Short Communication

Role of Kaempferol on gemcitabine lipid interaction in goat liver model

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Abstract

Objective: The study was done in vitro to explore antiperoxidative potential of kaempferol on gemcitabine-induced lipid peroxidation. **Material and methods:** Goat liver tissue homogenate was used as source of lipid. Two common laboratory markers such as 4-hydroxy-2-nonenal and reduced glutathione were used for the model. **Results and conclusion:** It was observed that for 4-HNE content, gemcitabine-treated group is statistically different from gemcitabine and kaempferol-treated and only kaempferol-treated groups, but there is no difference between gemcitabine and kaempferol-treated and only kaempferol-treated groups. For GSH content gemcitabine-treated group, gemcitabine and kaempferol-treated and only kaempferol-treated groups are statistically significantly different from each other. The result showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent and it was also found that kaempferol has the ability to suppress the gemcitabine-induced toxicity.

Keywords*:* Gemcitabine, kaempferol, lipid peroxidation, 4-hydroxy-2-nonenal, reduced glutathione

Introduction

In cancer chemotherapy, drugs are used to kill the cancerous cell with little damage to normal cell. But most of the cytotoxic drugs affect the rapidly dividing normal tissue, and produce to a greater or lesser extent toxic side effects such as bone marrow toxicity, impaired wound healing, loss of hair, damage to gastrointestinal epithelium, depression of growth in children, sterility, teratogenicity, kidney damage, reversible liver abnormalities etc (Rang et al., 1999). Gemcitabine is a pyrimidine nucleoside prodrug. Mainly it is used in breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer and bladder cancer. However along with its desired effect it produces several side effects such as pale skin, diarrhea, headache, skin rash etc to mention a few (Zhang et al., 2017). Lipid peroxidation is oxidative deterioration of poly unsaturated fatty acid and producing some toxic end products like malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc (Esterbaueret al., 1998). Measurement of these end products may act as marker of lipid peroxidation.Gemcitabine showed improved efficacy when used along with alpha tocopherol (Abu-Fayyad et al., 2017**)**. In view of the above findings and ongoing search of antioxidants by the author (Dey Ray, 2018, Dey Ray 2018**)**, the present work has been carried out *in vitro* toevaluate the antiperoxidative potential of kaempferolon gemcitabine-induced lipid peroxidation by measuring 4-hydroxy-2- nonenal (4-HNE) and reduced glutathione (GSH) content.

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Materials and methods

Materials

Trichloroacetic acid (TCA) was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi. The standard sample of 4-HNE was purchased from ICN Biomedicals Inc., Aurora, Ohio.Kaempferol, 2, 4-dinitrophenyl hydrazine (DNPH) and reduced glutathione 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 Corporationapproved 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

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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.

Group I: One equal part of the homogenate was kept as control (C).

Group II: Second equal part was treated with the gemcitabine (D) at a concentration of 1.2mg/g tissue homogenate.

Group III: Third equal part was treated with both gemcitabine at a concentration 1.2mg/g tissue homogenate and kaempferol at a concentration of 0.1666 mg / g homogenate (DA).

Group IV: Fourth portion was treated only with kaempferolat a concentration of 0.1666 mg / g tissue homogenate (A).

After drug and/or antioxidant treatment tissue homogenates of various groups were shaken for two hours and the 4-hydroxy-2-nonenal and reduced glutathione content of various portions were determined. For each parameter (4-HNE /GSH) three animal sets were used.

Estimation of 4-hydroxy-2-nonenal (4-HNE) level in tissue homogenate

In each case three samples of 2 ml of incubation mixture was treated with 1.5 ml of 10% (w/v) TCA solution then centrifuged at 3000 rpm for 30 min. 2 ml of the filtrate was treated with 1 ml of 2, 4-dinitrophenyl hydrazine (DNPH) (100 mg / 100 ml of 0.5 M HCl) and kept for 1 hour at room temperature. After that, the samples were extracted with hexane, and the extract was evaporated to dryness under argon at 40^0 C. After cooling to a room temperature, 2 ml of methanol was added to each sample and the absorbance was measured at 350 nm against methanol as blank(Kinter, 1996). The values were determined from the standard curve. The best-fit equation is: Nanomoles of 4-HNE = $(A_{350} - 0.005603185) / 0.003262215$, where A_{350} = absorbance at 350nm, r = 0.999, SEM = 0.007

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). 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.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 kaempferol (DA) and only kaempferol-treated (A) with respect to control group of corresponding time.

Results

The percent changes in 4-HNE 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 figure 1, it was evident that tissue homogenates treated with gemcitabine showed an increase in 4-HNE (11.07 %) 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. 4-HNE is formed due to lipid peroxidation occurring during episodes of oxidant stress, readily forms adducts with cellular proteins; these adducts can be assessed as a marker of oxidant stress in the form of lipid peroxidation (Esterbaueret al., 1991). But the 4-

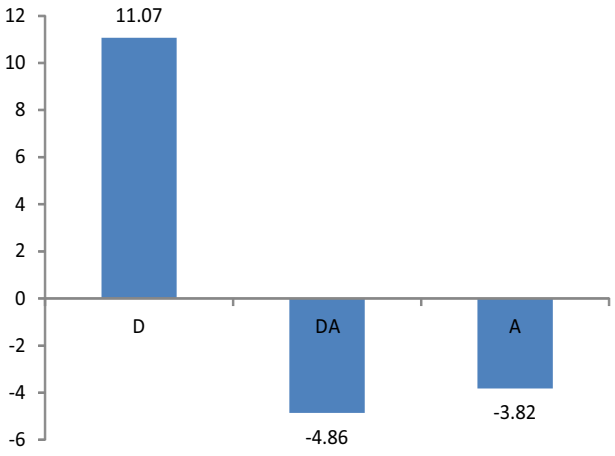

Figure 1. Effects of kaempferolon gemcitabine-induced changes in 4-HNE content (n=3); D, DA & A indicate only gemcitabine-treated, gemcitabine &kaempferol-treated and only kaempferol–treated samples

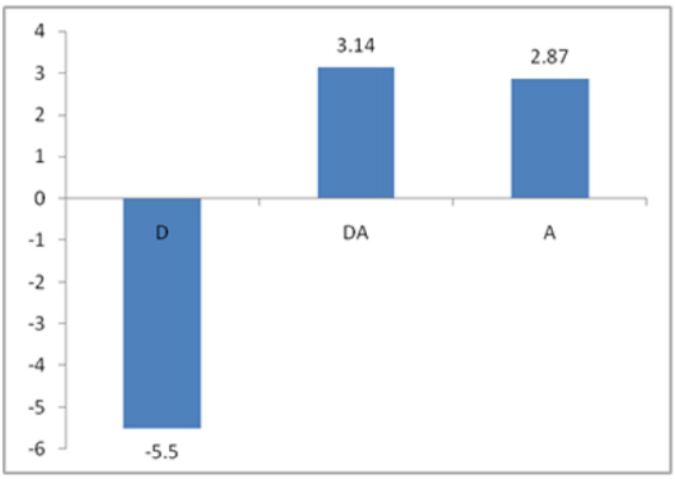

Figure 2. Effects of kaempferolon gemcitabine-induced changes in GSH (n=3); D, DA & A indicate only gemcitabine-treated, gemcitabine &kaempferol-treated and only kaempferol–treated samples

HNE(-4.86 %) content were significantly reduced in comparison to gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with kaempferol. Again the tissue homogenates were treated only with the kaempferol then the 4-HNE(-3.82%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging property of the kaempferol.

Figure 2 showed that tissue homogenates treated with gemcitabine showed a decrease in GSH (-5.50%) content in samples with respect to control to a significant extent. The observations suggest that gemcitabine could significantly induce the lipid peroxidation process. It is an important antioxidant and plays a very important role in the defence mechanism for tissue against the reactive oxygen species(Wilkinson, 2001). But the GSH content was significantly increased (3.14%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with kaempferol. Again the tissue homogenates was treated only with kaempferol then the GSH level was increased (2.87%) in comparison to the control and the gemcitabine treated group. This increase may be explained by the free radical scavenging property of the kaempferol.

The table 1 also indicates that for 4-HNE content, gemcitabine-treated group is statistically different from gemcitabine and kaempferol-treated and only kaempferol-treated groups, but there is no difference between gemcitabine and kaempferol-treated and only kaempferol-treated groups. For GSH content gemcitabine-treated group, gemcitabine and kaempferol-treated and only kaempferol-treated groups are statistically significantly different from each other.

Table 1. Analysis of variance & multiple comparisons

Name of markers	Analysis of variance & multiple comparisons
4-HNE	F1=682.32 [df=(2,4)], F2=0.834 [df=(2, 4)] Pooled variance $(S^2)^*$ =0.349 Critical difference,(p=0.05)# LSD =1.11 Ranked means** (D) (DA, A)
GSH	F1=1342.31 [df=(2,4)], F2=0.339 [df=(2, 4)] Pooled variance $(S^2)^*$ =0.054 Critical difference,(p=0.05)# LSD =0.44 Ranked means** (D) (DA) (A)

Theoretical values of F: p=0.05 level F1=4.46 [df=(2,8)], F2=3.84 [df=(4,8)]; P=0.01 level F1=8.65 [df=(2,8)], F2=7.01 [df=(4,8)]. F1 and F2 corresponding to variance ratio between groups and within groups respectively. D, DA, A indicate gemcitabine-treated, gemcitabine and kaempferol-treated, kaempferol-treated respectively. 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

Conclusion

The results indicated lipid peroxidation induction potential of gemcitabine, which may be related to its toxic potential. The results also suggest the antiperoxidative effects of kaempferol and demonstrate its potential to reduce gemcitabine induced toxic effects. However a detailed study is required to advance such hypothesis.

Conflict of Interest

The author declares no conflicts of interest

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