ROLE OF NARINGIN ON GEMCITABINE-INDUCED LIPID PEROXIDATION
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DOI: 10.7897/2277-4572.07385

Received on: 23/05/18 Revised on: 14/06/18 Accepted on: 17/06/18

ABSTRACT

Aim: The aim of the present study was to investigate the antiperoxidative property of naringin on gemcitabine-induced lipid peroxidation. Methods: The in vitro work 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: The data generated from the work showed that gemcitabine increase the MDA level and reduce the GSH level. But when gemcitabine was used in combination with naringin then there is decrease in MDA level and increase in GSH level. Conclusion: The results showed that gemcitabine has the ability to induce lipid peroxidation to a significant extent and it was also found that naringin has antiperoxidative potential to suppress the gemcitabine-induced toxicity.

Keywords: Gemcitabine, naringin, lipid peroxidation, malondialdehyde, reduced glutathione

INTRODUCTION

Naringin is a phenolic compound and exhibit antioxidant and antigenotoxic properties. Recent study shows that naringin has protective role against cisplatin induced oxidative stress. Gemcitabine falls under the class of antimitabolite. Chemically it is a pyrimidine nucleoside prodrug. Beside its activity against various types of cancer, it also produces several side effects such as pale skin, easy bruising or bleeding, numbness or tingling feeling, weakness, nausea, vomiting, upset stomach, diarrhea, constipation, headache, skin rash, drowsiness, hair loss etc.

Lipid peroxidation is considered as a possible marker of drug toxicity. The toxic end products of lipid peroxidation in tissues are exemplified by malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) etc. Considering the above and the ongoing search of the present author for antioxidant that may reduce drug induced lipid peroxidation the present work has been carried out in vitro to evaluate the antiperoxidative potential of naringin on gemcitabine-induced lipid peroxidation.

MATERIALS AND METHODS

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. Naringin was obtained from CDH Pvt. Ltd., New Delhi. Pure sample of gemicitabine 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. 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 naringin at a concentration of 0.1666 mg/g homogenate (DA) and the fourth portion was treated only with naringin at a concentration of 0.1666 mg/g tissue homogenate (A). After gemcitabine and/or naringin 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. 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-tetrahydroxypropylamine (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 \( A = 0.007676M + 0.003467 \), where \( M \) = nanomoles of MDA, \( A \) = absorbance, \( r = 0.996 \), \( S.E. = 0.0037 \), \( F = 1068.76 \) (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\(^7\). 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, \( r = 0.995 \), \( S.E. = 0.0067 \), \( F = 1638.83 \) (df=1,8).

**STATISTICAL ANALYSIS**

Analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure\(^11\) were also performed on the percent changes data of various groups such as gemcitabine-treated (D), gemcitabine and naringin (DA) and only naringin -treated (A) with respect to control group of corresponding time.

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

<table>
<thead>
<tr>
<th>Hours of incubation</th>
<th>Animal sets</th>
<th>% Changes in MDA content</th>
<th>Analysis of variance &amp; multiple comparison</th>
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<tr>
<td></td>
<td></td>
<td>D</td>
<td>DA</td>
</tr>
<tr>
<td>2</td>
<td>AL1</td>
<td>10.70(^{a})</td>
<td>-9.20(^{a})</td>
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<tr>
<td></td>
<td>AL2</td>
<td>16.33(^{a})</td>
<td>-10.68(^{a})</td>
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<tr>
<td></td>
<td>AL3</td>
<td>17.22(^{b})</td>
<td>-11.97(^{b})</td>
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<tr>
<td></td>
<td>AV.</td>
<td>14.75 (^{(±0.04)})</td>
<td>-10.61 (^{(±0.80)})</td>
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% Changes with respect to controls of corresponding hours are shown: \(a=99\%\); \(b=97.5\%-99\%\); \(c=95-97.5\%\); \(d=90-95\%\); \(e=80-90\%\); \(f=70-80\%\); \(g=60-70\%\); \(h=60\%\); \(p=0.05\) level \(F_1=6.94 \) [df=(2,4)], \(F_2=6.94 \) [df=(2, 4)], \(F_1\) and \(F_2\) corresponding to variance ratio between groups and within groups respectively.

D, DA, A indicate gemcitabine-treated, gemcitabine and naringin-treated, naringin-treated respectively. \(AV.\)= Averages of three animal sets; \(S.E.\)= Standard Error (df=2); \(\ast\) Error mean square, \# Critical difference according to least significant procedure \(^7\) ** Two means not included within same parenthesis are statistically significantly different at \(p=0.05\) level

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

<table>
<thead>
<tr>
<th>Hours of incubation</th>
<th>Animal sets</th>
<th>% Changes in GSH content</th>
<th>Analysis of variance &amp; multiple comparison</th>
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<tr>
<td></td>
<td></td>
<td>D</td>
<td>DA</td>
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<tr>
<td>2</td>
<td>AL1</td>
<td>-8.24(^{c})</td>
<td>7.34(^{c})</td>
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<td></td>
<td>AL2</td>
<td>-7.82(^{c})</td>
<td>6.12(^{c})</td>
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<td></td>
<td>AL3</td>
<td>-9.28(^{c})</td>
<td>6.85(^{c})</td>
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<tr>
<td></td>
<td>AV.</td>
<td>-8.45 (^{(±0.43)})</td>
<td>6.77 (^{(±0.35)})</td>
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% Changes with respect to controls of corresponding hours are shown: \(a=99\%\); \(b=97.5\%-99\%\); \(c=95-97.5\%\); \(d=90-95\%\); \(e=80-90\%\); \(f=70-80\%\); \(g=60-70\%\); \(h=60\%\); \(p=0.05\) level \(F_1=6.94 \) [df=(2,4)], \(F_2=6.94 \) [df=(2, 4)], \(F_1\) and \(F_2\) corresponding to variance ratio between groups and within groups respectively.

D, DA, A indicate gemcitabine-treated, gemcitabine and naringin-treated, naringin-treated respectively. \(AV.\)= Averages of three animal sets; \(S.E.\)= Standard Error (df=2); \(\ast\) Error mean square, \# Critical difference according to least significant procedure \(^7\) ** Two means not included within same parenthesis are statistically significantly different at \(p=0.05\) level
RESULTS & 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 (14.75 %) 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 (-10.61 %) content were significantly reduced in comparison to gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with naringin. Again the tissue homogenates were treated only with the naringin then the MDA (-12.01%) level were reduced in comparison to gemcitabine treated group. This decrease may be due to the free radical scavenging property of the naringin. So the decrease in MDA content of samples, when treated with gemcitabine and naringin implies the free radical scavenging property of naringin.

It was also evident from Table 2 that tissue homogenates treated with gemcitabine showed a decrease in GSH (-8.45%) 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\(^1\). But the GSH content was significantly increased (6.77%) in comparison to control and gemcitabine-treated group when tissue homogenates were treated with gemcitabine in combination with naringin. Again the tissue homogenates was treated only with naringin then the GSH level was increased (6.23%) in comparison to the control and the gemcitabine treated group. This increase may be explained by the free radical scavenging property of the naringin.

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 naringin -treated and only naringin -treated. But within a particular group, differences (F2) are insignifiant which shows that there is no statistical difference in animals in a particular group (Tables 1 & 2). The Table 1 and 2 also indicate that for MDA and GSH content, gemcitabine-treated group is statistically different from gemcitabine and naringin-treated and only naringin -treated groups. But there is no difference between gemcitabine and naringin-treated and only naringin -treated groups.

CONCLUSION

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 naringin and demonstrate its potential to reduce gemcitabine induced toxic effects. However a detailed study is required to advance the hypothesis.

REFERENCES


How to cite this article:

Source of support: Nil, Conflict of interest: None Declared

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