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Effect of salidroside on bone marrow haematopoiesis in a mouse model of myelosuppressed anaemia

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ABSTRACT

This study was designed to investigate the effect of salidroside (SAL) on bone marrow haematopoiesis in a mouse model of myelosuppressed anemia. After the mouse model was established by ^{60}Co γ irradiation and cyclophosphamide, pancytopenia and a sharp reduction in bone marrow stromal cells and bone marrow haematopoietic stem cells (lineage$^-$Sca1$^+$c-kit$^+$) were observed. This was greatly alleviated by SAL (25 mg/kg, 50 mg/kg, 100 mg/kg) in a dose-dependent manner (50% effective dose value of 35.7 mg/kg and 61.2 mg/kg, respectively), followed by a distinct increment in anti-apoptotic protein Bcl-2. For cell culture *in vitro*, treatment with SAL resulted in a significant recovery of burst-forming unit–erythroids, and colony-forming unit–granulocyte macrophages on Day 7, and colony-forming unit–erythroids on Day 3, dose-dependently, but not of colony-forming unit–megakaryocyte macrophages. Inoculation of bone marrow cells derived from SAL-administrated donor mice resulted in a 60% survival of recipient mice at the high dose of 100 mg/kg SAL at 2 months after surgery. SAL appeared to be able to stimulate the restoration of bone marrow haemopoietic regulation in myelosuppressed anemia. Based on the down-regulation of Fas ligand associated with the expression of Caspase-3 at the protein level, it was suggested that SAL might have an anti-apoptotic effect on bone marrow cells in the Fas-apoptotic pathway of Fas/FasL–caspase-3.

Keywords: salidroside; bone marrow haematopoiesis; myelosuppressed anemia; hematopoietic progenitors

INTRODUCTION

Currently, various strategies are applied in the clinical treatment of malignant cancer, such as resection, radiotherapy, chemotherapy, target gene therapy, conservative treatment and adjuvant intervention. However, myelosuppression may occur during the interventions of radiotherapy and chemotherapy, followed by an obvious decrease in neutrophils and other severe side-effects. Therefore, to effectively prevent the potential side-effects caused by radiotherapy and chemotherapy, especially severe myelosuppression, positive regulation of bone marrow haematopoiesis is a critical step in adjuvant treatment for malignant cancer.

Salidroside (SAL) is a glucoside extracted from *Rhodiola rosea*, and has been used for hundreds of years by Chinese and Russian doctors in traditional herbal medicine. SAL has important functions and properties, such as antioxidant, anti-inflammatory, anti-tumor, cytoprotection, and positive immunomodulation [1, 2]. An accumulating literature reports that SAL repairs the bone marrow cell cycle and reduces the expression of apoptosis-related proteins (such as Bcl-2 and matrix metalloproteinase-9) in bone marrow–depressed anemic mice [3, 4]. In addition, it also exhibits a positive dose–effect relationship on the expression of substance P and neurokinin-1 receptor, which might contribute to the restoration of bone marrow hematopoiesis in anemic mice [5]. Based on the aforementioned literature, it is believed that SAL has an important effect on haematopoietic function in bone marrow–depressed anemic mice.

Myelosuppressed anemic mice were treated with SAL to further study its effect on changes in pancytopenia, hematopoietic progenitors, bone marrow stromal cells (BMSCs) and other cells at different time points. The effect of SAL on the related survival rate of myelosuppressed anemic mice after bone marrow transplantation was studied as well. Our results provided evidence that SAL might have therapeutic potential in relation to myelosuppressed anemia by not only restorring the bone marrow haematopoietic microenvironment by promoting the proliferation of BMSCs, but also by enhancing the

related proliferation and further differentiation of hematopoietic stem cells (HSCs) to alleviate the pancytopenia observed in myelosuppressed anemia.

METHODS

Animals and grouping

Special pathogen-free Swiss male mice (weight: 18–22 g; age: 6–8 weeks) were obtained from the Experimental Animal Center of Jiangxi University of TCM (Jiangxi, China, Animal Response License No.: SCXK2015–0010). All mice were randomly separated into five marked subgroups ($n = 10$ for each group): normal group, myelosuppressed anemic model (vehicle), and SAL subgroups (25 mg/kg, 50 mg/kg and 100 mg/kg). The molecular structure of SAL is shown in Fig. 1D. After modeling, the mice were treated intraperitoneally with saline or various doses of SAL once a day for 7 consecutive days. All animal experiments were approved by the Committee on Animal Care and Use of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, and performed using protocols approved by the Animal Care and Use Committee of Jiangxi University of Technology.

Mouse model of myelosuppressed anemia

The myelosuppressed anemic mouse model was prepared according to a previous report, with minor changes [4]. The mice were irradiated (2.0 Gy ^{60}Co γ irradiation) via whole-body exposure. On Day 3, after the irradiating exposure, all mice were administrated with consecutive intraperitoneal injection of 40 mg/kg/day cyclophosphamide (Shanghai Hualian Pharmaceutical Co. Ltd, China, Batch No: 110325) and 50 mg/kg/day chloramphenicol (Southwest Pharmaceutical Stock Co. Ltd, China, Batch No: 94060031) over the next 3 days to suppress the immunologic function and depredate the hemopoietic system. IMDM medium, erythropoietin (EPO), thrombopoietin (TPO) and granulocyte macrophage colony-stimulating factor (GM-CSF) were all added to the cell culture. Lineage Ab mixture, PE-Cy7-conjugated anti-Sca1, fluorescent antibody PE-c-kit and FITC-CD45 (Beyotime Institute of Biotechnology, Shanghai, China) were used to label the haemopoietic stem cells and the mature leucocytes. The experiment was performed on BD FACS Calibur flow cytometry (Beckman Coulter, Inc., California, USA).

Peripheral blood cells

One hour after the last administration of SAL, 20 µl of peripheral blood was collected from the posterior orbital venous plexus, diluted and then analyzed using a fully automatic blood cell analyzer (Hitachi 7020, Japan). The counts of platelets (PLTs), red blood cells (RBCs) and white blood cells (WBCs) were recorded at various time points before and after treatment.

Culture of hemopoietic progenitor cells *in vitro*

On Day 7 after SAL administration, bone marrow cells (BMCs) were collected from the femur and cultured in a concentration of 5×10^5 cells/ml (37°C, 5% CO_2). Colony-forming unit–erythroids

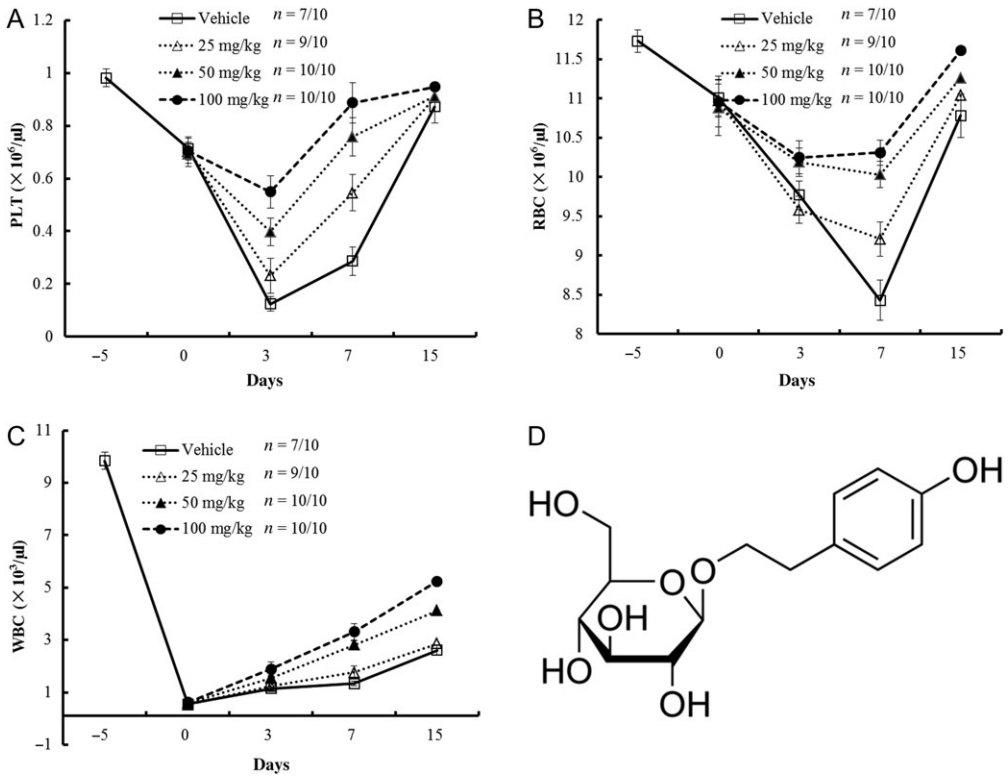

Fig. 1. Effects of SAL on pancytopenia in an irradiated mouse model of myelosuppressed anemia (A: PLT, platelet; B: RBC, red blood cells; C: WBC, white blood cells; D: molecular structure of SAL).

(CFU-Es) were counted using a blood counting chamber on Day 3 of cell culture, and the numbers of burst-forming unit–erythroids (BFU-Es), colony-forming unit–granulocyte macrophages (CFU-GMs) and colony-forming unit–megakaryocytes (CFU-Megs) were recorded on Day 7 of cell culture. Cell cultures were executed according to the previously reported method [6]. After culture for 7 days, agar disks were detached and moved to glass slides, and stained with acetylcholinesterase. CFU-Meg–derived colonies were counted based on a definition of at least three megakaryocytes. Cultures of CFU-GMs and BFU-Es were performed using a methylcellulose method for 7 days. Their colonies (at least 30 cells) were counted and scored under an inverted phase-contrast microscope. The total number of each type of hematopoietic progenitor cell per femur was calculated according to the formula below: total number of colonies = colonies per dish/cells seeded per dish × total number of cells obtained from one femur.

Measurement of the number of BMSCs and bone marrow haematopoietic stem cells

On Day 7 after SAL administration, BMSCs from the femur were harvested and diluted to a concentration of 1×10^6 cells/ml (37°C, 5% CO_2), before duplicate culturing with culture medium IMDM (containing 15% fetal calf serum) in 6-well plates. For the initial cell counting using a blood counting chamber, the BMSC suspension was prepared according to the routine method [7]. Sixty microliters of the suspension was centrifuged, followed by rinsing with PBS (5 min × 3 times). The form of the cells was observed under an inverted phase-contrast microscope every day, and the proportion of growing cells in the spreading area on the well bottom area of the plates was calculated on Days 0, 3, 7 and 15 of culture. For counting the bone marrow HSCs, using a flow cytometer, the HSCs were stained twice with fluorescent lineage Ab mixture, PE-Cy7–conjugated anti-Sca1, PE-conjugated anti-c-kit and FITC-conjugated anti-CD45 HSC lineage$^-$Sca1$^+$c-kit$^+$ cells were identified on the basis of a primary CD45/SSC (side scatter) positive gating [8].

Western blot analysis for the expression of apoptins

To investigate a potential mechanism that could be involved in cell-growth proliferation, we examined the expression of apoptins such as Bcl-2, Bax, procaspase-3 and caspase-3 on Day 7 after treatment. The main protocol used was that reported in the previous literature [9]. On Day 7 after SAL administration, BMCs were collected from the femur and cultured in a concentration of 5×10^5 cells/ml (37°C, 5% CO_2). All samples from each group were collected with the relative protein concentration tested by the Bradford method, and each 50 μg protein was loaded for western blot analysis. The separated proteins were transferred and blotted onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Non-specific binding was blocked with 5% fat-free milk in TBST (pH 7.5, 50 mM Tris-HCL, 0.15 NaCl, 0.1% Tween-20) for 2 h at room temperature, and the cells were incubated overnight at 4°C with the proper primary antibodies. Antibodies were purchased and diluted as follows: a rabbit anti-Bcl-2 (1:2000, Cell Signal), a rabbit anti-Bax antibody (1:2000, Santa Cruz Biotechnology), mouse anti-β-actin (1:5000, Abcam), anti-caspase-3 polyclonal antibody (1:1000, Santa Cruz Biotechnology) and rabbit anti-Fas ligand (FasL) (1:1000, Abcam). Detection of β-actin was carried out to confirm the equal loading of the proteins. Membranes were then washed three times for 10 min each with TBST buffer and incubated with the immunolabeled secondary antibody (1:6000) (Abcam) for 1 h at room temperature. After washing with TBST buffer, the membranes were dyed with ECL luminescent liquid (Bio-Red, California, USA) for 1 min and pressed with X-ray film (Beyotime Institute of Biotechnology, Shanghai, China) before ECL chemiluminescence detection. Finally, the gray value was scanned and densitometric analysis was taken by ImageJ Software. The results were analyzed by ANOVA and expressed as mean ± SEM for each experimental condition performed in triplicate.

Bone marrow transplantation and related survival rate

As mentioned in the previous study [10], 1 h after the irradiating exposure, donor mice were treated with SAL (25 mg/kg, 50 mg/kg, 100 mg/kg) or vehicle once a day for 3 days. One hour after the last intraperitoneal injection, the femoral BMCs from myelosuppressed anemic mice in each group were harvested, washed and resuspended, accordingly. Aliquots of cells (10^6 cells per mouse) were prepared and inoculated intravenously through the tail vein into syngeneic recipient mice that had suffered from a lethal dose of irradiation (8.6 Gy). The survival of the transplanted mice in each group were monitored and recorded every day for 2 months.

Statistical analysis

All data are presented in the form of mean ± SEM. The statistical significance of differences in the numbers of peripheral blood cells, hemopoietic progenitor cells, bone marrow stromal cells, HSCs and BMNCs, and data for the related protein expression comparing the vehicle- and SAL-treated groups was assessed by using one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test, using GraphPad Prism 5 (San Diego, CA, USA). Significance was set at $P < 0.05$.

RESULTS

Reduction of pancytopenia in the irradiated mouse model of myelosuppressed anemia

The counts of peripheral blood cells in the anemic model mice (vehicle) declined significantly in comparison with those in the normal control, which testified that the irradiation-induced mouse model of myelosuppression and anemia was successful and reliable. As shown in Fig. 1A–C, SAL resulted in pancytopenia being clearly reduced, with accelerated recovery of PLTs, RBCs and WBCs in a dose-dependent manner. The optimal efficacy of SAL was shown on Day 7 after treatment, the day on which there were the most changes in the indicators between each group ($P < 0.05$ compared with the model control). On Day 15 after onset, the counts of PLTs and RBCs had almost returned to basal levels. All data collected in each group were from living mice. Thirty percent (3/10) of all mice in the modeling control group were dead within the first week. The remarkable effect of SAL was to elevate the survival rate in the modeling mice.

The accelerated recovery of hematopoietic progenitors in the bone marrow

In addition, in the femur of the vehicle-treated modeling mice, extreme decreases in the counts of CFU-Megs, BFU-Es and CFU-GMs on Day 7, and CFU-Es on Day 3 of cell culture were noted (Fig. 2). For cell culture *in vitro*, treatment with SAL resulted in significant recovery of BFU-Es and CFU-GMs on Day 7, and CFU-Es on Day 3 of cell culture in a dose-dependent manner, but not of CFU-Megs.

Effect on the proliferation of BMSCs and the number of HSC lineage$^-$Sca1$^+$c-kit$^+$ cells

In *in vitro* BMSC culture, it was observed that the BMSCs tended to stick to the dish surface after culture for 24 h, but were rearranged into a fusiform shape after 48 h. After culture for 7 days, the proliferation of the BMSCs was obviously enhanced, and colonies of fibroblasts had formed as well. After culture for 15 days, the growing cells were spread over almost the whole bottom of the 6-well culture plate. As shown in Table 1, SAL promoted the proliferation of BMSCs.

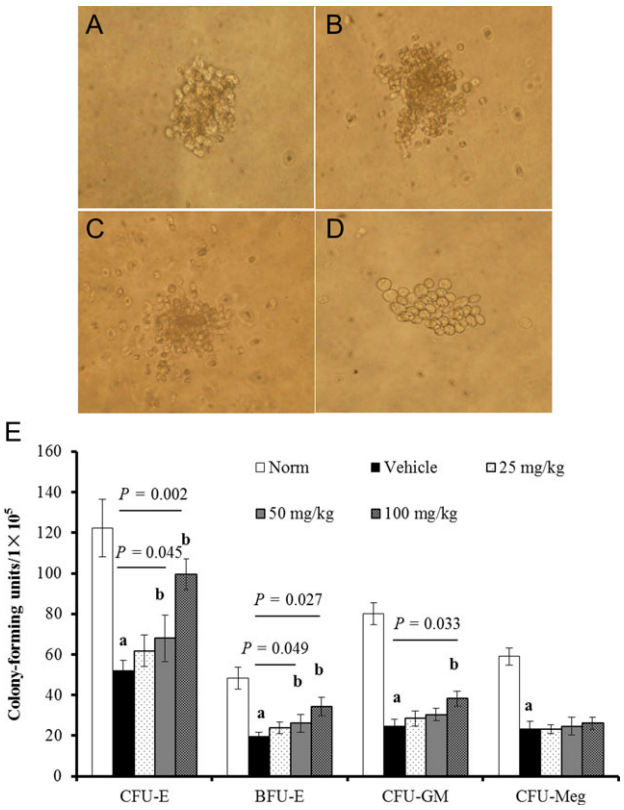

Fig. 2. Effects of SAL on the recovery of hematopoietic progenitors in the bone marrow (A: CFU-E; B: BFU-E; C: CFU-GM; D: CFU-Meg; E: the counting of colony-forming units). All data represented in the form of mean ± SEM (Norm: $n = 10$; vehicle: $n = 7$; 25 mg/kg SAL: $n = 9$; 50 mg/kg SAL: $n = 10$, 100 mg/kg SAL: $n = 10$). 'a' indicates a significant difference compared with the normal group, 'b' indicates a significant difference compared with the vehicle group.

Both the numbers of BMSCs and bone marrow HSCs were significantly decreased after modeling. However, after SAL administration for 7 days, both BMSCs and bone marrow HSCs of the lineage$^-$Sca1$^+$c-kit$^+$ showed a tendency to increase markedly with different doses, with an 50% effective dose value of 35.7 mg/kg and 61.2 mg/kg, respectively (Fig. 3).

Effect of SAL on the expression of apoptins in the irradiated mouse model of myelosuppressed anemia

According to the densitometric analysis of the western blots shown in Fig. 4, expressions of the proapoptic proteins Bax and Caspase-3 were inhibited dose-dependently after SAL treatment ($P < 0.05$). In contrast, the increment in the anti-apoptotic protein Bcl-2 was significant and dose-dependent, which might be related to its mechanism of inhibiting apoptosis of BMCs and promoting the recovery of hematopoietic progenitors in lethally irradiated recipient mice. On the other hand, based on the downregulation of FasL associated with the expression of Caspase-3, it was thought that SAL might have an anti-apoptotic effect on BMCs in the Fas-apoptotic pathway of Fas/FasL–caspase-3.

Bone marrow transplantation and the related survival rate

The viability of SAL treatment on the recovery of bone marrow hematopoietic progenitor cells after *in vivo* transplantation or intravenous inoculation of lethally irradiated recipient mice was also examined. One hour after the irradiating exposure, donor mice were treated with SAL (25 mg/kg, 50 mg/kg, 100 mg/kg) or vehicle once a day for 3 days. One hour after the last intraperitoneal injection, bone marrow was prepared from the donor mice and inoculated intravenously through the tail vein into syngeneic recipient mice that had suffered from a lethal dose of irradiation (8.6 Gy). Inoculation of BMCs derived from SAL-administrated donor mice resulted in a 60% survival of recipient mice at the high dose of 100 mg/kg SAL 2 months after surgery, and 30% survival at the middle dose of 50 mg/kg SAL. Furthermore, all recipient mice that received bone marrow cells derived from vehicle- or 25 mg/kg SAL-treated donor mice, died by 13 or 18 days after transplantation, respectively (Fig. 5).

DISCUSSION

Summarizing these *in vivo* and *in vitro* results, intraperitoneal administration of SAL produced a dose-dependent reduction in pancytopenia in myelosuppressed animals, with a great reduction in thrombocytopenia, anemia and leukopenia. It was believed that SAL not only decrease the apoptotic proteins of the Bcl-2/Bax and Fas-apoptotic pathways, but also activated or interacted with other endogenous cytokines to enhance the proliferation of erythroid and myeloid progenitors, thereby resulting in the elevation of hematopoietic restoration after irradiation in mice.

Our results on the timing of the SAL treatment were consistent with those of a previous report declaring that *Rhodiola* polysaccharide extract performed its activity on the recovery of peripheral blood

cells and the BMC cycle in myelosuppressed anemic mice dose-dependently [11]. It suggested that *Rhodiola* polysaccharide might stimulate the hematopoietic recovery of mice with myelosuppressed anemia by promoting the transition of G_0/G_1–S and S–G_2/M phases, and (finally) accelerating the proliferation of bone marrow hemopoietic cells [11]. In another study, hematopoietic progenitor cell culture was used to observe the effect of SAL on the proliferation of hematopoietie progenitor cells and on the number of BMCs in myelosuppressed anemic mice [12]. SAL could only promote the formation of BFU-Es and CFU-Es at the low concentration of 10 μg/ml, but was effective in a concentration range of 10–80 μg/ml on the formation of CFU-GMs and CFU-Megs *in vitro* ($P < 0.05$). This suggested that SAL might accelerate hematopoietic recovery of myelosuppressed anemic mice by stimulating the proliferation of hematopoietic progenitor cells.

Similarly, SAL enhanced cellular proliferation and angiogenic differentiation of bone marrow–derived endothelial progenitor cells (BM-EPCs) via Akt/mTOR/p70S6K and MAPK signaling pathways, and promoted secretions of VEGF and NO, which also mediated and attended their angiogenic differentiation. Besides, SAL statistically ameliorated H_2O_2-induced cell apoptosis, reversed the H_2O_2-induced phosphorylation of JNK and p38 MAPK, inhibited the obvious increase in the Bax/Bcl-xL ratio, decreased intracellular reactive oxygen species and restored the mitochondrial membrane potential of BM-EPCs [13].

Anti-c-kit is widely used as the antibody of protein c-kit, which is also 'stem cell factor receptor' or 'CD117', the main surface marker of mouse HSCs [14]. As leukocyte common antigen, CD45 is commonly used as a characteristic of mature cells, but it is weak in juvenile cells. In this study, HSC lineage$^-$Sca1$^+$c-kit$^+$ was identified on the basis of a primary CD45/SSC (side scatter) positive gating. Our results showed that both the numbers of BMSCs and bone marrow HSCs decreased significantly after modeling. However, after SAL administration for 7 days, both BMSCs and bone marrow HSC lineage$^-$Sca1$^+$c-kit$^+$ showed a tendency to increase markedly in various dose groups, with the ED_{50} value of 35.7 mg/kg and 61.2 mg/kg, respectively. For *in vitro* BMSC culture, it could be observed that the BMSCs tended to stick to the dish surface after culture for 24 h, and were rearranged into a fusiform shape after 48 h. After culture for 7 days, the proliferation of BMSCs was obviously improved and colonies of fibroblasts had formed as well. After culture for 15 days, the growing cells had spread over almost the

Table 1. Effect of Salidroside on the proliferation of bone marrow stromal cells in the mouse model of myelosuppressed anemia

Groups	0 days	3 days	7 days	15 days
Normal control	±	++	+++±	++++
Anemic vehicle	-	±	+	++
25 mg/kg	-	±	+ ±	++±
50 mg/kg	±	+	++	+++
100 mg/kg	±	++	+++	+++±

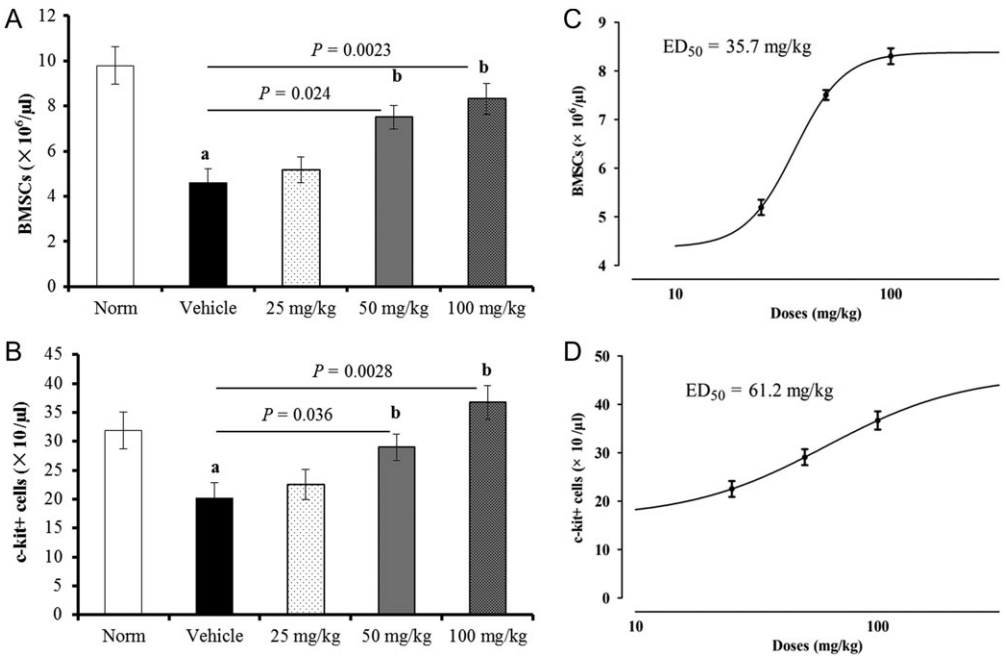

Fig. 3. Effect of SAL on the proliferation of BMSCs and the number of HSC lineage$^-$Sca1$^+$c-kit$^+$ cells (A: cell counting of BMSCs; B: ED_{50} of BMSCs; C: cell counting of HSC c-kit$^+$ cells; D: ED_{50} of HSC c-kit$^+$ cells). 'a' indicates a significant difference compared with the normal group, 'b' indicates a significant difference compared with the vehicle group.

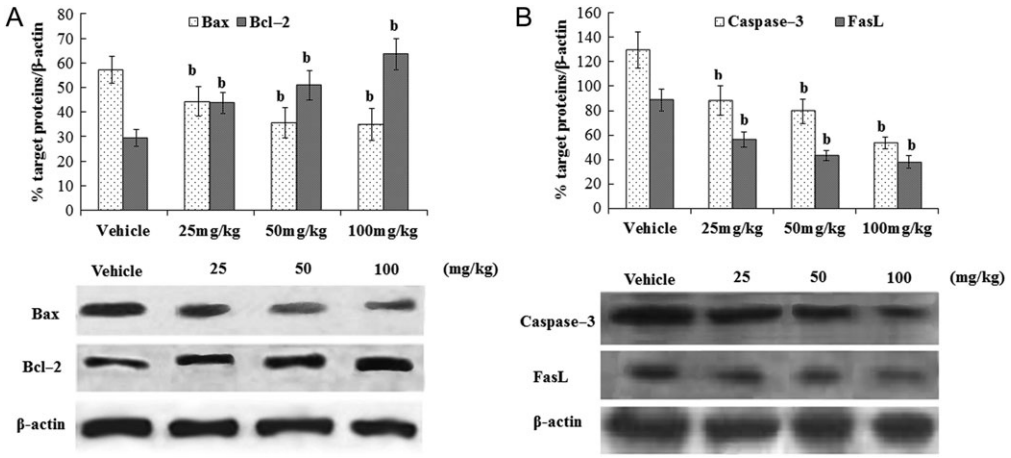

Fig. 4. The expressions of apoptins (A: Bcl-2 and Bax, B: Caspase-3 and FasL) in modeling mice after dose-dependent treatment. Data were normalized on the basis of β-actin levels. All data are represented in the form of mean ± SEM (Vehicle: $n = 7$; 25 mg/kg SAL: $n = 9$; 50 mg/kg SAL: $n = 10$, 100 mg/kg SAL: $n = 10$). 'b' indicates a significant difference compared with the vehicle group.

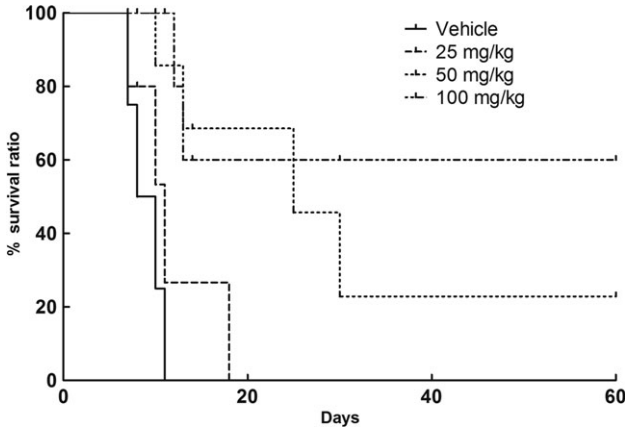

Fig. 5. Effects of SAL on the relative survival rate of myelosuppressed anemic mice after bone marrow transplantation ($n = 10$).

whole bottom of the 6-well culture plate. As shown in Table 1, SAL promoted the proliferation of BMSCs. As the major cells of the bone marrow haematopoietic microenvironment, BMSCs were established not only as adhesive sites for HSCs to be attached to for growth, but also as information transmitters via contact with HSCs directly or by promoting the release of a variety of growth factors [15, 16].

In summary, it was hypothesized that SAL could not only restore the bone marrow haematopoietic microenvironment by promoting proliferation of BMSCs, but also enhance the related proliferation and further differentiation of HSCs, thus significantly alleviating pancytopenia, with the accelerated recovery of PLTs, RBCs and WBCs and their supply of peripheral blood in a dose-dependent manner. Inoculation of BMCs derived from SAL-administrated donor mice resulted in 60% survival of recipient mice at the high dose of 100 mg/kg SAL 2 months after surgery, and 30% survival at the middle dose of 50 mg/kg SAL. In addition, all recipient mice that received BMCs derived from vehicle- or 25 mg/kg SAL-treated donor mice, died by 13 or 18 days after transplantation, respectively. Also, the expression of the proapoptic proteins Bax and Caspase-3 was inhibited dose-dependently after SAL treatment, followed by a remarkable increase in anti-apoptotic protein Bcl-2, which might be related to its functions of inhibiting apoptosis of BMCs and promoting the recovery of hematopoietic progenitors in lethally irradiated recipient mice. On the other hand, as the most studied membrane surface molecule and cytoplasmic molecule, the Fas/FasL system is a key regulator of apoptosis in T cells [17]. In our study, the downregulation of FasL was believed to be positively associated with the expression of Caspase-3. Therefore, it is suggested that SAL might have an anti-apoptotic effect on BMCs in the signaling pathway of Fas/FasL–caspase-3. SAL appeared to be able to stimulate the restoration of bone marrow haemopoietic regulation in myelosuppressed anemia.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Alotaibi NA, Slater NK, Rahmoune H. Salidroside as a novel protective agent to improve red blood cell cryopreservation. *PLoS One* 2016;11:e0162748.
2. Wang M, Luo L, Yao L et al. Salidroside improves glucose homeostasis in obese mice by repressing inflammation in white adipose tissues and improving leptin sensitivity in hypothalamus. *Sci Rep* 2016;6:25399.
3. Zhang X-S, Zhu B-D, Huang X-Q et al. Effect of salidroside on bone marrow cell cycle and expression of apoptosis-related proteins in bone marrow cells of bone marrow depressed anemia mice. *J Sichuan Univ (Med Sci Ed)* 2005;36:820–3 (in Chinese).
4. Zhang J, Li H, Lu L et al. The Yiqi and Yangyin Formula ameliorates injury to the hematopoietic system induced by total body irradiation. *J Radiat Res* 2017;58:1–7.
5. Zhang XS, Chen H, Xu MM et al. Effects of Salidroside on expression of substance P and neurokinin-1 receptor of bone marrow cells in bone marrow depressed anemia mice. *Chin J Immunol* 2015;31:1195–9.
6. Long W, Zhang G, Dong Y et al. Dark tea extract mitigates hematopoietic radiation injury with antioxidative activity. *J Radiat Res* 2018,59:387–94.
7. Chen Y, Zhu B, Zhang L et al. Experimental study of the bone marrow protective effect of a traditional Chinese compound preparation. *Phytother Res* 2009;23:823–6.
8. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. *Blood* 1997;90:2863–92.
9. Xu M, Fan Q, Zhang J et al. NFAT3/c4-mediated excitotoxicity in hippocampal apoptosis during radiation-induced brain injury. *J Radiat Res* 2017;58:827–33.
10. Kovalenko OA, Azzam EI, Ende N. Human umbilical-cord-blood mononucleated cells enhance the survival of lethally irradiated mice: dosage and the window of time. *J Radiat Res* 2013;54:1010–4.
11. Li J, Zhu BD, Chen YF et al. Effect of *Rhodiola* polysaccharide on peripheral blood cells and bone marrow cell cycle of myelosuppressed anemia mice. *Sichuan J Anat* 2006;14:4–6 (in Chinese).
12. Zhang XS, Wang YB, Zhu BD et al. Effect of salidroside on proliferation of hematopoietic progenitor cell in myelosuppressed anemia mice. *Sichuan J Zool* 2008;27:926–8 (in Chinese).
13. Tang Y, Vater C, Jacobi A et al. Salidroside exerts angiogenic and cytoprotective effects on human bone marrow–derived endothelial progenitor cells via Akt/mTOR/p70S6K and MAPK signalling pathways. *Br J Pharmacol* 2014;171:2440–56.
14. Katayama N, Shih JP, Nishikawa S et al. Stage-specific expression of c-kit protein by murine hematopoietic progenitors. *Blood* 1993;82:2353–60.
15. Verfaillie CM. Soluble factor(s) produced by human bone marrow stroma increase cytokine-induced proliferation and maturation of primitive hematopoietic progenitors while preventing their terminal differentiation. *Blood* 1993;82:2045–53.
16. Oki M, Ando K. Hematopoietic growth factors, cytokines, and bone-marrow microenvironment. *Nihon Rinsho* 2008;66:444–52 (in Japanese).
17. Rodríguez V, Plavnik L, Tolosa de Talamoni N. Naringin attenuates liver damage in streptozotocin-induced diabetic rats. *Biomed Pharmacother* 2018;105:95–102.