toxic effects.

Conflict of Interest

The author declares no conflicts of interest

References

Altintas R, Ciftci O, Aydin M, Akpolat N, Oguz F, Beytur A. 2015. Quercetin prevents docetaxel-induced testicular damage in rats. Andrologia, 47(3):248-56.

Abu-Fayyad A, Nazzal S. Gemcitabine-vitamin E conjugates. 2017. Synthesis, characterization, entrapment into nanoemulsions, and in-vitro deamination and antitumor activity. International Journal of Pharmaceutics, 528:463-70.

Bolton S. 2000. Statistics. In: Gennaro AR, (ed.) Remington: The Science and Practice of Pharmacy, pp. 124-58, Philadelphia, Lippincott Williams & Wilkins.

Benet LZ, Schewartz JB. 1996. In Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., pp. 707-16, New York, Mc Graw- Hill.

Gutteridge JMC, Halliwell B. Free radicals and antioxidants in the year. 2000. A historical look to the future. Annals of the New York Academy of Sciences, 899:136-47.

George EL. 1959. Tissue Sulfhydryl Groups. Archives of Biochemistry and Biophysics, 82(1):70-7.

Hilditch TP, Williams PN. 1964. The Chemical Constituents of Fats. London, Chapman & Hall.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analytical Biochemistry, 95(2):351-58.

Romero RJ, BoschMorell F, Romero MJ, Jareno EJ, Romero B, Marin N, Roma J. 1998. Lipid peroxidation products and antioxidants in human disease. Environmental Health Perspective, 106 (5):1229-34.

Stohs SJ. 1995. The role of free radicals in toxicity and disease. Journal of Basic and Clinical Physiology and Pharmacology, 6(3-4):205-28.

Snedecor GW, Cochran WG. 1967. Statistical Methods. New Delhi, Oxford & IBH Publishing Co Pvt Ltd.

Vendrely V, Peuchant E, Buscail E, Moranviller I, Rousseau B, Bedel A, Brillac A, de Verneuil H, Moreau-Gaudry F, Dabernat S. 2017. Resveratrol and capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model. Cancer Letters, 390:91-102.

Wilkinson GR. 2001. In Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed., pp. 10-20, New York, Mc Graw- Hill.

Yahya MD, Pinnsa JL, Meinke GC, Lung CC. 1996. Antibodies against malondialdehyde (MDA) in MLR / lpr / lpr Mice: Evidence for an autoimmune mechanism involving lipid peroxidation. Journal of Autoimmunity, 9:3-9.

Zhang XW, Ma YX, Sun Y, Cao YB, Li Q, Xu CA. 2017. Gemcitabine in Combination with a Second Cytotoxic Agent in the First-Line Treatment of Locally Advanced or Metastatic Pancreatic Cancer: a Systematic Review and Meta-Analysis. Targeted Oncology, 12(3):309-21.