# Combination of quercetin and Adriamycin effectively suppresses the growth of refractory acute leukemia

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**Abstract.** The present study aimed to investigate the effect of combined treatment with quercetin and Adriamycin (doxorubicin) on the development of refractory acute leukemia. Primary leukemic cells were isolated from patients with refractory drug-resistant acute leukemia. The Cell Counting Kit-8 assay was used to detect the proliferation of cells treated with a range of doses of Adriamycin, quercetin and a combination of the two drugs. Non-irradiated mice were used to establish a T cell acute lymphoblastic leukemia (T-ALL) model, which was subsequently treated with Adriamycin, quercetin and a combination of the two drugs. The survival time was recorded, and white and red blood cells and platelets in mouse peripheral blood were counted. Superoxide dismutase (SOD) activity and malondialdehyde (MDA) content of cardiac tissues were measured as indicators of oxidative stress and damage. Proliferation of primary leukemic cells was reduced by Adriamycin depending on the dose (0.06, 0.6 or 6 $\mu$g/ml) and treatment duration (24, 48 or 72 h) compared with the vehicle treated group. Co-treatment with quercetin achieved a similar suppression of leukemic cell proliferation when a lower dose of Adriamycin (0.03, 0.3 or 3 $\mu$g/ml) was administered for the same duration. The survival of non-irradiated mice with T-ALL was improved by co-treatment with a high dose of Adriamycin and quercetin compared with either treatment alone. Compared with treatment with Adriamycin alone, the combined treatment with Adriamycin and quercetin significantly enhanced the SOD activity and reduced the MDA content in the heart. Therefore, quercetin may enhance the effects of Adriamycin on refractory acute leukemia.

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*Key words:* Adriamycin, oxidative stress, quercetin, refractory acute leukemia, T cell acute lymphoblastic leukemia

## Introduction

Acute leukemia (AL) is a malignant clonogenic disease that results from the uncontrolled proliferation of abnormal hematopoietic stem cells. Based on the types of cells affected, AL can be divided into two categories: Acute myelocytic leukemia and acute lymphoblastic leukemia (ALL) (1). Treatments for AL include single-drug chemotherapy, molecular targeting therapy, hematopoietic stem cell transplantation and cellular immunotherapy. Chemotherapy is currently the main treatment strategy due to limited sources of donor bone marrow, high cost and severe complications associated with transplantation; however, a considerable number of patients exhibit no remission or early recurrence after chemotherapy, which may be caused by drug resistance, minimal residual disease after remission and persistence of leukemic stem cells (2,3).

Anthracycline-type drugs, such as Adriamycin (also termed doxorubicin), are frequently used for AL chemotherapy. Adriamycin kills leukemic cells by inhibiting DNA replication and RNA transcription. The dose of anthracyclines correlates with its efficiency in the clearance of leukemic cells, but increases in the dose are also associated with elevated toxic side effects (4,5). The heart is a major organ affected by the side effects of anthracycline drugs, including acute heart failure, congestive cardiomyopathy and occult ventricular dysfunction (6,7). In addition, the efficacy of the drug is also restricted by the development of drug resistance in leukemic cells (8). Therefore, it is crucial to develop a novel strategy

for treating AL to bypass the resistance of leukemic cells to anthracycline-type drugs and limit the side effects.

Quercetin is a plant flavonol derived from the flavonoid group of polyphenols; it is found in a number of fruits, vegetables, leaves and grains (9). It is commonly used as an ingredient of dietary supplements, beverages and foods. It is a potential anti-oxidant capable of modulating the activity of various cellular enzymes (10,11). Quercetin has an anti-tumor effect on a variety of tumor cells, including leukemia, lymphoma, colon, ovarian, cervical, prostate and breast cancer cells (12-16). However, whether quercetin is able to modify the therapeutic efficacy of anthracycline-type drugs (such as Adriamycin) for leukemia is yet to be determined.

The present study explored the combined AL treatment with quercetin and Adriamycin, and the results suggested that quercetin and Adriamycin may be used in combination to treat malignant hematopathy.

## Materials and methods

*Clinical samples*. The study recruited 20 patients with refractory, Adriamycin-resistant acute leukemia (17) (excluding acute promyelocyte leukemia) treated at the Department of Hematology of the Affiliated Hospital of Inner Mongolia Medical University (Hohhot, China) between November 2015 and December 2016 (Table I). The inclusion criteria were: i) Patients treated with two courses of induction chemotherapy; ii) patients with recurrence 6-12 months following complete response (CR) or patients with ineffective re-treatment using a standard chemotherapy regimen; and iii) patients with two or more recurrences. Patients who met any one of the aforementioned criteria were determined to be cases of refractory AL. A 5-ml vacuum heparin sodium tube was used to collect peripheral blood from the patients. This study was approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University. All patients signed the informed consent form.

*Cell Counting Kit-8 assay (CCK-8)*. Ficoll lymphocyte separation solution (Tianjin Hao Yang Biological Products Technology Co., Ltd, Tianjin, China) was used to separate lymphocytes in the peripheral blood of patients, cell density was adjusted and $5x10^4$ cells/well were placed in a 96-well cell culture plate. The maximum drug concentrations of Adriamycin (Shenzhen Wanle Pharmaceutical Co., Ltd., Shenzhen, China) and quercetin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) were set as 6 and 75.5 $\mu$g/ml, respectively, based on a preliminary experiment (data not shown). The samples were separated into one negative control group (Con) and six experimental groups (6 wells/group). Three experimental groups were treated with 0.06, 0.6 or 6 $\mu$g/ml Adriamycin, and three groups were treated with 0.03, 0.3 or 3 $\mu$g/ml Adriamycin combined with 75.5 $\mu$g/ml quercetin, based on a preliminary experiment (data not shown). The cells were incubated at 37˚C with saturated humidity and 5% $CO_2$. At 24, 48 and 72 h, 20 $\mu$l of CCK-8 solution (lot no. JM754; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each well. Cells were incubated for an additional 4 h at 37˚C, and the optical density (OD) of each well at 450 nm was detected using a BioTek ELX800 Automatic Enzyme Labeling Instrument microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Cell proliferation inhibition rate was calculated as follows: Proliferation inhibition rate=[(OD value of the Con group-OD value of the experimental group)/OD value of the Con group] x 100.

*Mouse model of T-ALL*. C57BL/6J ($CD45.2^+$) and B6.SJL-PtprcaPepcb/BoyJ (B6.SJL;$CD45.1^+$; donor mice for leukemia cells) mice (all female; age, 6-8 weeks; weight, 15-18 g; n=28 mice/group) were purchased from the Academy of Military Medical Sciences (Beijing, China) and maintained in the Central Laboratory of the Affiliated Hospital of Inner Mongolia Medical University. The mice received food and water *ad libitum*. They were kept under specific pathogen-free conditions at 20±2˚C and 40-60% relative humidity under a 12:12 h light/dark cycle. All animal experiments were approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University. A non-irradiated, Notch1-induced T-ALL model was established as previously described (18). Briefly, the mice were sacrificed, disinfected with ethanol and the spleens and the femurs of the mice were removed. The bone marrow cells were repeatedly flushed out using a 1-ml syringe, and the spleen cells were sieved by grinding. Finally, the cells were collected by centrifugation and stored frozen. Green fluorescent protein $(GFP)^+CD45.1^+$ leukemic cells were isolated from either the spleen or the bone marrow of mice with B6.SJL leukemia and transplanted by tail vein injection into C57BL/6 J female mice ($1x10^6$ cells/mouse) without irradiation. Successful transplantation was confirmed by flow cytometry, as previously described (19). The mice were randomly divided into four groups (n=7 mice/group): Untreated, quercetin, Adriamycin or Adriamycin combined with quercetin. For the low-dose Adriamycin experiments, the mice were intraperitoneally (i.p.) administered 0.9% saline, 50 mg/kg quercetin, 1 mg/kg Adriamycin, or 1 mg/kg Adriamycin + 50 mg/kg quercetin, respectively, daily for 10 days, starting at day 4 post-transplantation. For the high-dose Adriamycin experiments, the mice were i.p. administered 0.9% saline, 50 mg/kg quercetin, 2 mg/kg Adriamycin or 2 mg/kg Adriamycin + 50 mg/kg quercetin, respectively, daily for 10 days, starting at 4 days after transplantation. The survival duration of each group was recorded. Additionally, peripheral blood was collected from the tail in EDTA-modified tubes on days 4 (first day of dosing), 7 (starting point of leukemic accumulation in untreated mice), 11 (first death in untreated mice), 16 (end of dosing), 23 (negligible drug levels, based on the half-life of Adriamycin) and 31 (end of study) following transplantation, and blood cells and platelets were counted using an XN2000 blood cell analyzer (Sysmex Corporation, Kobe, Japan).

*Determination of superoxide dismutase (SOD) activity and malondialdehyde (MDA)content in the mouse heart*. Non-irradiated T-ALL mice were administered the allocated treatment daily for 10 days, starting 4 days following transplantation. On day 31 post-transplantation, the mice were sacrificed and heart tissue was collected. Following homogenization and dilution with nine times the volume of saline, the tissue samples were centrifuged at 800 x g for 5 min and

Table I. Clinical characteristics of 20 cases of refractory drug-resistant acute leukemia.

| No. | Sex | Age (years) | Diagnosis | Immunophenotyping | Chromosome | Fusion gene and gene mutation | Initial leukocyte number ($x10^9$cells/l) | Chemotherapy history | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Female | 82 | M5 | CD14/CD13/CD33/ CD64/CD117$^+$ | 48,XX,+8,+m | WT1$^+$ | 103.17 | DA,AA,FA, low dose Ara-C | Non-remission |
| 2 | Female | 61 | M2 | CD7CD13/CD33/ CD117$^+$ | 44,XX,dup(12)(q12q21),-15,-18 | WT1$^+$ | 96.28 | CAG,DA, HA | Non-remission |
| 3 | Female | 75 | M4 | CD11b/CD13/CD33/ CD34$^+$ | 49,XX,+8,+13,+22,inv(16)(p13;q22) | MLL-AF6$^-$ | 30.89 | FA,IA,AA,CAG | Non-remission |
| 4 | Female | 39 | M2 | CD13/CD33/MPO$^+$ | 46,XX,t(8;21)(q22;q22) | AML1/ETO$^-$ | 108.45 | DA,AA,MA,FA | Recurrence |
| 5 | Female | 52 | M4 | CD13/CD33/CD34$^+$ | 46,XX,6p-,t(8:11)(q23;q14) | MLL-SEPT6$^+$ | 50.22 | DA,AA,HAD,CAG,IA | Recurrence |
| 6 | Female | 68 | M2 | CD13/CD33/CD34$^+$ | 46,XX,i(1q),7q- | AML1/ETO$^-$ | 348.13 | DA,FM, CAG,IA, DCAG | Recurrence |
| 7 | Female | 52 | M2 | CD13/CD33/CD7/ MPO$^+$ | 47,XX,+19 | AML1/ETO$^-$ | 84.04 | DA,MA, AA | Non-remission |
| 8 | Female | 27 | M5 | CD14/CD13/CD33/ CD64/CD117$^+$ | 46,XX,add(1)(p36),t(8;16)(p11;p13) | WT1$^+$ | 30.27 | HAD,FA, DCAG,FIA,MAC | Non-remission |
| 9 | Female | 31 | M2 | CD13/CD33/CD34/ MPO$^+$ | 46,XX,t(8;21)(q22q22) | AML1/ETO$^-$ | 20.09 | DA,AA,IA,MA | Recurrence |
| 10 | Male | 60 | M5 | CD13/CD33/CD64/ CD117$^+$ | 46,XY,del(11)(q23),del(12)(p13) | WT1$^-$ | 129.33 | DA,FA, IA,CAG | Non-remission |
| 11 | Male | 58 | M5 | CD11b/CD13/CD33/ CD34/ CD117$^+$ | 46,XY,del(11)(q23) | WT1$^+$ | 46.32 | MA,CAG, HA,AA | Non-remission |
| 12 | Male | 40 | M5 | CD14/CD11b/CD13/ CD33/ CD117$^+$ | 46,XY,del(7)(q22),t(s;21)(q22;q22),del(9)(q11q22) | WT1$^+$ | 110.16 | DA,AA, FA,IA,MA | Recurrence |
| 13 | Male | 56 | M2 | CD13/CD33/CD7/ MPO$^+$ | 46,XY,t(8;21)(q22;q22) | AML1/ETO$^+$ | 29.74 | MA,HA, DA,CAG | Non-remission |
| 14 | Male | 68 | M2 | CD117/CD34/CD13/ CD123/CD33$^+$ | 46,XY,inv(16)(p13;q22) | FLT3-ITD$^+$ | 19.55 | DA,MA, CAG | Non-remission |
| 15 | Male | 76 | M2 | CD13/CD33/CD34/ MPO$^+$ | 45,X,-Y,t(8;21)(q22;q22) | AML1/ETO$^+$ | 44.02 | IA,CAG,low dose Ara-C | Non-remission |
| 16 | Female | 58 | B-ALL | CD19/CyCD22/CD34/ HLA-DR$^+$ | Normal karyotype | BCR/ABL$^-$ | 10.18 | CAG,HA, VDLP,V DCLP,HD-MTX | Recurrence |
| 17 | Female | 27 | B-ALL | CD10/CD19/CyCD22/ CD20/CD34/HLA-DR$^+$ | 46,XX,t(9;22)(q34;q11) | BCR/ABL$^+$ | 94.29 | VDLP,VDCLP, Hyper-CVAD combined with Imatinib, Dasatinib | Recurrence |

Table I. Continued.

| No. | Sex | Age (years) | Diagnosis | Immunophenotyping | Chromosome | Fusion gene and gene mutation | Initial leukocyte number ($x10^9$cells/l) | Chemotherapy history | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 18 | Female | 58 | B-ALL | CD19/CyCD22/CD34/ CyCD79a/HLA-$DR^+$ | 46,XX,t(11;12) (p10;p10),t(4;6)(q26;p2) | BCR/ABL$^-$ | 26.01 | VDLP,FLAG,MA | Recurrence |
| 19 | Female | 55 | B-ALL | HLA-DR/CD38/CD123/ CD19/CD9/$CD79a^+$ | 46,XX,2q-t(9;22) | BCR/ABL$^-$ | 118.34 | VDCLP,HD-MTX,CAG | Non-remission |
| 20 | Male | 34 | B-ALL | HLA-DR/CD19/CD10/ TDT/$CD79a^+$ | 46,XY,t(9;22)(q34;q11) | BCR/ABL$^-$ | 209.58 | VDLP,FLAG,MA$^*$ | Non-remission |

WT1, Wilms tumor 1; MLL, mixed-lineage leukemia; AML1, acute myeloid leukemia 1; ETO, eight twenty one; SEPT6, septin-6; FLT3, fms-like tyrosine kinase 3; ITD, internal tandem duplication; BCR, breakpoint cluster region protein; ABL, Abelson murine leukemia viral oncogene homolog 1; AA, Aclarubicin + Cytarabine; Ara-C, Cytrabine; B-ALL, B cell acute lymphoblastic leukemia; CAG, Cytarabine + Aclarubicin + recombinant human granulocyte colony-stimulating factor; DA, Daunorubicin + Cytarabine; DCAG, Decitabine + Cytarabine + Aclarubicin + recombinant human granulocyte colony-stimulating factor; FA, Fludarabine + Cytarabine; FIA, Fludarabine + I-darubicin + Cytarabine; FLAG, Fludarabine + Cytarabine + recombinant human granulocyte colony-stimulating factor; FM, Fludarabine + Mitoxantrone; HA, Homoharringtonine + Cytarabine; HAD, Homoharringtonine + Cytarabine + Daunorubicin; HD-MTX, high dose methotrexate; Hyper-CVAD, Cyclophosphamide + Vincristine + Adriamycin + Dexamethasone; IA, I-darubicin + Cytarabine; M2, acute myeloblastic leukemia with maturation; M4, acute myelomonocytic leukemia; M5, Acute monocytic leukemia; MA, Mitoxantrone + Cytarabine; MA$^*$, Methotrexate + Cytarabine; VDCLP, Vincristine + Daunorubicin + Cyclophosphamide + L-asparaginase + Prednisone; VDLP, Vincristine + Daunorubicin + L-asparaginase + Prednisone.

supernatant was collected. SOD activity and MDA content in the homogenate were measured using a SOD Activity Detection kit (lot no. A064; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and an MDA Detection kit (lot no. A003-2; Nanjing Jiancheng Bioengineering Institute), respectively.

*Statistical analysis*. Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation of at least three independent tests. For cell culture data, the comparison among groups was performed using the Wilcoxon rank-sum test, and α was adjusted according to the number of comparisons (α'=α/number of comparisons). Analysis of variance with the Student-Newman-Keuls test was used to analyze the differences among the groups in mouse experiments. Survival curves were compared using Kaplan-Meier and log-rank (Mantel-Cox) test, and α was adjusted according to the number of comparisons (α'=α/number of comparisons). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Quercetin enhances the cytotoxicity of Adriamycin to leukemic cells from patients*. A number of available leukemic cell lines have been selected based on pre-existing intrinsic features that are favorable to the establishment of *in vitro* culture, which may influence drug resistance and survival (20). Therefore, primary leukemic cells directly isolated from the peripheral blood of patients with drug resistance were used in the present study to represent the actual conditions observed in the clinical setting.

Primary leukemic cells were treated with a series of concentrations of Adriamycin or quercetin. The results demonstrated that the inhibition rate of Adriamycin at 6 $\mu$g/ml was close to 50% ($IC_{50}$, 5.6 $\mu$g/ml; Fig. 1A). Treatment with 75.5 $\mu$g/ml or 151 $\mu$g/ml quercetin did not affect cell proliferation (Fig. 1B). Regarding the concentration of the quercetin solution that could interfere with the measurement of cell viability, 75.5 $\mu$g/ml was chosen for the following experiments, as derived from Fig. 1.

A gradient dose of Adriamycin was combined with quercetin to investigate whether quercetin enhanced the cytotoxic effect of Adriamycin. The results revealed that Adriamycin efficiently suppressed the proliferation of primary leukemic cells (Fig. 2); this effect depended on treatment duration (24, 48 or 72 h) and dose (0.06, 0.6 or 6 $\mu$g/ml). Longer treatment and higher drug dose suppressed cell growth more efficiently. When half doses of Adriamycin (0.03, 0.3 or 3 $\mu$g/ml) were co-administered with quercetin (75.5 $\mu$g/ml), the treatment resulted in similar suppression of cell growth (Fig. 2), which indicated that quercetin may increase the sensitivity of leukemic cells to Adriamycin.

*Quercetin and high-dose Adriamycin co-treatment enhances the survival of mice with leukemia*. The mouse model of non-irradiated T-ALL was established to further explore the potential of quercetin to enhance Adriamycin efficiency in treating AL. Following the transplantation of leukemic cells into recipient mice, the survival duration was recorded. The analysis demonstrated that administering low-dose

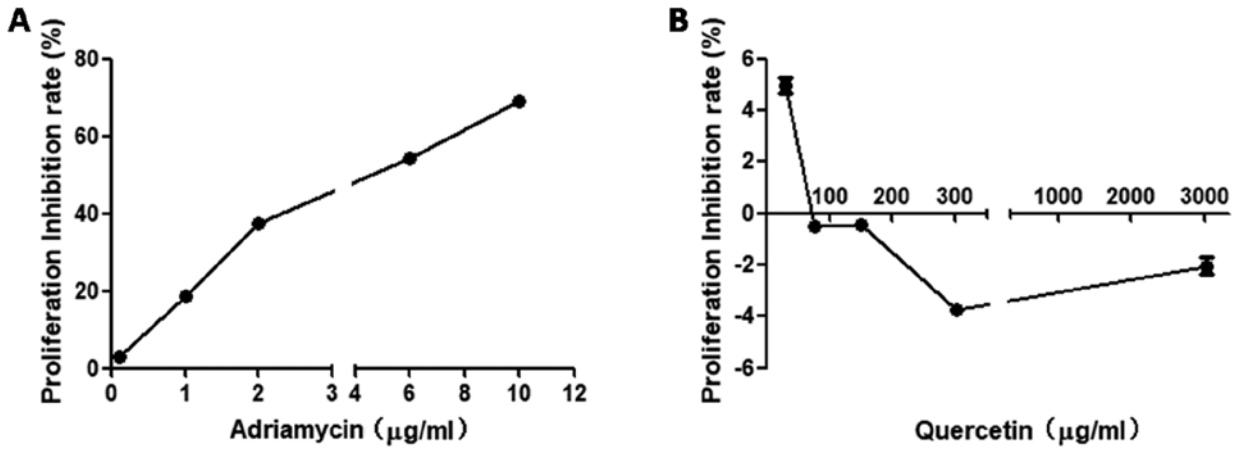


Figure 1. Adriamycin and quercetin suppress leukemia cell growth. (A and B) Primary leukemia cells isolated from patients with Adriamycin-resistant acute leukemia were treated with gradient concentrations of (A) Adriamycin or (B) quercetin for 72 h. Cell proliferation was detected using the Cell Counting Kit-8 assay, and the proliferation inhibition rate was calculated accordingly.

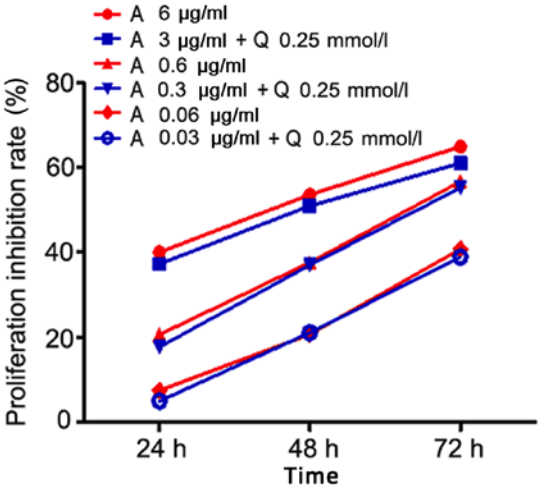


Figure 2. Quercetin increases the sensitivity of primary leukemic cells to Adriamycin. Primary leukemic cells isolated from patients with Adriamycin-resistant acute leukemia were treated with different concentrations of Adriamycin with or without quercetin for 24, 48, or 72 h. The Cell Counting Kit-8 assay was conducted to detect cell proliferation, and the rate of proliferation inhibition was calculated accordingly. A, Adriamycin; Q, quercetin.

Adriamycin alone, quercetin alone or low-dose Adriamycin combined with quercetin did not significantly alter the survival duration of mice with T-ALL (Fig. 3A). Administration of high-dose Adriamycin alone reduced the median survival time to 14 days, although administering high-dose Adriamycin combined with quercetin (median survival time 51 days) markedly extended the survival of mice with T-ALL compared with untreated mice ($P<0.05$; Fig. 3B). The mice in the low-dose Adriamycin group exhibited a longer survival time compared with the Adriamycin + quercetin group, but the difference was not significant (Fig. 3A).

Peripheral blood from T-ALL model mice was analyzed to determine the mechanism of enhanced survival with high-dose Adriamycin + quercetin combination treatment. The analysis revealed that the numbers of white blood cells (WBCs) and red blood cells (RBCs) were altered by drug treatment following transplantation, as determined by flow cytometry. There were no differences in the changes in blood cells at 4 days among the groups, with the exception of an increase in RBCs in the Adriamycin + quercetin group compared with the untreated group. The number of WBCs in peripheral blood was significantly reduced in the Adriamycin and Adriamycin + quercetin groups compared with the untreated group on days 7, 11, and 16 following transplantation ($P<0.01$ and $P<0.001$); however, no differences were observed at 23 and 31 days (Fig. 4A). Quercetin alone did not significantly change the number of WBCs compared with the untreated group (Fig. 4A). The RBC count indicated that Adriamycin significantly ($P<0.01$) reduced the number of RBCs at an earlier stage (before day 23) following transplantation compared with the untreated group (Fig. 4B). Platelets (PLTs) began to decrease significantly in the untreated and quercetin-alone groups from day 11 after transplantation, whereas Adriamycin and Adriamycin + quercetin co-treatment restored this reduction to the level comparable to healthy C57BL/6 mice (normal group) (Fig. 4C), which indicated that Adriamycin may alleviate leukemia by preventing the dramatic decrease in the number of PLTs.

*Quercetin attenuates Adriamycin-induced oxidative stress in the heart*. Several toxic side effects of Adriamycin have been reported, including the induction of oxidative injury in the heart, which is monitored based on SOD activity and MDA content (4,21). In the present study, quercetin was demonstrated to be beneficial in reducing the side effects of Adriamycin; in cardiac tissues of mice, SOD activity in the Adriamycin (low dose or high dose) + quercetin combination treatment group was higher compared with the respective Adriamycin-alone (low dose or high dose) group (Fig. 5A). MDA content in the Adriamycin (low dose or high dose) + quercetin combination treatment group was lower compared with the Adriamycin-alone (low dose or high dose) group (Fig. 5B). Higher SOD activity and lower MDA content indicated enhanced anti-oxidant capacity and reduced oxidative damage, respectively. Thus, the results suggested that quercetin may reduce the cardiac toxicity of Adriamycin by reducing oxidative injury to cardiac tissue.

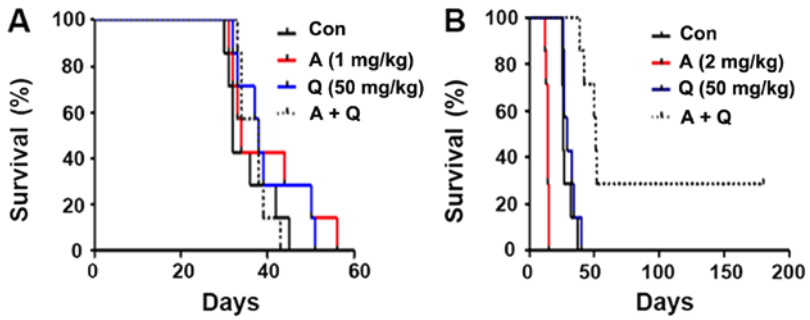


Figure 3. Survival time of mice with T-ALL is enhanced by co-treatment with high-dose Adriamycin and quercetin. (A) Mice with T-ALL leukemia were treated with low-dose Adriamycin alone, quercetin alone or their combination, and the median survival duration was calculated from the survival record. Median survival: Con, 32 days; A, 34 days; Q, 38 days; A+Q, 38 days. (B) Mice with T-ALL leukemia were treated with high-dose Adriamycin alone, quercetin alone or their combination, and the median survival duration was calculated. Median survival: Con, 26 days; A, 14 days; Q, 29 days; A+Q, 51 days. A, Adriamycin; Con, untreated control; Q, quercetin; T-ALL, T cell acute lymphoblastic leukemia.

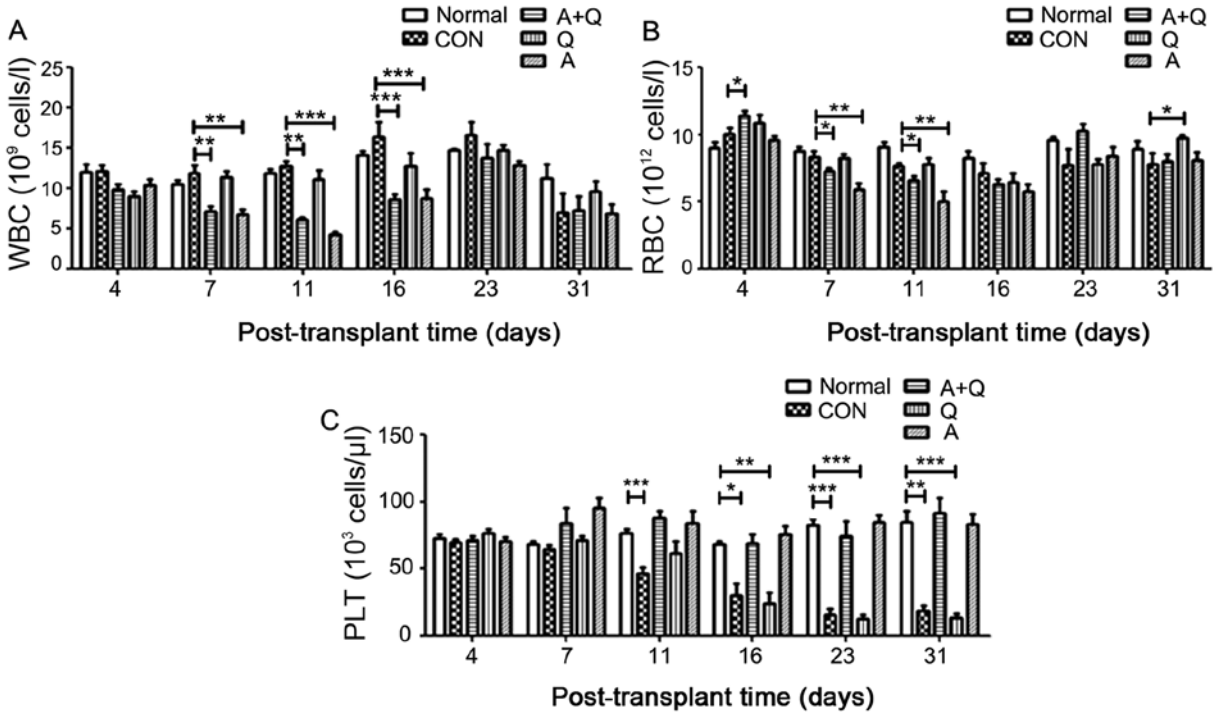


Figure 4. Peripheral blood cells and components are altered by Adriamycin and quercetin in mice with T-ALL. (A) WBCs, (B) RBCs and (C) PLTs were counted in peripheral blood from normal healthy C57BL/6 mice, untreated CON mice with T-ALL or mice with T-ALL treated with A (2 mg/kg), Q (50 mg/kg) or their combination. n=7 for each group. $^{*}$P<0.05,$^{**}$P<0.01 and $^{***}$P<0.001. A, Adriamycin; CON, untreated control; PLT, platelets; Q, quercetin; RBC, red blood cells; T-ALL, T cell acute lymphoblastic leukemia; WBC, white blood cells.

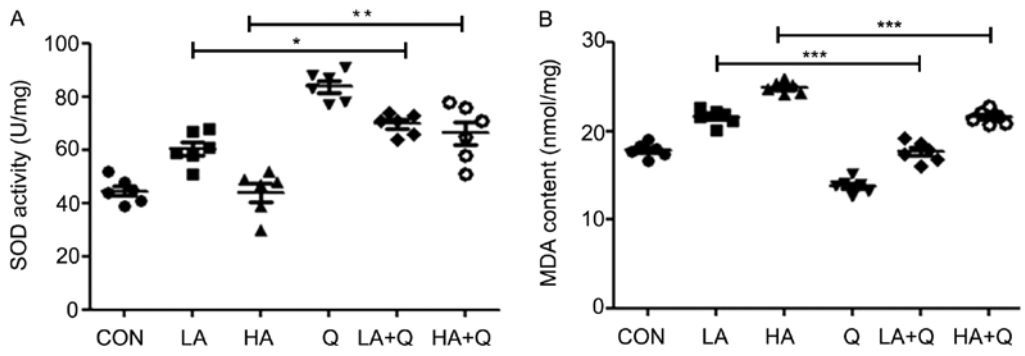


Figure 5. Quercetin attenuates Adriamycin-induced oxidative stress in the heart. (A) SOD activity and (B) MDA content in the heart were monitored on day 31 post-transplantation in CON mice with T-ALL without treatment or treated with LA (1 mg/kg), HA (2 mg/kg), Q (50 mg/kg), or their combination (LA+Q or HA+Q). $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. CON, untreated; HA, high-dose Adriamycin; LA, low-dose Adriamycin; MDA, malondialdehyde; Q, quercetin; SOD, superoxide dismutase (SOD); T-ALL, T cell acute lymphoblastic leukemia.

## Discussion

Leukemia is a severe malignancy of the hematopoietic system. AL displays rapid progression and high mortality (1,5). Chemotherapy is currently the main strategy for treating AL (22). Adriamycin is a commonly used anthracycline-type chemotherapy drug that has toxic side effects when a high dose is administered (4). In addition, drug resistance to Adriamycin in cancer cells is widely reported, limiting the effectiveness of chemotherapy (6,7). Originally developed as a food supplement, quercetin has been reported to exert an anti-tumor effect on a variety of cancers, including leukemia, lymphoma, colon, ovarian, cervical, prostate and breast cancer (23,24). In addition, quercetin protects the cardiovascular system from damage during aging or disease (25). A previous study has demonstrated that quercetin inhibits the proliferation of P388 leukemic cells (12). The aim of the present study was to investigate the anti-leukemic effect of quercetin on cultured leukemic cells and a mouse model of leukemia, with a particular focus on enhancing the therapeutic effect of Adriamycin and reducing its side effects.

The present study used primary leukemic cells isolated from patients with AL exhibiting drug resistance to determine the effect of drug treatment. The results demonstrated that quercetin, Adriamycin and their combination inhibited the proliferation of primary leukemic cells; the suppression depended on the concentration and duration of drug treatment. Notably, when quercetin was co-administered, a two-fold lower dose of Adriamycin was required to achieve a similar growth-inhibitory effect on leukemic cells to that of Adriamycin alone. This result indicated that quercetin may enhance the inhibitory activity of Adriamycin.

The enhancing effect of quercetin on Adriamycin was also indicated in a mouse model of leukemia. Most animal models of leukemia are generated in immunodeficient mice; for example, mice in which the immune system has been destroyed by irradiation (26,27). However, these models do not reflect immune alteration, which is crucial in patients during tumorigenesis or drug response. Therefore, the present study was conducted in a non-irradiated, immunocompetent mouse model.

NOTCH1 is a type I transmembrane receptor involved in signal transduction; activation of the NOTCH1 signaling pathway is present in >50% of patients with T-ALL (18). In agreement with this clinical observation, the transplantation of hematopoietic leukemic cells harboring mutant Notch1 effectively induces T-ALL in recipient mice (18). Taking advantage of this model, the present study explored the therapeutic effect of Adriamycin and quercetin. Of note, based on the Kaplan-Meier survival curves, low-dose Adriamycin treatment was more effective in extending mouse survival time compared with high-dose Adriamycin treatment, which was contrary to the results obtained from primary leukemic cells and indicated that *in vitro* experiments cannot replace the biological complexity of a whole organism for the study of diseases. In addition, high-dose Adriamycin combined with quercetin effectively inhibited leukemia development, reflected by the extended survival. This was consistent with the result observed in primary leukemic cells that quercetin enhanced the treatment effect of Adriamycin.

The anti-leukemic effect of Adriamycin was dose-dependent. However, with the increase in the dose of Adriamycin, its toxic side effects became more severe. The most severe side effect is cardiac injury (7,28). A previous study has found that administering Adriamycin to BALB/c nude mice transplanted with the leukemic cell line P388 causes cardiac injury, which is alleviated following co-treatment with Adriamycin and quercetin (12). The mechanism underlying the cardiac toxicity of Adriamycin may be oxidative damage to cardiomyocytes, which is associated with various types of heart injury (24,28). SOD scavenges superoxide radicals in cells to protect them from damage. MDA is the product of lipid peroxidation in the cell membrane, which is a marker for oxidative damage in cells (12). The present study demonstrated that quercetin increased SOD activity and decreased MDA content in the hearts of non-irradiated mice with T-ALL, which indicated that quercetin may reduce the cardiac toxicity of Adriamycin by attenuating oxidative stress, thus promoting the survival of mice with leukemia under high-dose Adriamycin treatment.

In conclusion, high-dose Adriamycin effectively inhibited the proliferation of leukemic cells, but the toxic side effects of high-dose Adriamycin limited the efficacy of the drug in mice with T-ALL. Quercetin was beneficial for both cultured primary leukemic cells and mice with T-ALL treated with Adriamycin by enhancing the pharmacological effect of Adriamycin and/or restricting oxidative damage to the heart. These results shed light on the development of a novel strategy for treating AL. To further clarify the molecular mechanism of quercetin and Adriamycin co-treatment in the suppression of growth in refractory acute leukemia, transcriptome sequencing is planned for future experimental studies.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

YH and HD conceived and supervised the study. YS designed the experiments. YS, XS, HC, LY and HD performed the experiments. YS, HD and YH analyzed the data. YS wrote

the manuscript. YS, HD and YH revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

**Ethics approval and consent to participate**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments. Ethical approval was received from the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University All patients signed informed consent. All animal experiments were approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Chan KW: Acute lymphoblastic leukemia. Curr Probl Pediatr Adolesc Health Care 32: 40-49, 2002.
2. Ma JJ, Chen Y and Yu L: Research progress on drug-resistance of acute lymphoblastic leukemia-review. Zhongguo Shi Yan Xue Ye Xue Za Zhi 24: 261-265, 2016 (In Chinese).
3. Jing H and Feng R: Research progress on minimal residual disease in acute leukemia detected by multiparametric flow cytometry. Zhongguo Shi Yan Xue Ye Xue Za Zhi 22: 847-851, 2014 (In Chinese).
4. Johnson-Arbor K and Dubey R: Doxorubicin. StatPearls, Treasure Island, FL, 2019.
5. Mehta-Shah N, Ratner L and Horwitz SM: Adult T-cell leukemia/lymphoma. J Oncol Pract 13: 487-492, 2017.
6. Ballmann C, Denney T, Beyers RJ, Quindry T, Romero M, Selsby JT and Quindry JC: Long-term dietary quercetin enrichment as a cardioprotective countermeasure in mdx mice. Exp Physiol 102: 635-649, 2017.
7. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD and Colan SD: Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. J Clin Oncol 23: 2629-2636, 2005.
8. Padron E and Fernandez H: Anthracycline dose intensification in young adults with acute myeloid leukemia. Ther Adv Hematol 3: 17-27, 2012.
9. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H and Yin Y: Quercetin, inflammation and immunity. Nutrients 8: 167, 2016.
10. Darband SG, Kaviani M, Yousefi B, Sadighparvar S, Pakdel FG, Attari JA Mohebbi I, Naderi S and Majidinia M: Quercetin: A functional dietary flavonoid with potential chemo-preventive properties in colorectal cancer. J Cell Physiol 233: 6544-6560, 2018.
11. Francaux M and Deldicque L: Using polyphenol derivatives to prevent muscle wasting. Curr Opin Clin Nutr Metab Care 21: 159-163, 2018.
12. Han Y, Yu H, Wang J, Ren Y, Su X and Shi Y: Quercetin alleviates myocyte toxic and sensitizes anti-leukemic effect of Adriamycin. Hematology 20: 276-283, 2015.
13. Alam RT, Zeid EH and Imam TS: Protective role of quercetin against hematotoxic and immunotoxic effects of furan in rats. Environ Sci Pollut Res Int 24: 3780-3789, 2017.
14. Brito AF, Ribeiro M, Abrantes AM, Pires AS, Teixo RJ, Tralhão JG and Botelho MF: Quercetin in cancer treatment, alone or in combination with conventional therapeutics? Curr Med Chem 22: 3025-3039, 2015.
15. Lamson DW and Brignall MS: Antioxidants and cancer therapy II: Quick reference guide. Altern Med Rev 5: 152-163, 2000.
16. Chen C, Zhou J and Ji C: Quercetin: A potential drug to reverse multidrug resistance. Life Sci 87: 333-338, 2010.
17. Belli JA and Harris JR: Adriamycin resistance and radiation response. Int J Radiat Oncol Biol Phys 5: 1231-1234, 1979.
18. Hu X, Shen H, Tian C, Yu H, Zheng G, XuFeng R, Ju Z, Xu J, Wang J and Cheng T: Kinetics of normal hematopoietic stem and progenitor cells in a Notch1-induced leukemia model. Blood 114: 3783-3792, 2009.
19. Ma S, Shi Y, Pang Y, Dong F, Cheng H, Hao S, Xu J, Zhu X, Yuan W, Cheng T and Zheng G: Notch1-induced T cell leukemia can be potentiated by microenvironmental cues in the spleen. J Hematol Oncol 7: 71, 2014.
20. Garnett MJ and McDermott U: The evolving role of cancer cell line-based screens to define the impact of cancer genomes on drug response. Curr Opin Genet Dev 24: 114-119, 2014.
21. Cappetta D, De Angelis A, Sapio L, Prezioso L, Illiano M, Quaini F, Rossi F, Berrino L, Naviglio S and Urbanek K: Oxidative stress and cellular response to doxorubicin: A common factor in the complex milieu of anthracycline cardiotoxicity. Oxidative medicine and cellular longevity 2017: 1521020, 2017.
22. Brown PA, Shah B, Fathi A, Wieduwilt M, Advani A, Aoun P, Barta SK, Boyer MW, Bryan T, Burke PW, *et al*: NCCN Guidelines Insights: Acute Lymphoblastic Leukemia, Version 1.2017. J Natl Compr Canc Netw 15: 1091-1102, 2017.
23. Kim HS, Wannatung T, Lee S, Yang WK, Chung SH, Lim JS, Choe W, Kang I, Kim SS and Ha J: Quercetin enhances hypoxia-mediated apoptosis via direct inhibition of AMPK activity in HCT116 colon cancer. Apoptosis 17: 938-949, 2012.
24. Maso V, Calgarotto AK, Franchi GC Jr, Nowill AE, Filho PL, Vassallo J and Saad ST: Multitarget effects of quercetin in leukemia. Cancer Prev Res (Phila) 7: 1240-1250, 2014.
25. Shen Y, Croft KD, Hodgson JM, Kyle R, Lee IL, Wang Y, Stocker R and Ward NC: Quercetin and its metabolites improve vessel function by inducing eNOS activity via phosphorylation of AMPK. Biochem Pharmacol 84: 1036-1044, 2012.
26. Rivina L, Davoren M and Schiestl RH: Radiation-induced myeloid leukemia in murine models. Hum Genomics 8: 13, 2014.
27. Almosailleakh M and Schwaller J: Murine models of acute myeloid leukaemia. Int J Mol Sci 20: pii: E453, 2019.
28. Segredo MP, Salvadori DM, Rocha NS, Moretto FC, Correa CR, Camargo EA, de Almeida DC, Reis RA, Freire CM, Braz MG, *et al*: Oxidative stress on cardiotoxicity after treatment with single and multiple doses of doxorubicin. Hum Exp Toxicol 33: 748-760, 2014.