Exp. Anim. 65(4), 345–351, 2016

—Original—

A swine model of acute thrombocytopenia with prolonged bleeding time produced by busulfan

Tomoyuki ABE[1, 2)], Shota KONO[1,2)], Takahiro OHNUKI[1)], Shuji HISHIKAWA[3)], Satoshi KUNITA[4)], and Yutaka HANAZONO[1,2)]

[1)]*Division of Regenerative Medicine, Center for Molecular Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan*
[2)]*Division of Stem Cell Research and Drug Development, Center for Development of Advanced Medical Technology, Jichi Medical University, Tochigi, Japan*
[3)]*Division for Medical Skill Training, Center for Development of Advanced Medical Technology, Jichi Medical University, Tochigi, Japan*
[4)]*Division of Development of Animal Resource, Center for Development of Advanced Medical Technology, Jichi Medical University, Tochigi, Japan*

Abstract: Animal models of thrombocytopenia are indispensable for evaluating the *in vivo* efficacy of hemostatic agents, cryopreserved platelets, and artificial platelets, but no large animal models are available. In this study, we generated a swine model of acute thrombocytopenia with prolonged bleeding times by administering the chemotherapeutic drug busulfan. First, we tested multiple doses of busulfan (4, 6, and 8 mg/kg) in pigs, and found that 6 mg/kg of busulfan is an optimal dose for producing a safe and moderate thrombocytopenia, with a platelet count of less than 30,000/μl. The pigs administered 6 mg/kg of busulfan (n=8) reached half their initial counts at day 7, counts below 30,000/μl at day 12, and their nadirs at day 15 (on average). The minimal platelet count was 14,000/μl. With this dose of busulfan (6 mg/kg), bleeding times were significantly prolonged in addition to the decrease in platelet counts (r=−0.63, P<0.01), while there were no cases of apparent hemorrhage. White blood cell counts were maintained at over 5,000/μl, and there were no infections or other adverse events including anemia or appetite or body weight loss. All pigs were sacrificed on day 16, with subsequent examination showing a significant reduction in cellularity and colony-forming units in the bone marrow, indicating that thrombocytopenia was the result of myelosuppression. In summary, administration with 6 mg/kg of busulfan induces safe and moderate thrombocytopenia with a prolonged bleeding time in swine.

Key words: large animal model, busulfan, pig, prolonged bleeding time, thrombocytopenia

Introduction

Thrombocytopenia, defined as peripheral blood platelet count of less than 30,000/μl, is often observed in hematological malignancies and as an adverse side effect of chemotherapy or radiotherapy [2, 9, 15]. It increases the risk for bleeding complications and often results in prolonged hospitalization, an impaired quality of life, and an increase in healthcare costs. Artificial platelets, cryopreservation of platelets, and various hemostatic agents have been developed to treat bleeding complications [4, 17, 19, 26]. In order to evaluate their hemostatic efficacy and safety, animal models of thrombocytopenia are needed. However, only small animal models

(Received 5 March 2016 / Accepted 21 April 2016 / Published online in J-STAGE 21 June 2016)
Address corresponding: Y. Hanazono, Director and Professor, Center for Development of Advanced Medical Technology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan
T.A. and S.K. contributed equally to this work.

(rodents and rabbits) have been characterized to date [5]. Large animal models are useful in bridging the gap between small animal studies and clinical investigations in human subjects. The swine has been increasingly used in preclinical research as a large animal model [3, 23]. Because of the similarities in anatomy and physiology between humans and swine, the data obtained from swine can be reliably translated to humans, facilitating the development of clinical techniques and treatments.

In order to produce thrombocytopenic swine, we used busulfan, an alkylating agent in chemotherapy. In clinical situations, thrombocytopenia is often seen in patients treated with busulfan [2, 6, 11, 25]. Although busulfan has been used for myeloablation in piglets [22], there have been no reports on producing thrombocytopenic pigs. In the present study, we performed a dose-response study of busulfan in miniature swine and evaluated the hematological and hemostatic parameters and general conditions.

Materials and Methods

Pigs

Ten micro-mini pigs (female, aged 6 to 12 months, weighing 8.5 to 14.4 kg, Fuji Micra, Inc., Shizuoka, Japan) were used in this study. All surgical procedures on pigs were performed under general anesthesia. Pigs were anesthetized with midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan) and medetomidine (Domitor, Orion, Finland), followed by inhalation of sevoflurane (Pfizer Japan Inc., Tokyo, Japan). An ear vein was cannulated, and buprenorphine hydrochloride (Lepetan, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was administrated for pain relief. An indwelling 14-gauge central venous catheter was inserted into the right external jugular vein for administration of busulfan and collection of blood samples. Pigs were euthanized on day 16 after administration of busulfan by injection of potassium chloride (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) through the venous catheter. The experimental protocols were in accordance with the Jichi Medical University Guide for Laboratory Animals and approved by the animal care committee of Jichi Medical University.

Busulfan administration

The solution of busulfan (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was prepared as previously described [1]. Busulfan was dissolved in dimethylacetamide (*N*, *N*-Dimethylacetamide, dehydrated, Wako Pure Chemical Industries, Ltd.). A twofold volume of polyethylene glycol 400 (Wako Pure Chemical Industries, Ltd.) was then added to stabilize the solution. The busulfan solution was diluted; a ninefold volume of sterile distilled water was added to the solution before administration to the pigs. Pigs were weighed, and the solutions of busulfan at 4, 6, and 8 mg/kg were prepared. The busulfan solution was divided into halves, and each aliquot was injected intravenously at an interval of 12 h.

Peripheral blood counts

Peripheral blood samples were obtained from pigs at the indicated time points and examined for platelet, white blood cell (WBC), and red blood cell (RBC) counts and the hemoglobin and hematocrit values with an automatic blood-cell counter (MEK-6308, Nihon Kohden, Tokyo, Japan), which was adjusted for swine samples. Differential WBC counts including neutrophils and lymphocytes were determined by Wright-Giemsa staining of peripheral blood smears. Coagulation parameters including prothrombin time, activated partial thromboplastin time, and fibrinogen in the plasma were measured using 0.38% (w/v) sodium citrated blood.

Bleeding time measurement

Bleeding time was measured before and after the administration of busulfan at days 7 and 16. Bleeding time was defined as the time from incision to cessation of bleeding at a shaved inner site on the ear using a standard cutting device (QuikHeel Lancet, BD, Franklin Lakes, NJ, USA). Shed blood was carefully removed at exactly 15-second intervals with a filter paper. At each time point, the means of independent triplicate measurements was recorded as the bleeding time. If the bleeding time exceeded 900 seconds, the bleeding time was recorded as 900 seconds and further bleeding was stopped.

Bone marrow examinations

Bone marrow was aspirated from the proximal humerus before and after the administration of busulfan (on day 16) and collected in blood collection tubes (Terumo Corporation, Tokyo, Japan) containing EDTA-2K as an anticoagulant. Hematopoietic progenitors in the bone marrow were assessed by colony-forming unit (CFU) assay. The mononuclear cells were separated by density gradient centrifugation, using Ficoll-Paque Plus

(GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) according to manufacturer protocol, and 2×10^4 cells were plated in 35-mm dishes in MethoCult GF+ H4435 (StemCell Technologies Inc., Vancouver, BC, Canada). After incubation for 14 days at 37°C with 5% CO_2, the number of colonies containing 50 or more cells was counted in triplicated dishes using an inverted light microscope. Bone-marrow biopsy was performed using a 13-gauge needle (Jamshidi, CareFusion Japan, Tokyo, Japan). For light microscopic examination, the biopsy specimens were fixed in 10% neutral buffered formalin, paraffin-embedded, and stained with hematoxylin and eosin.

Statistical analysis

Data are presented as means ± standard deviation (SD). The numbers of bone marrow nucleated cells or CFUs before and after busulfan administration at day 16 were compared by Student's *t*-tests. Correlations between platelet counts and bleeding times were analyzed by Pearson's product-moment correlation coefficient. All statistical analyzes were performed with the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13]. *P* values less than 0.05 were considered statistically significant.

Results

Dose-dependent decrease of platelets by busulfan

First, we tested multiple doses (4, 6, and 8 mg/kg) of busulfan in pigs. Overall, administration of busulfan showed dose-dependent effects on platelet counts in the peripheral blood (Fig. 1). In a pig administered 4 mg/kg of busulfan (n=1), a mild decrease in platelet counts with a nadir of 76,000/μl was observed at day 12. In a pig administered 8 mg/kg of busulfan (n=1), the platelet counts reached almost zero by day 11. The pig treated with this dose (8 mg/kg) of busulfan suffered from hematuria, poor appetite, and decreased activity around day 10 and was euthanized on day 14. The pigs administered 6 mg/kg of busulfan (n=8) developed moderate thrombocytopenia with half their initial counts at day 7 and reached nadirs below 30,000/μl in the second to third week, at day 12 on average. The minimal platelet count in the pigs treated with 6 mg/kg of busulfan was 14,000/μl at day 15. There were no cases of hemorrhage in the pigs given 6 mg/kg of busulfan.

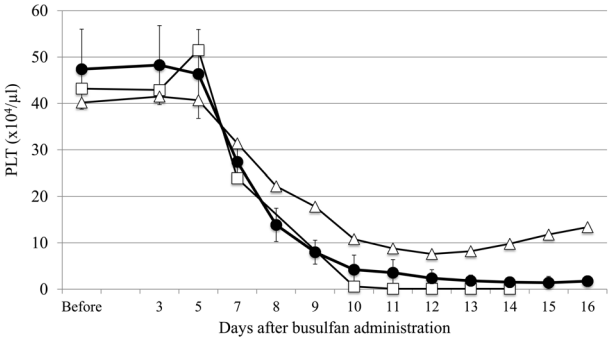

Fig. 1. Dose response effects of busulfan on platelet counts in pigs. Busulfan was intravenously administered to pigs at 4 (△, *n*=1), 6 (●, *n*=8), and 8 mg/kg (□, *n*=1). Blood samples were obtained at the indicated time points. Platelet, PLT. Data represent the mean ± SD.

No clinical signs with the optimal dose of busulfan

Based on the results described above, we evaluated the effects of 6 mg/kg of busulfan on pigs (n=8) in further detail. The total WBC counts gradually decreased for the first week after administration of busulfan, but the minimal WBC count stayed over 5,000/μl (Fig. 2). The neutrophil counts declined in parallel with the WBC counts, and the minimal neutrophil count was 440/μl. On the other hand, there were no significant changes in the counts of lymphocytes and RBCs or in the levels of hemoglobin and hematocrit after administration of busulfan for the observation period of 16 days. No significant changes were observed in the coagulation parameters including the prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen values (FIBG) (Fig. 3). No infections or other clinical signs related to treatment with busulfan, such as fever, diarrhea, poor appetite, weight loss, or depressed activity were observed in any of the eight pigs.

Prolonged bleeding time

The relationship between platelet counts and bleeding times in the thrombocytopenic pigs is shown in Fig. 4. In the eight pigs given 6 mg/kg of busulfan, the mean platelet count was $474 \pm 86 \times 10^3$/μl, and the mean bleeding time was 100 ± 22 s at baseline (before busulfan administration). The bleeding times were prolonged in parallel with a decrease in the platelet counts (r=−0.63, P<0.01, analyzed with the EZR software). When the platelet counts were less than 1,000/μl, the bleeding times were always greater than 900 s.

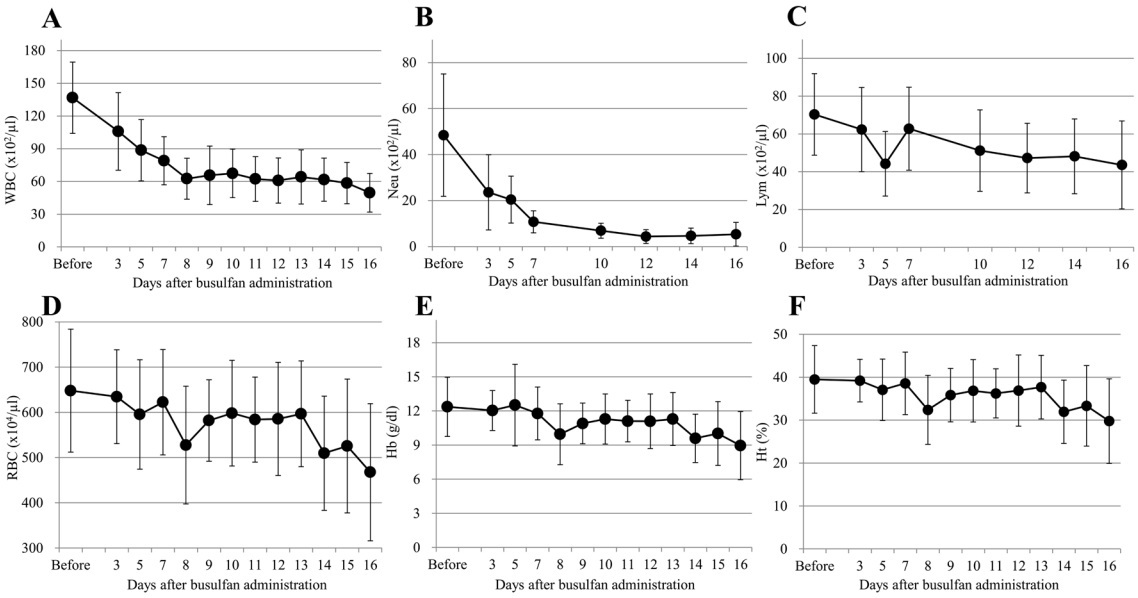

Fig. 2. Effects of busulfan on hematologic parameters. The hematologic parameters of the pigs administered busulfan at 6 mg/kg in Fig. 1 (n=8) are shown. Values are shown for (A) white blood cell (WBC) counts, (B) neutrophil (Neu) counts, (C) lymphocyte (Lym) counts, (D) red blood cell (RBC) counts, (E) hemoglobin (Hb), and (F) hematocrit (Ht). Data represent the mean ± SD.

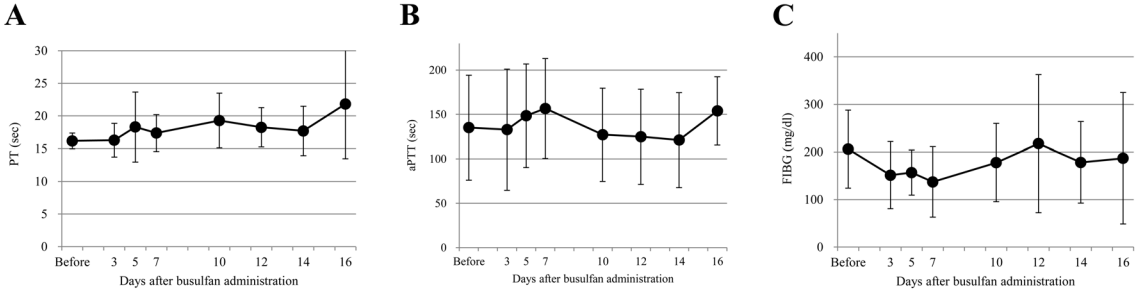

Fig. 3. Effects of busulfan on coagulation parameters. The coagulation parameters of the pigs administered busulfan at 6 mg/kg in Fig. 1 (n=8) are shown. (A) Prothrombin time (PT), (B) activated partial thromboplastin time (aPTT), and (C) fibrinogen concentration (FIBG). Data represent the mean ± SD.

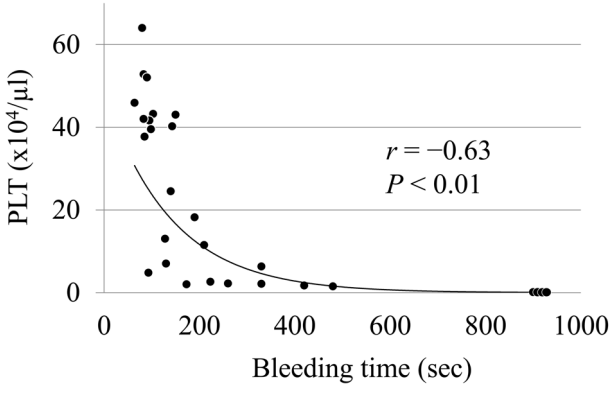

Fig. 4. A scatterplot of the relationship between the platelet count and the bleeding time. The relationship between the platelet (PLT) counts and bleeding times of the pigs administered busulfan at 6 mg/kg in Fig. 1 (n=8) is shown. Bleeding time was measured at an incision made at an inner ear site by a standard cutting device in triplicate. Correlations between platelet counts and bleeding times were analyzed by Pearson's product-moment correlation coefficient. Statistical significance was determined with the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

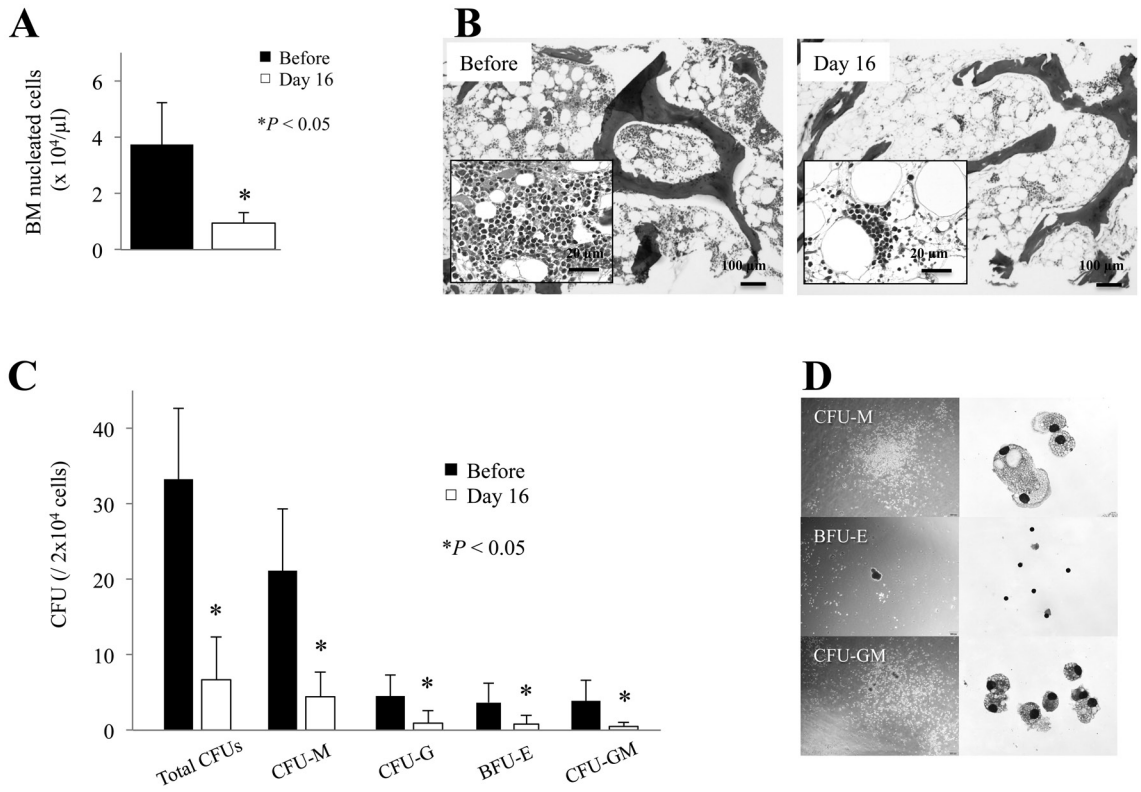

Fig. 5. Effects of busulfan on the bone marrow progenitor cells. (A) Numbers of bone marrow nucleated cells of pigs before and after the treatment with 6 mg/kg of busulfan on day 16. Total numbers of nucleated cells were counted in Turk's solution. The numbers of nucleated cells before and after busulfan administration on day 16 were compared by Student's *t*-tests. Statistical significance was determined with the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan). (B) Representative histological sections of a bone marrow biopsy specimen subjected to hematoxylin and eosin staining. The scale bars represent 100 μm. The inserts show images with a higher magnification (scale bars=20 μm). (C) Colony-forming unit (CFU) assays of the porcine bone marrow were also examined before and after busulfan administration at day 16. The numbers of CFU-macrophages (CFU-Ms), CFU-granulocytes (CFU-Gs), burst-forming unit-erythroids (BFU-Es), and CFU-GMs colonies are shown. The numbers of CFUs before and after busulfan administration on day 16 were compared by Student's *t*-tests. Statistical significance was determined with the EZR software. (D) Cytospin specimens of CFU-Ms, BFU-Es, and CFU-GMs colonies were examined by Wright-Giemsa staining.

Myelosuppressive effects of busulfan

We examined the bone marrow of pigs before and after the administration with 6 mg/kg of busulfan at day 16. The numbers of nucleated cells in the bone marrow were significantly decreased after the administration of busulfan (P<0.05, Fig. 5A). Histological examination of the biopsy specimens also revealed a marked reduction in the cellularity of bone marrow after administration (Fig. 5B). At day 16 after administration, the numbers of hematopoietic progenitor cells, that is, CFU-macrophages (CFU-Ms), CFU-granulocytes (CFU-Gs), CFU-GMs, and burst-forming unit-erythroids (BFU-Es), were significantly decreased compared with those before administration (P<0.05, Fig. 5C). These results clearly showed the myelosuppressive state of the bone marrow induced by busulfan.

Discussion

In this study, we produced a porcine model of acute thrombocytopenia with a prolonged bleeding time by administering busulfan. The platelet counts decreased during the first week and reached nadirs in the second to third weeks. Similar time-course profiles of decreased platelets have been documented in other animals, including mice, rabbits, dogs, sheep, monkeys, and humans [1, 8, 14, 16, 21, 24]. The doses of busulfan required to achieve platelet counts of less than 50,000/μl, however,

differed widely among animal species; for example, the dose was 20 mg/kg in mice [21], 40 mg/kg in rabbits [16], 15 mg/kg in dogs [8], and 7.5 mg/kg in sheep [1]. In this study, the optimal dose of busulfan for moderate thrombocytopenia with a platelet count of less than 30,000/μl was determined to be 6 mg/kg in pigs, close to that of humans. The variations in sensitivity to busulfan among animal species may be attributed to the distinct activities of drug-metabolizing enzymes such as glutathione S-transferase [20]. Busulfan is converted into inactive metabolites mostly by this enzyme, the activity of which differs among species [7, 12]. Therefore, the optimal dose of busulfan for platelet reduction should be determined for each animal species.

In large animals, cases of thrombocytopenia induced by total body irradiation and by hemodilution have been reported in swine [10, 18]. Total body irradiation and hemodilution, however, are difficult for pigs in actual practice. Therefore, we took advantage of busulfan to induce thrombocytopenia in pigs. The effects of busulfan on swine platelet counts have not previously been characterized, although there is a report on myeloablation induced by busulfan and cyclophosphamide in piglets [22]. In the present study, we found that 6 mg/kg of busulfan is the optimal dose for producing a moderate reduction in platelets to less than 30,000/μl with a prolonged bleeding time. Although moderate neutropenia was also observed, lymphopenia, anemia, prolonged coagulation times, fever, and other clinical signs were not observed in any of the eight animals during the 16-day observation period. Unlike mice, pigs are not inbred. Thus, their genetic backgrounds are not identical, and there should be certain levels of individual differences among pigs. The hematological data obtained in the present study using pigs, however, did not vary widely. Therefore, administration of 6 mg/kg of busulfan safely and reproducibly induces moderate thrombocytopenia with a platelet count of less than 30,000/μl and a prolonged bleeding time in pigs.

Administration of busulfan induced platelet reduction through its myelosuppressive effects on the bone marrow in pigs. Unfortunately, we could not detect porcine megakaryocytic colonies (CFU-MKs) in the bone marrow even before the administration of busulfan. The medium we used to produce CFU-MKs indeed supports the production of human and mouse CFU-MKs, but it does not seem to support the production of porcine CFU-MKs. For instance, interleukin (IL)-3 is required for the production of CFU-MKs, but human or mouse IL-3 included in the medium does not act on porcine cells. Although we could not demonstrate the decrease of CFU-MKs after administration of busulfan, the platelet reduction was apparently a result of myelosuppression by busulfan given that the cellularity of the bone marrow was significantly decreased in the pigs, as shown in Fig. 5.

In conclusion, we successfully developed a large animal model of acute thrombocytopenia in pigs by administration of busulfan. Busulfan-induced thrombocytopenia in pigs is easy to produce and should be useful for the evaluation of human platelet products and putative platelet substitutes.

Conflict of Interest

The authors declare that they have no competing financial interests.

Acknowledgments

We wish to thank Yoshihisa Mizukami, Cheng Jung, Suvd Byambaa, Yukitoshi Makimura (Jichi Medical University), and Sachi Okabayashi (Tsukuba Primate Research Center, Ibaraki, Japan) for their excellent technical assistance. This study was supported in part by a grant from the Japan Agency for Medical Research and Development (AMED) Research Project for Practical Application of Regenerative Medicine (formerly KAKENHI from the Ministry of Health, Labour and Welfare of Japan) to Y.H.

References

1. Abe, T., Masuda, S., Tanaka, Y., Nitta, S., Kitano, Y., Hayashi, S., Hanazono, Y., and Nagao, Y. 2012. Maternal administration of busulfan before in utero transplantation of human hematopoietic stem cells enhances engraftments in sheep. *Exp. Hematol.* 40: 436–444. [Medline] [CrossRef]
2. Aiuti, A., Cattaneo, F., Galimberti, S., Benninghoff, U., Cassani, B., Callegaro, L., Scaramuzza, S., Andolfi, G., Mirolo, M., Brigida, I., Tabucchi, A., Carlucci, F., Eibl, M., Aker, M., Slavin, S., Al-Mousa, H., Al Ghonaium, A., Ferster, A., Duppenthaler, A., Notarangelo, L., Wintergerst, U., Buckley, R.H., Bregni, M., Marktel, S., Valsecchi, M.G., Rossi, P., Ciceri, F., Miniero, R., Bordignon, C., and Roncarolo, M.G. 2009. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N. Engl. J. Med.* 360: 447–458. [Medline] [CrossRef]
3. Bassols, A., Costa, C., Eckersall, P.D., Osada, J., Sabrià, J.,

and Tibau, J. 2014. The pig as an animal model for human pathologies: A proteomics perspective. *Proteomics Clin. Appl.* 8: 715–731. [Medline] [CrossRef]
4. Blajchman, M.A. 1999. Substitutes for success. *Nat. Med.* 5: 17–18. [Medline] [CrossRef]
5. Blajchman, M.A. and Lee, D.H. 1997. The thrombocytopenic rabbit bleeding time model to evaluate the in vivo hemostatic efficacy of platelets and platelet substitutes. *Transfus. Med. Rev.* 11: 95–105. [Medline] [CrossRef]
6. Cavo, M., Bandini, G., Benni, M., Gozzetti, A., Ronconi, S., Rosti, G., Zamagni, E., Lemoli, R.M., Bonini, A., Belardinelli, A., Motta, M.R., Rizzi, S., and Tura, S. 1998. High-dose busulfan and cyclophosphamide are an effective conditioning regimen for allogeneic bone marrow transplantation in chemosensitive multiple myeloma. *Bone Marrow Transplant.* 22: 27–32. [Medline] [CrossRef]
7. Chasseaud, L.F. 1979. The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Adv. Cancer Res.* 29: 175–274. [Medline] [CrossRef]
8. Deeg, H.J., Schuler, U.S., Shulman, H., Ehrsam, M., Renner, U., Yu, C., Storb, R., and Ehninger, G. 1999. Myeloablation by intravenous busulfan and hematopoietic reconstitution with autologous marrow in a canine model. *Biol. Blood Marrow Transplant.* 5: 316–321. [Medline] [CrossRef]
9. Grill, J., Dufour, C., and Kalifa, C. 2006. High-dose chemotherapy in children with newly-diagnosed medulloblastoma. *Lancet Oncol.* 7: 787–789. [Medline] [CrossRef]
10. Ha, C.T., Li, X.H., Fu, D., Moroni, M., Fisher, C., Arnott, R., Srinivasan, V., and Xiao, M. 2014. Circulating interleukin-18 as a biomarker of total-body radiation exposure in mice, minipigs, and nonhuman primates (NHP). *PLOS ONE* 9: e109249. [Medline] [CrossRef]
11. Hassan, M. 1999. The role of busulfan in bone marrow transplantation. *Med. Oncol.* 16: 166–176. [Medline] [CrossRef]
12. Igarashi, T. and Satoh, T. 1989. Sex and species differences in glutathione S-transferase activities. *Drug Metabol. Drug Interact.* 7: 191–212. [Medline] [CrossRef]
13. Kanda, Y. 2013. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* 48: 452–458. [Medline] [CrossRef]
14. Kang, E.M., Hsieh, M.M., Metzger, M., Krouse, A., Donahue, R.E., Sadelain, M., and Tisdale, J.F. 2006. Busulfan pharmacokinetics, toxicity, and low-dose conditioning for autologous transplantation of genetically modified hematopoietic stem cells in the rhesus macaque model. *Exp. Hematol.* 34: 132–139. [Medline] [CrossRef]
15. Kantarjian, H., Thomas, D., Jorgensen, J., Jabbour, E., Kebriaei, P., Rytting, M., York, S., Ravandi, F., Kwari, M., Faderl, S., Rios, M.B., Cortes, J., Fayad, L., Tarnai, R., Wang, S.A., Champlin, R., Advani, A., and O'Brien, S. 2012. Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol.* 13: 403–411. [Medline] [CrossRef]
16. Kuter, D.J. and Rosenberg, R.D. 1995. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. *Blood* 85: 2720–2730. [Medline]
17. Levi, M., Friederich, P.W., Middleton, S., de Groot, P.G., Wu, Y.P., Harris, R., Biemond, B.J., Heijnen, H.F., Levin, J., and ten Cate, J.W. 1999. Fibrinogen-coated albumin microcapsules reduce bleeding in severely thrombocytopenic rabbits. *Nat. Med.* 5: 107–111. [Medline] [CrossRef]
18. McLoughlin, T.M., Fontana, J.L., Alving, B., Mongan, P.D., and Bünger, R. 1996. Profound normovolemic hemodilution: hemostatic effects in patients and in a porcine model. *Anesth. Analg.* 83: 459–465. [Medline] [CrossRef]
19. Nakamura, S., Takayama, N., Hirata, S., Seo, H., Endo, H., Ochi, K., Fujita, K., Koike, T., Harimoto, K., Dohda, T., Watanabe, A., Okita, K., Takahashi, N., Sawaguchi, A., Yamanaka, S., Nakauchi, H., Nishimura, S., and Eto, K. 2014. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell* 14: 535–548. [Medline] [CrossRef]
20. Nau, H. 1986. Species differences in pharmacokinetics and drug teratogenesis. *Environ. Health Perspect.* 70: 113–129. [Medline] [CrossRef]
21. Pitchford, S.C., Yano, H., Lever, R., Riffo-Vasquez, Y., Ciferri, S., Rose, M.J., Giannini, S., Momi, S., Spina, D., O'connor, B., Gresele, P., and Page, C.P. 2003. Platelets are essential for leukocyte recruitment in allergic inflammation. *J. Allergy Clin. Immunol.* 112: 109–118. [Medline] [CrossRef]
22. Pontoppidan, P.L., Shen, R.L., Petersen, B.L., Thymann, T., Heilmann, C., Müller, K., and Sangild, P.T. 2014. Intestinal response to myeloablative chemotherapy in piglets. *Exp. Biol. Med. (Maywood)* 239: 94–104. [Medline] [CrossRef]
23. Schook, L.B., Collares, T.V., Darfour-Oduro, K.A., De, A.K., Rund, L.A., Schachtschneider, K.M., and Seixas, F.K. 2015. Unraveling the swine genome: implications for human health. *Annu. Rev. Anim. Biosci.* 3: 219–244. [Medline] [CrossRef]
24. Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben-Tal, O., Eldor, A., and Or, R. 1998. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 91: 756–763. [Medline]
25. Tutschka, P.J., Copelan, E.A., and Klein, J.P. 1987. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. *Blood* 70: 1382–1388. [Medline]
26. Vadhan-Raj, S., Kavanagh, J.J., Freedman, R.S., Folloder, J., Currie, L.M., Bueso-Ramos, C., Verschraegen, C.F., Narvios, A.B., Connor, J., Hoots, W.K., Broemeling, L.D., and Lichtiger, B. 2002. Safety and efficacy of transfusions of autologous cryopreserved platelets derived from recombinant human thrombopoietin to support chemotherapy-associated severe thrombocytopenia: a randomised cross-over study. *Lancet* 359: 2145–2152. [Medline] [CrossRef]