Immunomodulatory Effects of Blood Transfusion on Tumor Size, Metastasis, and Survival in Experimental Fibrosarcoma

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Abstract In spite of efforts, blood transfusion is still accompanied with adverse effects such as transfusion-related immunomodulation (TRIM). The current study aimed to evaluate the effects of allogeneic, syngeneic, fresh and storage blood transfusion on the growth and metastasis of tumors and survival in fibrosarcoma bearing BALB/c mice. Twenty-five BALB/c mice were grouped into five groups of equal size. All groups were injected 1.2×10^6 WEHI-164 cells subcutaneously to induce fibrosarcoma tumor. After expansion of the tumor, in four groups (except for the control group), hemorrhage-induced anemia was developed. Twenty-four hours later, blood deficit was replaced by fresh allogeneic, storage allogeneic, fresh syngeneic and storage syngeneic blood transfusion, respectively. After a blood transfusion, for 13 days, the tumor size and survival of the mice were evaluated. In the day 20, the mice were sacrificed and their spleen tissues were evaluated for TRIM induced metastasis. Tumor size increase in the groups that received allogeneic (fresh and storage) and storage

syngeneic blood transfusion was significantly higher than the control group (P value < 0.05). However, no significant difference was present in survival between the experiment groups and the control group. There was no metastasis in none of groups at the end of the study. Allogeneic and storage blood transfusion could have immunomodulatory effects such as increased tumor size. However, it seems that fresh and syngeneic blood transfusion have no effects on tumor growth in fibrosarcoma bearing mice. Further evidence may prove that more attention is warranted in blood transfusion into cancer cases.

Keywords Blood transfusion · Fibrosarcoma · Immunomodulation

Introduction

Blood transfusion is transplantation of cells and considerable amount of antigens. It could be allogeneic (obtained from a subject for injection to another of the same species) or autologous (obtained from a subject for injection to himself/itself) [1, 2]. In laboratory animals, syngeneic blood transfusion (from inbred laboratory animals to another inbred animals of the same species) is also performed [3]. Since many years ago, blood transfusion has found an important place in medicine. Its life-saving and condition-improving effects is well known in various clinical circumstances [4, 5]. The therapeutic effects of blood transfusion is undoubted. However, despite its advantages, blood transfusion is accompanied by some adverse effects and complications such as post-transfusion infections, HLA immunization, recurrent febrile non-hemolytic transfusion reactions (FNHTR) and transfusion-

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related immunomodulation (TRIM) [2, 6–9]. Variety of measures is adapted to minimize the adverse effects of blood transfusion. Despite research efforts, effects of TRIM are still not completely known. This phenomenon was first described in 1953 and has been studied by many researchers so far [2, 7, 10–14]. Different studies in the 1990s demonstrated that the immunosuppressive effects of blood transfusion in the host would be reduced when the white blood cells count in the transfused blood is decreased [6, 8]. In 1994, the results of two large randomized studies suggested the role of leukocyte-reduced or autologous blood transfusion in prevention of cancer relapse and recurrence of post-surgical infection [15]. Increased prevalence of cancers and sudden relapse or growth of tumors are some consequences associated with TRIM. However, studies on immunomodulatory effects of transfusion of blood products on the growth and metastasis of tumors are not frequent [2, 3, 8, 12, 14, 16–19]. Therefore, the current study aimed to evaluate effects of allogeneic, syngeneic, fresh and storage blood transfusion on the growth and metastasis of tumors and survival in fibrosarcoma bearing BALB/c mice.

Materials and Methods

Experimental Animals

Twenty-five male mice of BALB/c strain and three male mice of C57BL/6 strain were purchased from the Iran Pasture Institute, Tehran, Iran and kept under standard conditions. The mice were 6–8 week old with average weight of 25–35 g at the time of experiment. At least 24 h before the start of the experiment, the mice were transported to the laboratory for being accustomed to the lab environment. To avoid alloimmunization effects of pregnancy, mice of male sex were used in the current study.

Induction of Fibrosarcoma and Grouping

We used WEHI-164 mouse fibrosarcoma cells for tumor induction under laboratory conditions. A flask of WEHI-164 cells was obtained and cultured in RPMI-1640 containing 10% fetal calf serum (FCS). After achieving desired number of cells, WEHI-164 cells were harvested using 0.25% Trypsin–EDTA solution. The cells viability percentage was determined by 0.4% Trypan blue and the living cells were counted on the Neubauer slide. Induction of tumor was performed by subcutaneous injection of 1.2×10^6 living WEHI-164 cells in a specific region of the right flank of BALB/c mice (in 200 μ l). The mice were kept under appropriate conditions and examined for the

development of palpable subcutaneous fibrosarcoma on a daily basis.

The BALB/c mice were randomly allocated into five groups of equal size, i.e. five groups with five mice in each group. The three C57BL/6 mice were used to collect blood samples for allogeneic transfusions. Syngeneic blood samples were obtained from healthy BALB/c mice. In the first group (the control group), only the tumor was induced and no blood transfusion was done. In the second group, fresh allogeneic blood (obtained from C57BL/6 mice and kept at 4 °C for 1 day) was transfused. In the third group, storage allogeneic blood transfusion (collected from C57BL/6 mice and kept at 4 °C for 3 weeks) was performed. The fourth group received fresh syngeneic blood (obtained from healthy BALB/c mice and kept at 4 °C for 1 day), and the fifth group received storage syngeneic blood (obtained from healthy BALB/c mice and kept at 4 °C for 3 weeks). Design of the experiment is shown in Fig. 1.

Blood Transfusion in Experimental Animal Model

Allogeneic blood was obtained from healthy C57BL/6 mice, and the blood sampling was performed under anesthesia (Ketamine 100 mg/kg + Xylazine 20 mg/kg, intraperitoneal administration) from the hearts of animals under the sterile condition using insulin syringes. The blood samples were mixed with anti-coagulant CPD-A1 (seven units of blood and one unit of CPD-A1) and were kept in cryotubes at the temperature of 2–6 °C with daily temperature control. The prepared allogeneic and syngeneic blood samples were kept under sterile condition and appropriate temperature for 1–2 days and used as fresh blood. The samples were also stored for 3 weeks and used as storage blood.

In the day 7 after the induction of fibrosarcoma, to develop hemorrhage-induced anemia in mice bearing a palpable subcutaneous tumor, we obtained 200–300 μ l blood under anesthesia (Ketamine 100 mg/kg + Xylazine 20 mg/kg, intraperitoneal administration) from medial eye corner using heparinized microhematocrit tubes. Obtaining this volume of blood from a male BALB/c mouse at the age of 6–8 weeks and an average weight of 35–35 g is approximately equivalent to 600–700 cc blood loss in an adult person of weight 70 kg.

Twenty-four hours after induction of anemia in fibrosarcoma bearing BALB/c mice, 250 μ l fresh and storage allogeneic/syngeneic blood were transfused via tail vein in all four experiment groups.

Fig. 1 Study design

Evaluation of Tumor Size

To evaluate the TRIM effects, tumor size was determined from day 8 to day 20 after blood transfusion. In all experiment groups, every 48 h, the tumor size was recorded using a digital caliper. Tumor size was calculated according to the following formula provided by the Cancer Institute International [20]:

$$V = \frac{D \times W^2}{2}$$

where V is the tumor size in mm^3 , D is the length or large diameter (mm), and W is the width or small diameter (mm).

Measurement of Survival

The death of the mice in any group, from any cause and at any stage of the experiment was recorded on a daily basis. The survival of each mouse was calculated until the day 20.

Histopathological Examination

At the end of the experiment (day 20), all animals in the experiment groups were sacrificed (under absolute anesthesia “Ketamine 100 mg/kg + Xylazine 20 mg/kg; intraperitoneal administration” and by observing the ethics of working with laboratory animals) and the spleen tissues were removed. In this stage, after dissecting the mice, the

spleen was excised from the animals’ left side abdomen. The obtained samples were fixed in 10% formalin. Then the tissue sections (5 μm) were prepared and stained with hematoxylin and eosin (H&E) and the ten randomly selected sections for each tissue sample were analyzed for the presence of metastasis after staining. Histological evaluation was performed by a pathologist blind to the study.

Statistical Analysis

Data were analyzed using GraphPad Prism statistical software (version 5.00). Descriptive statistics were calculated as mean $\pm$ SD. Groups were compared using repeated measures ANOVA and Bonferroni tests for pair wise comparisons. Kaplan–Meier method and log-rank (Mantel–Cox) test were used to estimate survival probabilities and compare survival curves. The significance level was set at 0.05.

Results

Morphology of WEHI-164 cells after serial passage was adherent spindle-shaped and percentage of viable cells after staining with Trypan blue was reported as 90%. Induction of tumor in BALB/c mice by subcutaneous injection of 1.2×10^6 living WEHI-164 cells demonstrated that in the day 7 after injection, palpable

subcutaneous fibrosarcoma was developed in more than 70% of the mice.

The Effect of Blood Transfusion on Tumor Size

Tumor size increase in the experiment groups was significantly higher than the control group ($P < 0.0001$). Furthermore, increase in tumor size was significantly higher in the groups that received allogeneic (fresh and storage) and storage syngeneic blood transfusion compared with the control group ($P < 0.0001$). The difference between tumor size in the fresh syngeneic group and the control group was not significant ($P > 0.05$) (Fig. 2 right). Tumor size was highest on the day 16, 18 and 20 after the blood transfusion (Fig. 2 left).

The Effect of Blood Transfusion on Metastasis and Survival

At the end of the study (day 20), no metastasis was observed in none of the groups under study. Survival curves for BALB/c mice bearing fibrosarcoma tumor in the control and blood transfusion groups are shown in Fig. 3. The control group and the blood transfusion groups had similar time to death after injection (P value = 0.15).

Discussion

In this study, immunomodulatory effects of blood transfusion were assessed. The results suggested that allogeneic and storage blood transfusion significantly increase tumor size. However, blood transfusion did not alter survival and metastasis in experimental fibrosarcoma model. Many

Fig. 3 Kaplan–Meier survival curves for control and blood transfusion groups

studies have employed various techniques to help overcome the obstacles in preparation, storage, and transfusion of blood. However, blood transfusion is still accompanied with adverse effects such as TRIM. Even though TRIM may lead to clinical complications by altering patient immune system and stimulating tumor growth and metastasis, the results from preclinical studies are not consistent. Also, results of studies on the immunomodulatory (pro and anti-inflammatory) effects of types of blood products (allogeneic or syngeneic, fresh or storage) on the growth and metastasis of tumors are heterogeneous [19, 21–25].

In the late 1970s, a research team in New Castle carried out several studies and hypothesized that allogeneic blood transfusion could be harmful in cancer patients undergoing the operation. They explained that intact immune system is required for elimination of any type of cancer [26]. Lin

Fig. 2 The effect of blood transfusion on tumor size between control and overall (the left) and each (the right) blood transfusion group. Mean $\pm$ SD are shown. *Significant at 0.05, **significant at 0.01

et al. [3] observed that, in animal models, tumor size increase in allogeneic blood transfusion could be higher than syngeneic blood transfusion. However, syngeneic and autologous blood transfusion did not have different effects on tumor growth. The results of our study suggested significant increase in tumor size after allogeneic blood transfusion while tumor size increase in the fresh syngeneic group was not significant. None of the previous studies evaluated the effects of fresh and storage blood transfusion on the tumor growth. We observed that tumor size increased significantly in the groups that received storage allogeneic and storage syngeneic blood transfusion compared with the control group. As the storage time becomes longer, the concentration of some soluble molecules such as FasL would increase. Thus, transfusion of storage blood products could lead to apoptosis of activated T cells in the recipients' body, and thus would modulate or suppress the immune system in different levels [27].

All mice in the control survived until the end of the experiment, while the survival was 80% in mice undergoing blood transfusion. Meng et al. [17] reported that allogeneic transfusion increased the postoperative tumor mortality in patients with stage II colon cancer. They also observed significant reverse association between blood transfusion volume and survival rate that could be attributed to the immunomodulatory effects of blood transfusion. In the current study, no significant difference was present in survival between control and blood transfusion groups.

Bordin et al. [19] reported that allogeneic and nonleuko reduced blood transfusion led to a five-fold increase in the number of nodules in the lung of animals with cancer. Also, Meng et al. [17] showed that allogeneic transfusion enhanced the local recurrence and distant metastasis in patients with stage II colon cancer. In histological examinations, we observed no metastasis in none of the groups at the end of the study. Many previous studies showed that lung tissue the main site of metastasis in fibrosarcoma bearing mice. Spleen metastasis would develop only when a tumor reaches its advanced stages [19, 28–30]. Therefore, the absence of metastasis in the experiment groups in the current study seems to be reasonable. Our study had some limitations as well. The study period was relatively short due to an increase of cannibalism in mice during the experiment. Extension of time would be a desirable property in future research that could reveal other aspects of blood transfusion.

Conclusion

There is growing evidence on immunomodulatory effects of allogeneic and storage blood transfusion in experimental fibrosarcoma model. Therefore, it is necessary to prepare

guidelines rather than a routine hospital procedure for using these types of blood products in patients with different clinical circumstances such as cancer. Ideally, transfusion of blood components should be considered as a personalized medicine. Furthermore, comprehensive data analysis and well-designed larger studies are required to highlight the other aspects of the immunomodulatory effects of blood transfusion. Also, further clinical research is required to verify the preclinical findings. Increasing awareness of clinicians can make an effective contribution to the correct use of blood products and reduce complications such as TRIM.

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Author Contributions AAP and ZMH and KA designed the study. KA performed laboratory works. KA and TM and TJK performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and Animal Rights All procedures performed in studies involving animals were in accordance with the ethical standards of the Tarbiat Modares University.

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