Investigation of Protective Effects of Quercetin on Oxidative Stress Induced by Vinblastine in Bone Marrow of Rats

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Abstract

Background: Chemotherapy drugs such as vinblastine cause oxidative stress in the bone marrow resulting in changes in blood cell production and anemia. In this study, the antioxidant and therapeutic potential of quercetin was evaluated.

Methods: Twenty-one male Wistar rats were divided into three groups; The Control group received a daily dose of normal saline, group 2 received a single dose of 2 mg/kg b.w vinblastine intraperitoneally (i.p.) on the first day of study, and group 3 received a single dose of vinblastine (2 mg/kg b.w. i.p.) along with quercetin (20 mg/kg b.w. i.p.) for 14 days. To evaluate oxidative stress in bone marrow; malondialdehyde (MDA), Total Antioxidant Capacity (TAC) and Pro-Oxidant/ Antioxidant Balance (PAB) were also measured using specific methods.

Results: The blood analysis showed that the mean level of RBC, Hemoglobin, and Hematocrit were significantly higher in the vinblastine group compared to the control group. Treatment with quercetin could elevate them into the normal range. Administration of vinblastine elevated the levels of bone marrow MDA and PAB significantly (p<0.05) compared to the control group but had no effect on total antioxidant capacity. The use of quercetin with vinblastine showed a decrease in the levels of bone marrow MDA and PAB compared to the vinblastine group alone.

Conclusion: The findings of this study showed that quercetin at a dose of 20 mg/kg could improve the anemia induced by vinblastine chemotherapy, and it can also be useful in improving vinblastine-induced lipotoxicity.

Introduction

Vinblastine (C46H58N4O9) as a natural alkaloid derived from Catharanthus roseus is one of the most effective anticancer drugs currently used in chemotherapy independently or in combination with other drugs. It is used to treat Hodgkin’s and non-Hodgkin’s lymphomas as well as some solid tumors such as bladder, brain, melanoma, and testicular tumors. The action mechanism of vinca alkaloids depends on beta-tubulin and microtubule dysfunction during mitosis, leading to mitosis stoppage at stages of metaphase and cell death. Also, vinblastine interferes with spindle formation, causing chromosome segregation.

Side effects of this drug include hair loss, white blood cell and platelet loss, anemia, gastrointestinal problems, hypertension, sweating, depression, muscle cramps, dizziness, and headache. As mentioned before, anemia is one of the side effects of taking vinblastine and some other chemotherapy drugs, which is prevalent in about 40% of patients with cancer, and it reaches up to 90% in patients undergoing chemotherapy. Treatment to reduce anemia involves the use of blood-cell-boosting hormones such as erythropoietin, use of iron supplementation, and ultimately blood transfusion. Blood transfusion is not a safe process and can cause many problems for the recipient, especially when this person has other underlying problems. Oxidative stress is one of the factors causing and aggravating the anemia. Of course, the underlying cause may not be oxidative stress, but it damages stem cells playing a vital role in hematopoiesis, due to its effect on erythrocyte production in the bone marrow and the life span of red blood cells in a cycle.

Quercetin is a flavonoid found in various food products and plants including fruits, vegetables, beans, and tea; onions and broccoli are common foods containing significant amounts of quercetin. Numerous applications of quercetin have made it a known potent anticancer agent. Quercetin, with specific structural features including one hydroxyl group in carbon 3 and a carbonyl group in carbon 4 facilitating binding to iron ions and contributing to antioxidant activity has all the properties required for inhibition of free radical process. Also, the presence of catechol in the β-ring of quercetin is...
samples were obtained by centrifugation of blood samples containing EDTA and the rest was kept in test tubes. Serum puncture. 2 mL of the blood sample was collected in tubes for 2 weeks. The injection volume never exceeded 0.6 mL.

In this study, the rats were randomly divided into the following three groups (n=7). Group I, the control group which received vinblastine drug. Group II received 2 mg/kg vinblastine (dissolved in 0.9% sodium chloride solution) intraperitoneally on the first day of study along with 20 mg/kg quercetin per day (dissolved in water) intraperitoneally. Group III received vinblastine (2 mg/kg) intraperitoneally on the first day of study along with 20 mg/kg quercetin per day (dissolved in water) intraperitoneally for 2 weeks. The injection volume never exceeded 0.6 mL. The water and food intake of each group of rats was recorded at the beginning and end of the study.

Collection of the samples
At the end of the study, the rats were anesthetized with ketamine (80 mg/kg) and xylazine (100 mg/kg) after 12-hour fasting, and their blood was collected through cardiac puncture. 2 mL of the blood sample was collected in tubes containing EDTA and the rest was kept in test tubes. Serum samples were obtained by centrifugation of blood samples for 25 minutes at 2500 rpm. Also, bone marrow samples of rats from each group were separated from the femur bone of rats and were transferred to the bone marrow culture medium.

Materials and Methods

Chemicals and Reagents
Trichloroacetic acid, Thiobarbituric acid, 1-Butanol (Merck KGaA, 64271 Darmstadt, Germany), Quercetin powder (Sigma-Aldrich, St. Louis, Missouri, United States), Vinblastine (Gedeon Richter LTD, Budapest, Hungary), TMB powder (3, 3, 5, 5-tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), N-chloro 4-methylbenzenesulfonamide, sodium salt (chloramine T trihydrate) (Applichem: A4331, Darmstadt, Germany), and hydrogen peroxide (30%) (Merck Company), as well as all the other reagents used were of analytical grade and were prepared in double-distilled water.

Experimental animal groups
Twenty-one male Wistar rats were used in this study with a mean bodyweight of 268.3 ± 9.78 g. The rats were kept in the animal house under the stable physical condition at 25 ± 2 °C and on a 12-hour light-dark cycle with free access to standard food (Javaneh Khorasan Company) and drinking water. The animals were randomly grouped into 3 groups (n=7) 72 hours before starting the study and were placed in appropriate cages to adapt to new conditions. Attempts were made to perform the tests at the same time of day and night within 14 days, as much as possible, to avoid the possible effects of the day-night cycle.

In this study, the rats were randomly divided into the following three groups (n=7). Group I, the control group received 0.5 mL normal saline (0.9%) intraperitoneally. Group II received 2 mg/kg vinblastine (dissolved in 0.9% sodium chloride solution) intraperitoneally on the first day of the study. Group III received vinblastine (2 mg/kg) intraperitoneally on the first day of study along with 20 mg/kg quercetin per day (dissolved in water) intraperitoneally for 2 weeks. The injection volume never exceeded 0.6 mL. The water and food intake of each group of rats was recorded at the beginning and end of the study.

Pro-oxidant/antioxidant balance (PAB) assay
Tetramethyl benzidine (TMB) and TMB cation were used as the indicators of oxidation-reduction (due to their electrochemical and optical properties) in order to evaluate the oxidant-antioxidant balance. In this method, the oxidant-antioxidant balance was simultaneously measured in one trial by two different reaction types. In an enzymatic reaction, the TMB chromogen was oxidized to a colored cation, using pro-oxidants, and in a chemical reaction, the TMB cation was converted to a colorless composition, using antioxidants. The photometric adsorption is then compared with the specific adsorption of a series of standard solutions (a mixture of different ratios (0-100%) of hydrogen peroxide and uric acid). The PAB scale was expressed in a contract unit which is called HK (Hamidi-Koliakos) unit.
Statistical analysis

SPSS software version 16.00 was used to analyze data. To compare results in the study groups, the results were subdivided into normal and abnormal variables by one-sample Kolmogorov-Smirnov test. One-way Analysis of Variance (ANOVA) was used for examining normal variables and the non-parametric equivalent of one-way ANOVA-Kruskal-Wallis test was used for evaluating non-normal variables. All tests were used for one-way variance, and a P-value of 0.05 or less was considered statistically significant.

Results

The effects of vinblastine and quercetin on food and water intake, and weight of rats

As shown in Figure 1, a significant decrease was observed in the amount of water intake in the group receiving vinblastine (GII) compared to the control group (GI). Treatment with quercetin (GI) significantly increased the level of water intake compared to the vinblastine group (GII). According to Figure 2, the weight of rats decreased significantly in the vinblastine group. Treatment with quercetin improved weight loss in the treatment group (GIII) (P < 0.05). Results showed that treatment with vinblastine and/or quercetin had no effect on food intake level.

The effects of vinblastine and quercetin on blood components

As shown in Table 1, the use of vinblastine elevated the level of WBC compared to the control group (GI). Quercetin reduced the rate increase of WBC in the group receiving quercetin (GIII) compared to the vinblastine group (GII). RBC level was significantly lower in the vinblastine group (GII) compared to the control group (GI). Quercetin was able to increase RBC levels in the vinblastine associated with the quercetin group (GIII) compared to the vinblastine group (GII). Hemoglobin (Hb) level was significantly lower in the vinblastine group (GII) compared to the control group (GI). Quercetin was able to increase the hemoglobin level in the vinblastine associated with the quercetin group (GIII) compared to the vinblastine group (GII). The level of hematocrit (HCT) was significantly lower in the vinblastine group than the control group. Quercetin also increased hematocrit level in the vinblastine associated with the quercetin group compared to the vinblastine group. This rate was higher in the vinblastine plus the quercetin group than the vinblastine group and had a smaller decrease compared to the vinblastine group. The level of MCV was significantly lower in the control group (GI) than the vinblastine group (GII).

Table 1. WBC, RBC, Hemoglobin and Hematocrit levels in controlled (GI) and treated rats for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII).

<table>
<thead>
<tr>
<th>Test</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^4.L)</td>
<td>5.15±2.37</td>
<td>8.08±3.78*</td>
<td>3.38±0.90</td>
<td>4.008</td>
<td>0.049</td>
</tr>
<tr>
<td>RBC (10^12.L)</td>
<td>7.06±0.44</td>
<td>5.75±1.25**</td>
<td>8.41±0.90</td>
<td>1306</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin (g.L^-1)</td>
<td>13.80±0.34**</td>
<td>12.68±1.30***</td>
<td>3.38±0.90</td>
<td>17.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>49.83±3.29**</td>
<td>39.48±2.15**</td>
<td>3.38±0.90</td>
<td>11.884</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 7): * P < 0.05 compared to control group. ** P < 0.01 compared to control group. *** P < 0.001 compared to control group.
Quercetin was able to slow down the increase in the rate of MCV in the vinblastine plus quercetin group (GIII) compared to the vinblastine group (GII). Also, MCH level was lower in the control group (GI) than the vinblastine group (GII) and quercetin was able to decline the increase in the rate of MCH in the vinblastine associated with the quercetin group (GII) compared to the vinblastine group (GII) (Table 2).

The effects of vinblastine and quercetin on markers of oxidative stress in blood and bone marrow

Results of the oxidative stress markers shown in Figure 3 indicated that the use of vinblastine increased lipid peroxidation in bone marrow cells, such that a significant increase was found in the level of malondialdehyde in the vinblastine group (GII) compared to the control group (GI). It was shown that the use of quercetin significantly decreased (p<0.05) malondialdehyde levels in bone marrow in the vinblastine associated with the quercetin group (GIII). Total antioxidant capacity did not change in the group receiving vinblastine (GII) compared to the control group (GI) in both the serum and the bone marrow. Also quercetin had no effect on total antioxidant capacity in both the serum and the bone marrow (Figure 4).

The effects of vinblastine and quercetin on pro-oxidant-antioxidant balance (PAB)

As shown in Figure 5, the PAB level was significantly higher in the control group (GI) than the vinblastine group (GII). Quercetin was able to decelerate the increase in the level of PAB in the vinblastine associated with quercetin group (GIII) compared to the vinblastine group (GII) and this rate was significantly lower in the vinblastine associated with the quercetin group than the vinblastine group (p=0.01) and increased to a lesser extent.

Discussion

Since antioxidant activity of flavonoids attenuates oxidative damage and cell death, in this study, it was attempted to investigate antioxidant and therapeutic effects of quercetin on the reduction of oxidative stress induced by vinblastine. Exposure to vinblastine generates Reactive Oxygen Species (ROS). It has been proved that flavonoids including quercetin neutralized ROS by directly reacting with superoxide anion, nitric oxide (NO), and peroxynitrite.

Vinblastine related oxidative stress leads to the production of lipid peroxidation in red blood cells as well as oxidative stress in the bone marrow. RBC damage caused by oxidative stress is generally thought to occur as a result of two procedures: oxidation of hemoglobin, followed by conversion of met hemoglobin to hemichromes, and

![Table 2. MCV and MCH levels in control (GI) and treatment groups for 14 days with vinblastine (GII) or with vinblastine along with quercetin (GIII).](image-url)

<table>
<thead>
<tr>
<th>Test</th>
<th>GI</th>
<th>GII</th>
<th>GIII</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV (pg)</td>
<td>56±5</td>
<td>71±15*</td>
<td>58±6</td>
<td>5.94</td>
<td>0.05</td>
</tr>
<tr>
<td>MCH (%)</td>
<td>19±1</td>
<td>23±3**</td>
<td>20±1</td>
<td>4.91</td>
<td>0.08</td>
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</table>
the membrane components disrupting due to free radical attack. These procedures can be prevented by the addition of flavonoids. Other effects of oxidative stress include decreasing blood components such as RBC, Hemoglobin, and Hematocrit, and increasing blood components like WBC, MCV, and MCH. Collectively, anemia is one of the side effects of this drug used in chemotherapy. The effects of the antioxidant defense mechanism of quercetin as well as its dose and tissue-dependent effects have been reported in previous studies. According to the literature preventive and therapeutic potential of quercetin in several research model has been proved. Furthermore, protective activity of quercetin against toxic agents and several drugs has been mentioned.

According to the results of this study, treatment with quercetin increased RBC, Hemoglobin, and Hematocrit. In contrast, these blood components decreased in the group receiving vinblastine compared to the control group, which was in agreement with those reported in the study by Cesquini et al. who showed that quercetin effectively prevented red blood cell oxidation, reduced hemoglobin oxidation by 30%, and increased oxyhemoglobin levels. Gargi Sen et al. reported that flavonoids were effective in repairing hemoglobin reduction in Leishmania-induced anemia in animals, with quercetin being the most successful compound among the 5 studied compounds regarding prevention of oxidation of cell membrane proteins and lipids in infected animals. It also had a more significant effect on Leishmania-induced anemia, increased life-span of red blood cells, which reduces due to osmotic fragility, and significantly increased hemoglobin levels as well compared to other compounds. It is noteworthy that, splenomegaly was observed in the group receiving quercetin along with vinblastine, indicating the body’s attempt for further hematopoiesis and anemia compensation.

Inhibition of lipid peroxidation is one of the other protective effects of quercetin. This observation is in agreement with our findings regarding the inhibition of lipid peroxidation after treatment with quercetin in rats. MDA, as an end-product of lipid peroxidation, may be used as an indicator of oxidative stress that may increase in the body after administration of vinblastine. Results also showed that the use of quercetin compound reduced malondialdehyde levels in the bone marrow compared to the group receiving vinblastine, which was similar to the results of previous studies showing that quercetin partially protects blood glutathione and leads to suppression of plasma malondialdehyde levels, as well as nitric oxide metabolites and the production of superoxide anion. Collectively, the results of the present study showed that chronic administration of quercetin in rats caused inhibition of lipid peroxidation. Quercetin also increased total antioxidant capacity (TAC) in the bone marrow compared to the group receiving vinblastine. Kumiko Ishige et al. showed that quercetin reduced the levels of free radicals and increased the levels of TAC. However, results of the Ferric Reducing Antioxidant Power (FRAP) tests of bone marrow and serum were not significant, which were in agreement with those obtained in the study by Agnieszka Cierniak et al. that elevated FRAP content in the quercetin-treated group might be due to its free radical scavenging property. Oxidative stress-induced by oxygen-derived free radicals is a disturbance in the pro-oxidant antioxidant balance.

Pro-oxidant antioxidant balance is important in the development of oxidative stress-related diseases such as anemia. Results of this study showed that the PAB parameter in the group receiving vinblastine was significantly different from the control group and the group receiving quercetin along with vinblastine, which was similar to the results obtained in the study by Choi et al. who stated that quercetin is considered not only as an antioxidant but also as a pro-oxidant in rats. One of the reasons for lack of finding significant results regarding FRAP, which is an oxidative stress marker may have been an inadequate dose of quercetin used or a small sample size, certainly disturbing significance indicators of statistical tests which is among limitations of this study. Therefore, researchers are certainly recommended to consider appropriate and adequate sample sizes in similar studies. Finally one of the side effects of chemotherapy agents is dehydration and our results are in line with the other studies. Treatment with quercetin could improve water intake that could be mentioned for ameliorating chemotherapy side effects.

**Conclusion**

The findings of the study showed that quercetin at a dose of 20 mg/kg reduced the effects of oxidative stress in rats receiving vinblastine, which could be effective in improving oxidative stress and subsequent anemia. Due to attention to flavonoids such as quercetin, preventive and therapeutic applications of the molecules is noteworthy. The multitude of pre-clinical studies has allowed more in-depth studies, including the development of increasingly specific and targeted clinical studies. To fully understand the mechanism of quercetin function, pre-clinical and clinical studies are suggested to be conducted at whole body, tissue and cell surface.

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**Ethical Issues**

The study was approved by the Deputy of Research and Technology and Ethics Committee of Birjand University of...
Medical Sciences (Ethics code: IR.BUMS.REC.1398.156). Ethical considerations were considered at all stages of the research. Ethical considerations related to laboratory animals included choosing right animal species for this study, using a minimum number of animals for the study as much as possible, providing appropriate living conditions for the animals, training researchers to understand the animal’s life and physiological conditions (nutrition, health, disease, discomfort and pain, and other physiological and pathological changes in the animal), not using sick animals in the experiment, and finally adhering to ethical protocols in blood sampling and providing conditions for euthanasia of the animals at the end of the study.

Author Contributions
HA and HEZ: Participated in the acquisition, analysis, or interpretation of data for the work. MH: Designed the study and participated in data analysis. All authors participated in drafting the work or revising it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest
The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References
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