

Glucose oxidase induces mobilization of long-term repopulating hematopoietic cells in mice

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Abstract

Hematopoietic stem progenitor cells (HSPCs) mobilized to peripheral blood, rather than those remaining in the bone marrow (BM), are commonly used as stem cell source in the clinic. As reactive oxygen species (ROS) are suggested as mediator of HSPC mobilization, we examined the impacts of glucose oxidase (GO) on peripheral mobilization of BM HSPCs and the associated mechanisms. Intravenous injection of GO induced HSPC mobilization even by single treatment, and the GO-mobilized cells maintained their long-term reconstituting and differentiating potentials in conditioned recipients. GO-injected mice lived a normal life without adverse effects such as stem cell senescence, hematopoietic disorders, and blood parameter alteration. The mobilization effect of GO was even evident in animal models showing poor mobilization, such as old, 5-fluorouracil-treated, or alendronate-treated mice. Importantly, combined injection of GO with granulocyte colony-stimulating factor (G-CSF) and/or AMD3100 enhanced more greatly HSPC mobilization than did G-CSF, AMD3100, or both. The GO-stimulated HSPC mobilization was almost completely attenuated by *N*-acetyl-L-cysteine treatment. Collectively, our results not only highlight the potential role of GO in HSPC mobilization via ROS signaling, but also provide a GO-based new strategy to improve HSPC mobilization in poorly mobilizing allogeneic or autologous donors via combination with G-CSF and/or AMD3100.

KEYWORDS

hematopoietic stem cell transplantation, long-term repopulation, mobilization, self-renewal

Significance statement

The authors demonstrate for the first time that glucose oxidase (GO) promotes peripheral mobilization of hematopoietic stem progenitor cells (HSPCs) that maintain their functions in conditioned recipients without hematopoietic and immunological disorders. This study highlights mobilizing potential of GO even in mice exhibiting poor HSPC mobilization and provides an evidence that the efficacy of commonly used mobilizers is synergistically enhanced in combination with GO without adverse events.

Han-Sol So and Min-Guk Kim contributed equally to this work.

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1 | INTRODUCTION

In recent years, bone marrow (BM) transplantation has been replaced to the transplantation of mobilized peripheral blood (PB) stem cells (PBSCs) in many cases of cancer patients. PBSCs engraft and provide faster recovery of white blood cell (WBC) count better than BM-derived hematopoietic stem progenitor cells (HSPCs), thereby protecting patients from the risk of infection during the early post-transplantation period.¹⁻³

Reactive oxygen species (ROS) are associated with HSPC mobilization induced by stem cell mobilizers, granulocyte colony-stimulating factor (G-CSF), and AMD3100.^{4,5} However, whether ROS are a determinant in peripheral mobilization of HSPCs still remains to be elucidated. Here we explore the impacts of glucose oxidase (GO) itself or in combination with the commonly used mobilizers on HSPC mobilization along with the associated mechanisms using various types of animal models. GO, approved by the federal Food and Drug Administration, is an oxidoreductase that converts glucose into glucuronic acid and hydrogen peroxide. Differently to hydrogen peroxide, GO induces a mild and prolonged generation of ROS and modulates intracellular redox state.⁶⁻⁸ Fungal GO is also widely applied in various areas of food industry.⁹ The results of our study show potential roles of GO on HSPC mobilization. This study also demonstrates that the use of GO enhances synergistically the efficacy of G-CSF and AMD3100 without adverse effects.

2 | MATERIALS AND METHODS

2.1 | Animals and treatment

The Animal Welfare Committee of Jeonbuk National University approved all experimental procedures. Unless otherwise specified, this study used BALB/c mice (8-week-old, Orient Bio, Daejeon, South Korea), and chemicals were purchased from Sigma-Aldrich Co. LLC (St. Louis, Missouri). Mice received intravenous injection of GO (0.250 U/g) dissolved in 0.05 M sodium acetate via tail vein for single or consecutive days (2-5 days). Mice received subcutaneous injection of G-CSF (250 µg/kg, Amgen, Thousand Oaks, California) for five consecutive days, AMD3100 (5 mg/kg) for single treatment, or both. To evaluate HSPC mobilization, PB was collected from mice 4 hours after the last injection of GO or the mobilizers. To explore the impact of GO on mobilizer-induced HSPC mobilization, mice that had received subcutaneous injection of G-CSF and/or AMD3100 were administered with GO via intravenous injection 12 hours before PB collection. To determine whether GO-stimulated HSPC mobilization is mainly due to ROS signaling, mice were subcutaneously administered with *N*-acetyl-L-cysteine (NAC; 100 mg/kg) 10 minutes before GO injection. We also examined the impact of GO on HSPC mobilization using old mice (12-month-old) or the mice that received HSPC mobilizing disturbers such as 5-fluorouracil (5-Fu), alendronate (ALN), or streptozotocin (STZ).¹⁰ To produce the poor HSPC mobilizing models,

mice were intraperitoneally treated with single injection of 5-Fu (50 mg/kg), 3-week consecutive injection of ALN (0.25 mg/kg), or single injection of STZ (150 mg/kg). The 5-Fu- and ALN-injected mice received a single GO (0.250 U/g) injection 3 weeks and 4 hours after the reagent injection, respectively. Diabetic phenotype in STZ-injected mice was evaluated 7 days after the injection, in which the mice showing more than 300 mg/dL of blood glucose level were used for GO treatment.

2.2 | Flow cytometric analysis

The frequencies of Lin⁻c-Kit⁺ (LK) and CD150⁺CD48⁻LK cells in PB and BM were assessed by multicolor flow cytometry (BD Aria, BD Bioscience, Franklin Lakes, New Jersey) at the Center for University-Wide Research Facilities of Jeonbuk National University. Those cells were phenotypically gated and further analyzed using the FlowJo software program (FLOWJO, Ashland, Oregon). LK and CD150⁺CD48⁻LK cells from PB and BM were phenotypically identified using the following antibodies (all antibodies were purchased from BD Biosciences [San Jose, California], unless specified otherwise): lineage markers phycoerythrin (PE)-Cy7-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD45R, anti-CD11b, anti-Gr-1, and anti-TER-119; allophycocyanin (APC)-conjugated anti-c-Kit; PerCP/Cy5.5-conjugated anti-CD150 (eBioscience, Waltham, Massachusetts); and APC-Cy7-conjugated anti-CD48. Gr-1⁺/CD11b⁺ granulocytes in BM were measured using PE-Cy7/FITC-conjugated antibodies. CXCR4⁺ cells were assessed using PE-conjugated anti-CXCR4 antibody (eBioscience). We also evaluated whether GO injection induces cellular oxidative stress and senescence several markers.¹¹ Briefly, levels of mitochondrial superoxide anion and hydrogen peroxide were measured using MitoSox Red (Invitrogen, Carlsbad, California) and 2',7'-dichlorodihydrofluorescein-diacetate (DCF-DA), respectively. The 5-Dodecanoylamino fluorescein Di-β-D-Galactopyranoside (C₁₂FDG; Molecular Probes, Eugene, Oregon) was used to analyze senescence-associated β-galactosidase activity.

2.3 | Transplantation assay

To evaluate competitive repopulation activity, an equal number of PB cells in experimental groups (phosphate-buffered saline vs GO, G-CSF vs GO+G-CSF, AMD3100 vs GO+AMD3100, G-CSF+AMD3100 vs GO+G-CSF+AMD3100, and CD45.1 vs CD45.2 groups) was transplanted into lethally irradiated recipient mice (CD45.1/2, 9.5 Gy) by tail vein injection. Donor-derived PB cells were collected from recipient mice after various times of transplantation, and the ratio of CD45.1 to CD45.2 was assessed by flow cytometry. To analyze the multilineage reconstitution and self-renewal potentials of GO-mobilized HSPCs, donor-derived CD150⁺CD48⁻LK cells (CD45.2⁺) sorted from primary recipient mice were transplanted into conditioned secondary and tertiary recipient mice. The PB and BM of tertiary recipient mice were examined at 8 weeks post-transplantation.

2.4 | Colony-forming cell assay

Mononuclear PB (1×10^6 /dish) and spleen cells (5×10^5 /dish) were seeded into 35-mm dishes containing MethoCult GF M3434 (STEMCELL Technologies, Vancouver, Canada). Numbers of burst forming unit-erythrocyte (BFU-E), colony-forming unit-granulocyte/macrophage (CFU-GM), and colony-forming unit-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM) colonies were counted after 12 days of incubation using standard criteria.

2.5 | Blood test

PB was collected into Vacutainer (BD Biosciences) plastic tubes coated with K₂EDTA, and various types of blood cells were quantified using an automated complete cell counter (Prokan PE-6800VET). For arterial blood gas analysis, PB was also collected into 29-gauge insulin syringes (Feeltech, Gwangdeok-myeon, South Korea) without heparin, and levels of pH, O₂ saturation, PCO₂, and PO₂ were measured.

2.6 | Enzyme-linked immunosorbent assay

BM supernatant and blood serum were used to measure the concentration of stromal cell-derived factor-1 (SDF-1) using an anti-mouse ELISA kit (Abcam, Cambridge, UK) according to the manufacturer's instructions.

2.7 | Microcomputerized tomographic analysis

Hind limbs from ALN-treated and control mice were scanned using a desktop scanner (1076 Skyscan Micro-CT; Skyscan, Kontich, Belgium) and analyzed with CTAn software (Skyscan).

2.8 | Immunohistochemistry and tartrate-resistant acid phosphatase staining

Immunohistochemistry was performed with the Histostain Plus Anti-Rabbit or -Goat Primary DAB Kit (Zymed Laboratories) according to the manufacturer's instructions. Briefly, the hind limbs were dissected from mice and then processed for fixation, decalcification, paraffin embedding, section (5 μ m in thickness), deparaffinization, and hydration.¹² Finally, the hydrated slides were incubated with anti-rabbit osteocalcin antibody (1:100 dilution, Takara Bio, Kyoto, Japan). Femoral bones were also fixed, decalcified, embedded, and sectioned into a thickness of 5.0 μ m, and the tissue sections were stained with tartrate-resistant acid phosphatase (TRAP) using a leukocyte acid phosphatase kit. Tissue sections were counterstained with hematoxylin before microscopic observation.

2.9 | Statistical analyses

All data are expressed as the mean \pm SD. Student's *t* test was used to determine significant differences between two sets of data, and one-way analysis of variance was used for multiple comparisons using SPSS statistical software for Windows, version 16.0 (Chicago, Illinois). A value of *P* < .05 was considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1 | GO mobilizes long-term repopulating hematopoietic cells without adverse events

Intravenous injection of GO at 0.250 U/g, that is a concentration considered reasonably safe for clinical trials,¹³ rapidly increased DCF-positive cells in PB, but not in BM cells, in a time-dependent manner (Figure 1A). Circulating lymphoid (B220⁺ and CD3⁺) and myeloid (CD11b⁺ and Gr-1⁺) cells also exhibited a time-dependent increase in DCF-positive cells after GO injection (Figure S1A). However, numbers of circulating lymphoid and myeloid cells were differently affected, such that GO injection decreased the percentage of B220⁺ cells, but increased CD3⁺, CD11b⁺, and Gr-1⁺ cells (Figure S1B). Number of peripheral WBCs was unaffected by GO injection (Figure S1C). GO injection increased significantly the numbers of LK (*P* < .001) and CD150⁺CD48⁻LK cells (*P* < .05) only at 12 hours postinjection (Figure 1B), along with their relative decreases in BM (Figure S2). When the efficacy of single or consecutive injection (2-5 days) of GO to mobilize LK cells was evaluated, no different effects among the injection times were found (Figure S3). PB cells collected from mice 12 hours after single GO injection revealed greater formation of BFU-E, CFU-GM, and CFU-GEMM colonies compared with the vehicle-injected mice (Figure 1C). Similarly, spleen-conserved cells in GO-injected mice exhibited significantly higher numbers (*P* < .001) of colony-forming unit cell (CFU-C) compared with the vehicle-injected mice (Figure S4). When C57BL/6 mice, a strain exhibiting a resistance against stem cell mobilization,¹⁵ were injected with GO, the mice received more than single GO injection exhibited higher numbers of PB-conserved LSK cells and CFU-C than did the vehicle-injected mice (Figure S5A-C). These results support the potential of GO to mobilize HSPCs that maintain their repopulating capacities. To further determine whether GO-mobilized HSPCs show functional properties, we conducted *in vivo* competitive repopulation experiments (Figure 1D). GO-mobilized cells displayed great reconstitution advantages in conditioned recipients even at the 4-month post-transplantation (Figure 1E). Significantly higher LK and CD150⁺CD48⁻LK cells were found in BM of the recipients transplanted with GO-mobilized PB cells compared with the mice transplanted with the vehicle-mobilized cells (Figure 1F). Donor-derived CD150⁺CD48⁻LK cells also exhibited durable multilineage differentiation in PB and engraftment in BM of tertiary transplanted recipient animals (Figure 1G). These results suggest that GO-mobilized HSPCs possess functionally long-term repopulating and multilineage differentiating potentials.

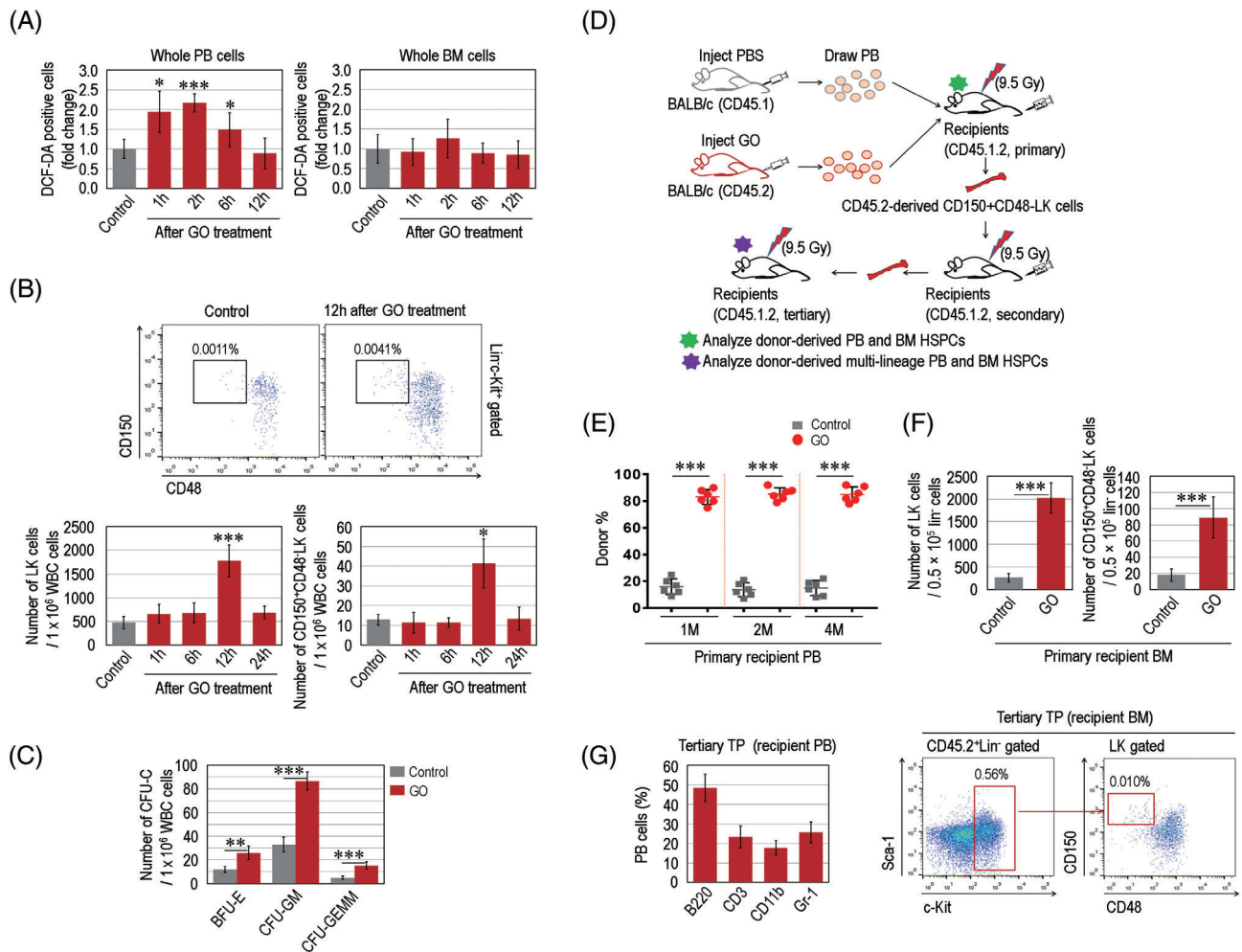


FIGURE 1 GO increases levels of hydrogen peroxide and induces mobilization of long-term repopulating hematopoietic cells in PB. A, Level of hydrogen peroxide in whole PB and BM cells was measured at the indicated times after intravenous injection of GO (0.250 U/g, $n = 6$). B, Numbers of LK and CD150⁺CD48⁻LK cells in PB were assessed by multicolor flow cytometry at the indicated times after GO injection ($n = 12$ /group). In this study, LK subsets in BALB/C mice were quantified as phenotypical markers for hematopoietic stem progenitor cells, because of their low or no expression of Sca-1.¹⁴ C, For analysis of hematopoietic stem progenitor cell activity, we performed colony-forming cell assays by incubating PB cells (1×10^6 /dish) in a methylcellulose-based medium for 12 days, followed by counting of the colonies (BFU-E, CFU-GM, and CFU-GEMM). Representative results from four independent experiments are shown. D, Schematic diagram of the transplantation protocol. E, For competitive transplantation experiments, an equal number of PB cells (2×10^6) collected from PBS- and GO-injected mice was transplanted into lethally irradiated recipient mice. The competitive repopulation capacity of donor cells was assessed in the recipient PB at the indicated times after post-transplantation ($n = 6$ /group). F, Four months post-transplantation, BM cells in primary transplanted recipient mice were assessed for LK and CD150⁺CD48⁻LK cells after gating CD45.1⁺ (PBS-injected) and CD45.2⁺ (GO-injected) cells ($n = 6$ /group). G, Donor-derived CD150⁺CD48⁻LK cells were transplanted from primary recipient mice into conditioned secondary and tertiary recipient mice. Multilineage reconstitution and engrafted hematopoietic stem progenitor cells were determined in the PB and BM of tertiary recipient mice at 2 months post-transplantation ($n = 5$ /group). All data are expressed as the mean \pm SD. * $P < .05$; ** $P < .01$; *** $P < .001$, as determined by one-way analysis of variance for multiple comparisons (A, B, G) or by Student's t tests for two sets of data (C, E, F). BFU-E, burst forming unit-erythrocyte; BM, bone marrow; CFU, colony-forming unit; CFU-GEMM, CFU-granulocyte/erythroid/macrophage/megakaryocyte; CFU-GM, CFU-granulocyte/macrophage; GO, glucose oxidase; PB, peripheral blood; PBS, phosphate-buffered saline; TP, transplantation; WBCs, white blood cells

While HSPCs in PB are major source for autologous and allogeneic transplantations, use of mobilizers may increase adverse effects. G-CSF, the most widely used mobilizer in the clinic, causes side effects such as spleen enlargement, bone pain, headaches, and a propensity for thrombosis in donors.^{16,17} A few of patients transplanted with G-CSF-mobilized peripheral cells are also suffered from chronic-graft-vs-host disease (GVHD).^{18,19} The incidence of GVHD is

associated with G-CSF-mediated dysregulation in the production of WBC and lymphocytes.^{20,21} However, our results show that GO injection does not alter components of PB cells, blood parameters, and spleen weight (Figure S6A-C). The current findings also reveal that BM numbers and senescence of LK and CD150⁺CD48⁻LK cells, circulating blood cell count, and spleen weight are unchanged by GO injection in old mice even after 6 months of injection (Figure S7A-E).

In addition, GO-injected mice have life span similar to that of the vehicle-injected mice (Figure S8). Together, these results indicate that GO induces HSPC mobilization without immunological or pathological disorders. Our results also imply that GO injection neither impairs maintenance of BM HSPCs, nor causes ROS-mediated stem cell senescence. Furthermore, the result showing a normal life span of GO-injected mice supports a safety of GO in a clinical application.

3.2 | GO stimulates HSPC mobilization in mice exhibiting poor mobilization and enhances the efficacy of G-CSF and AMD3100

Older individuals or patients with Fanconi anemia and impaired BM after cancer therapy exhibit poor HSPC mobilization in response to

mobilizers.^{22,23} We investigated whether GO injection stimulates HSPC mobilization in the mice that exposed to poor mobilizing inducers or in old mice. Similar to a previous report,²⁴ ALN injection induced osteopetrosis phenotypes showing increased bone density with decreased osteoclastic activity, while STZ highly increased blood glucose level compared with the vehicle-injected controls (Figure S9A-D). GO injection increased significantly ($P < .001$) number of LK cells in PB of the old, 5-Fu-, and ALN-treated mice, but not in STZ-treated mice, compared with the vehicle-injected controls (Figure 2A). PB cells derived from the old, 5-Fu-, or ALN-exposed mice also formed greater numbers of BFU-E, CFU-GM, and CFU-GEMM colonies compared with the control mice (Figure 2B). These results support the potential of GO to facilitate HSPC mobilization even in the mice expressing poor cell mobilization. However, our results indicate that STZ itself increases numbers of LK cells and CFU-C in PB,

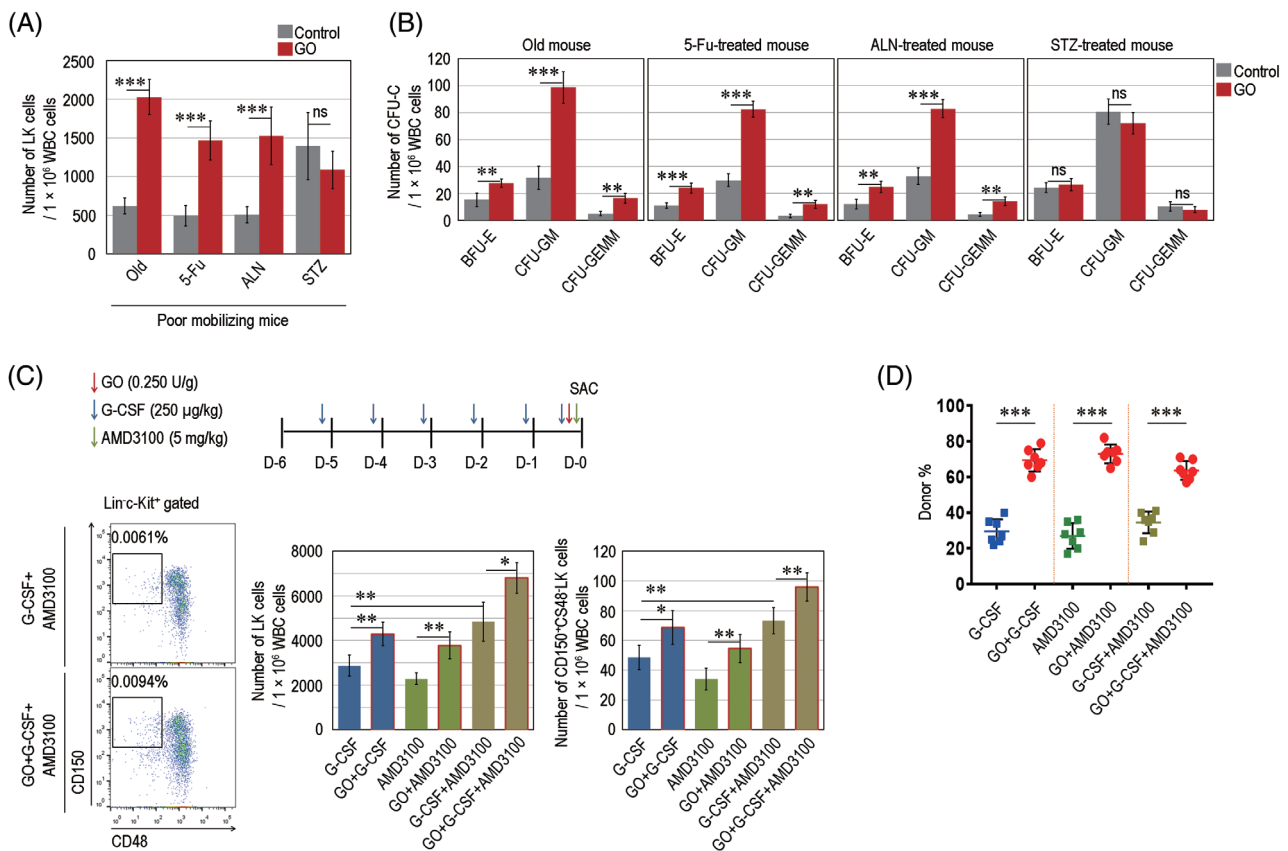


FIGURE 2 GO exerts stem cell mobilization in mice with poor mobilization and enhances the efficacy of mobilizers. A, To assess the mobilization effect of GO in animal models with poor mobilization, we prepared old mice (>12 months, $n = 5$), 5-fluorouracil-treated mice ($n = 5$), alendronate-treated mice ($n = 5$), and streptozotocin-treated mice ($n = 5$), and measured number of peripheral LK cells in the mice after vehicle or GO injection. B, PB cells collected from the mice injected with vehicle or GO were subjected to colony-forming unit assay using the same procedures mentioned in Figure 1C. Representative results from four independent experiments are shown. C, Injection schedules of GO, G-CSF, and AMD3100: G-CSF (250 μ g/kg) was injected subcutaneously daily for five consecutive days, while GO was injected intravenously 12 hours before the mice were euthanized (day 0). AMD3100 (5 mg/kg) was injected subcutaneously 4 hours before the mice were euthanized. Numbers of LK and CD150⁺CD48⁻LK cells were assessed in PB collected from these mice ($n = 6$ /group). D, For competitive repopulation experiments, an equal number of PB cells (2×10^6) was collected from mice injected with G-CSF, AMD3100, or both in combination with or without GO ($n = 7$). All data are expressed as the mean \pm SD. * $P < .05$; ** $P < .01$; *** $P < .001$, as determined by Student's *t* tests for comparison of two sets of data. 5-Fu, 5-fluorouracil; ALN, alendronate; BFU-E, burst forming unit-erythrocyte; CFU, colony-forming unit; CFU-GEMM, CFU-granulocyte/erythroid/macrophage/megakaryocyte; CFU-GM, CFU-granulocyte/macrophage; G-CSF, granulocyte colony-stimulating factor; GO, glucose oxidase; PB, peripheral blood; STZ, streptozotocin; WBCs, white blood cells

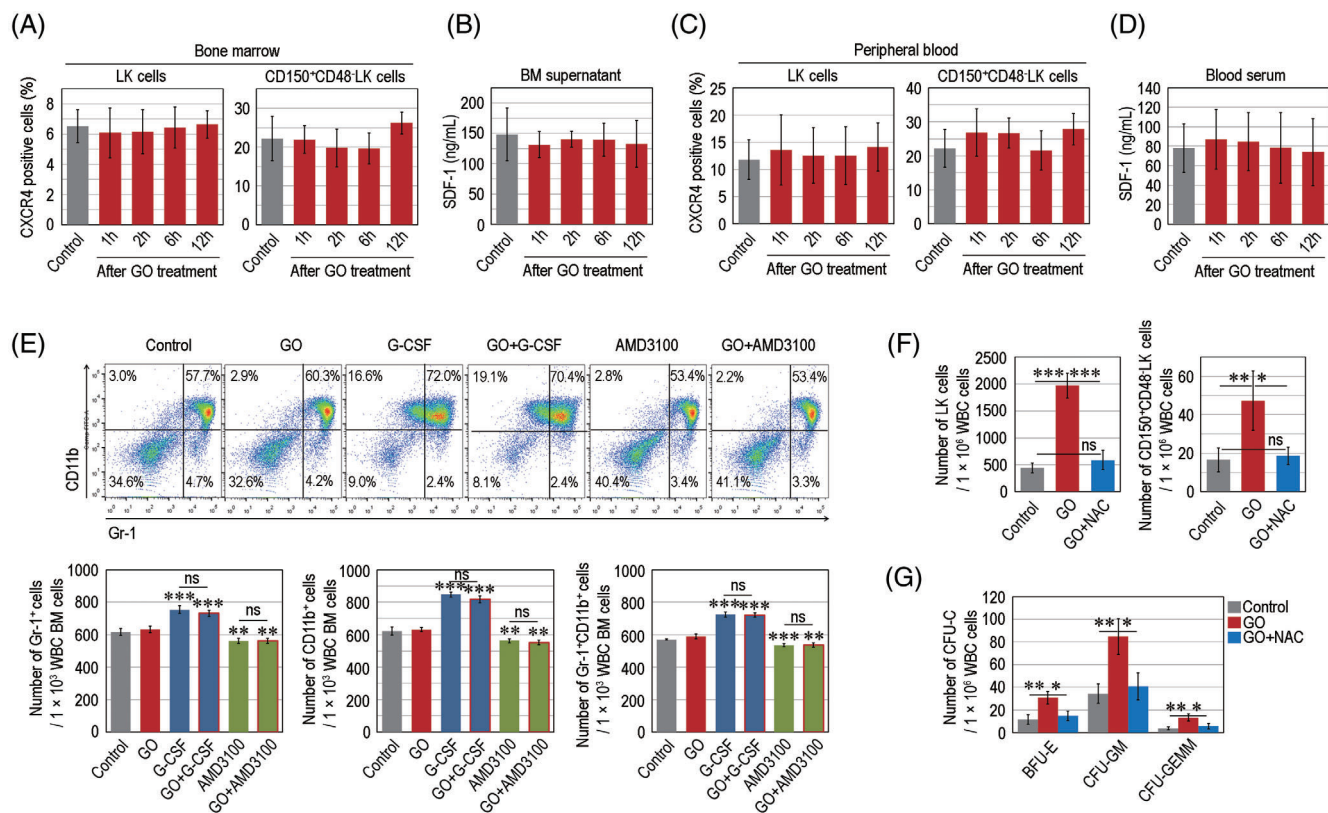


FIGURE 3 GO induces peripheral mobilization of hematopoietic stem progenitor cells via ROS signaling rather than alteration of the SDF-1/CXCR4 retention axis. A, The percentages of CXCR4⁺ LK and CD150⁺CD48⁻LK cells were measured in bone marrow of vehicle- and GO-injected mice ($n = 6/\text{group}$). B, Concentration of SDF-1 in bone marrow supernatant was determined by enzyme-linked immunosorbent assay; representative data from three independent experiments are shown. C, The percentages of peripheral CXCR4⁺ LK and CD150⁺CD48⁻LK cells in GO-injected mice were measured by flow cytometry ($n = 6/\text{group}$). D, SDF-1 level in blood serum; representative data from three independent experiments are shown. E, Numbers of CD11b⁺ and/or Gr-1⁺ cells in bone marrow of GO-injected mice were assessed ($n = 5/\text{group}$). Upper panel shows a representative flow diagram. F, G, To determine whether GO-induced mobilization of hematopoietic stem progenitor cells was mediated by generation of reactive oxygen species, mice were administered subcutaneously with NAC (100 mg/kg) before GO injection. Numbers of peripheral LK and CD150⁺CD48⁻LK cells (F, $n = 5/\text{group}$) and of colonies formed after PB cell incubation (G, three independent experiments) were counted. All data are presented as the mean \pm SD. * $P < .05$; ** $P < .01$; *** $P < .001$, as determined by one-way analysis of variance for multiple comparisons (A-D) and by Student's t tests for two sets of data (E-G). BFU-E, burst forming unit-erythrocyte; BM, bone marrow; CFU, colony-forming unit; CFU-GEMM, CFU-granulocyte/erythroid/macrophage/megakaryocyte; CFU-GM, CFU-granulocyte/macrophage; G-CSF, granulocyte colony-stimulating factor; GO, glucose oxidase; NAC, *N*-acetyl-L-cysteine; ns, not significant; PB, peripheral blood; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor-1; WBCs, white blood cells

and this is not affected by GO injection. Further experiments using other diabetic animals will be necessary to verify a relationship between GO treatment and diabetes.

Combined treatment of G-CSF with AMD3100 or cyclophosphamide induces synergistic effects on HSPC mobilization.²⁴⁻²⁷ Administration of G-CSF alone mobilized LK and CD150⁺CD48⁻LK cells more than did AMD3100 only, and this was significantly ($P < .01$) augmented in combination both with the mobilizers (Figure 2C). GO injection markedly enhanced mobilization of LK and CD150⁺CD48⁻LK cells in the mice that received G-CSF, AMD3100, or both (Figure 2C). Competitive repopulation assay also revealed that GO injection in combination G-CSF, AMD3100, or both significantly ($P < .001$) increased donor cell repopulating potential than did the mobilizer only (Figure 2D). These results support an additive mobilization effect of

GO in combination regimens involving conventionally used mobilizers. Our findings also indicate that peripheral mobilization of HSPCs in mice rapidly occurred even when treated with single GO injection like does AMD3100 rather than G-CSF.^{28,29}

3.3 | GO induces HSPC mobilization mainly via ROS signaling

To understand the mechanisms involved in GO-stimulated HSPC mobilization, we investigated stem cell mobilization-related factors in BM and PB. A temporal disruption of the C-X-C chemokine receptor type 4 (CXCR4)/C-X-C motif chemokine ligand 12 (CXCL12/SDF-1) retention axis in BM induces mobilization of HSPCs into PB, and this

is a cellular mechanism of G-CSF- and AMD3100-mediated mobilization.³⁰ Similar to whole BM cells (Figure 1A), DCF level in BM LK and CD150⁺CD45⁻LK cells of GO-injected mice were unchanged compared with the vehicle-injected mice (Figure S10). Compared with the vehicle-injected mice, GO-injected mice exhibited similar percentages of CXCR4⁺ LK and CD150⁺CD48⁻LK cells in BM and PB (Figure 3A, C), as well as SDF-1 levels in BM supernatant and blood serum throughout the postinjection times (Figure 3B,D). Activated granulocytes and monocytes in BM contribute to HSPC mobilization by increasing the production of proteolytic enzymes.^{25,31} While the G-CSF-injected mice showed greater numbers of Gr-1⁺, CD11b⁺, or both cells in BM, AMD3100-injected mice exhibited lower proportions of these cells compared with those in the vehicle-injected mice (Figure 3E). GO injection neither altered BM levels of Gr-1⁺ and CD11b⁺ cells, nor affected numbers of these cells regardless of combination with G-CSF or AMD3100 (Figure 3E).

Otherwise, the induction of HSPC mobilization is correlated partially with increased activity of osteoclasts.^{26,31} When number of osteoclasts was determined in femoral bones of GO-injected mice, no significant increase in TRAP-stained osteoclasts was found (data not shown). Based on the mobilizing effect of GO in ALN-induced osteopetrosis mice, it is postulated that GO-induced HSPC mobilization is not associated with a signaling pathway that mediates osteoclastic activation. As GO increases oxidative stress and alters ROS-related cellular signaling, we explored whether GO-stimulated HSPC mobilization is associated with increased ROS by treating mice with the antioxidant NAC prior to GO injection. NAC treatment almost completely inhibited the GO-stimulated peripheral mobilization of LK and CD150⁺CD48⁻LK cells (Figure 3F). NAC treatment also diminished the GO-induced increases in numbers of CFU-C up to the levels similar to that in the vehicle-injected mice (Figure 3G). These results indicate that GO-induced HSPC mobilization is closely associated with ROS signaling or an activation of ROS-mediated signaling. Considerable evidence highlights that activation of ROS signaling mediates G-CSF-induced mobilization of HSPCs, and this is diminished by treatment with the ROS inhibitor NAC.^{4,32} With these regards, we consider that GO stimulates HSPC mobilization with the mechanisms in part similar to that of G-CSF. However, GO injection alone neither changes numbers of BM Gr-1⁺ and CD11b⁺ cells, nor increases numbers of CXCR4⁺ HSPCs and SDF-1 levels in BM and PB along with numbers of granulocytes and osteoclasts, all of which are to be major contributors in G-CSF- and AMD3100-induced mobilization of HSPCs.^{28,29,33,34} Therefore, we may suggest that GO exerts its mobilizing role mainly by ROS signaling, while G-CSF- or AMD3100-induced mobilization of HSPCs is due to various cellular events derived from the SDF-1/CXCR4 axis together with ROS signaling. To clarify whether there is a specific intracellular redox molecule or ROS-unrelated pathway in GO-induced mobilization of HSPCs will be necessary. Consequently, our study indicates that the use of GO in combination with G-CSF and/or AMD3100 is to be a desirable strategy in PBSC transplantation, and the GO-related impacts on HSPC mobilization are controlled by treating antioxidant molecules.

4 | CONCLUSION

This study shows that single GO injection stimulates peripheral mobilization of HSPCs through ROS signaling and enhances synergistically the mobilizing efficacies of G-CSF and AMD3100 without adverse effects. GO-mobilized HSPCs show their reconstituting and differentiating potentials in conditioned recipients for long times. GO also stimulates HSPC mobilization even in the mice exhibiting poor stem cell mobilization. Collectively, our results provide an evidence that combined treatment of GO with commonly used mobilizers may enhance the efficacy and outcome in autologous and allogenic PBSC transplantation.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

J.-C.L.: conceived and designed the experiments, contributed reagents, materials, and analytical tools, wrote and/or edited the manuscript; S.-H.K.: conceived and designed the experiments, performed the experiments and analyzed the data, contributed reagents, materials, and analytical tools, wrote and/or edited the manuscript; H.-S.S.: performed the experiments and analyzed the data, wrote and/or edited the manuscript; M.-G.K.: performed the experiments and analyzed the data.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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