SHORT COMMUNICATION

The chemokine (C-X-C motif) receptor 4 inhibitor AMD3100 accelerates blood flow restoration in diabetic mice

C. Jiao · S. Fricker · G. C. Schatteman

Abstract

Aims/hypothesis Bone marrow cell mobilisation potently induces vascular growth in ischaemic tissue, possibly by mobilising endothelial cell progenitors. Thus, mobilising agents might not be therapeutic when endothelial cell progenitors are dysfunctional, as in diabetes mellitus. Local injection of autologous endothelial cell progenitors also stimulates vascular growth in ischaemic tissue, but endothelial cell progenitors from people with multiple cardiovascular risk factors and from obese diabetic mice are marginally therapeutic or inhibitory. We sought to identify possible strategies to improve vascularisation in patients with diabetes mellitus by determining if (1) mobilisation accelerates neovascularisation in diabetic animals, and (2) mobilised cells from a non-diabetic source accelerate vascularisation in diabetic animals.

Methods We tested whether systemic administration of the chemokine (C-X-C motif) receptor 4 inhibitor AMD3100 or local injection of human $CD34^+$ circulating cells mobilised by AMD3100 could speed or enhance blood flow restoration in ischaemic limbs of diabetic mice. The small-molecule-mobilising drug AMD3100 was selected because mobilisation and apheresis can be done on the same day.

Results Systemic administration of AMD3100 and local injection of cells mobilised by AMD3100 greatly accelerated the restoration of blood flow to ischaemic limbs of diabetic mice. $CD34^+$ cells mobilised by AMD3100 appeared to be more potent growth stimulators than their unmobilised counterparts.

Conclusions/interpretation Unlike other mobilising agents requiring multi-day mobilisation, AMD3100 enables mobilised donors to undergo mobilisation and apheresis on the same day. The combination of excellent therapeutic benefits as well as ease of use indicates that AMD3100 could be a powerful tool to ameliorate tissue ischaemia in the diabetic environment.

Keywords AMD3100 · Bone marrow · CD34 · CXCR4 · Diabetes mellitus · Ischaemia · Mobilisation · Neovascularisation · SDF-1

Abbreviations

ECPs	endothelial cell progenitors
G-CSF	granulocyte colony stimulating factor
$mCD34^+$	$CD34^+$ PBMCs mobilised by AMD3100
PBMC	peripheral blood mononuclear cell
$unCD34^+$	unmobilised $CD34^+$ PBMC

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Introduction

Agents that mobilise haematopoietic stem and progenitor cells can stimulate vascular growth [1–3]. For example, systemic injection of the mobilising agent granulocyte colony stimulating factor (G-CSF) promotes vascularisation of ischaemic tissue, possibly through mobilisation of endothelial cell progenitors (ECPs) from the bone marrow,

but this has never been proved [2, 3]. If true, however, mobilisation might not promote vascular growth in patients with cardiovascular risk factors, including diabetes mellitus, because these factors compromise bone marrow-derived ECP function. On the other hand, if the pro-angiogenic effects of mobilising drugs are mediated through mechanisms other than ECP mobilisation, such agents might be effective even in patients with compromised ECPs. G-CSF, probably the most commonly used mobilising agent, may not be an ideal choice for inducing leucocytosis because of its pleiotropic effects. Moreover, a clinical trial testing its ability to promote vascular growth was halted due to unanticipated adverse events [3]. AMD3100, a small-molecule drug that mobilises bone marrow cells by blocking chemokine (C-X-C motif) receptor 4 receptors, might be more suitable for promoting vascular growth in the setting of diabetes mellitus [4, 5].

A number of studies have demonstrated that local injection of ECPs from non-diabetic mice and humans promote vascular growth in diabetic mice, but those from obese type 2 diabetic mice and patients with ischaemic heart disease evoke a variety of responses, from potently stimulating to profoundly inhibiting angiogenesis in mice [6–9]. Thus, autologous ECP therapy may not be effective and could actually be harmful in some diabetic patients. However, locally injected ECPs rarely integrate into the neovasculature and are cleared rapidly, despite the fact that they have profound effects on vascular growth [9–11]. Hence, because the transplanted cells would not persist, it might be possible to couple allogenic transplantation with short-term immuno-suppression in order to enhance neovascularisation.

Human unmobilised CD34$^+$ (unCD34$^+$) peripheral blood mononuclear cells (PBMCs) that have been derived from non-diabetic donors are potent stimulators of vessel growth in diabetic mice, and may have similar properties in diabetic patients [6]. However, because they are relatively rare cells, mobilisation would be required to make their use clinically practical. Unfortunately, standard 5-day mobilisation protocols make it difficult to recruit donors. If CD34$^+$ PBMCs that have been mobilised by AMD3100 (mCD34$^+$) stimulate vascular growth, AMD3100 might be an effective alternative, especially as clinical trials demonstrate that a single dose of AMD3100 is as potent a mobilising agent as a 5-day regimen of G-CSF [12].

To identify possible ways of improving vascularisation in patients with diabetes mellitus, we examined whether neovascularisation is accelerated in diabetic animals treated with AMD3100, and whether AMD3100-mobilised cells from a non-diabetic source accelerate vascularisation in diabetic animals. Specifically, we tested whether systemic administration of AMD3100 or local injection of AMD3100 mCD34$^+$ PBMCs promotes vascular growth in ischaemic limbs of diabetic mice.

Subjects and methods

Procedures involving healthy adult volunteers were performed after informed consent had been given in accordance with protocols approved by the Institutional Review Board and with the principles of the Declaration of Helsinki. For unCD34$^+$ PBMCs, 50 to 100 ml human blood was collected and PBMCs were collected as described [6]. For mCD34$^+$ PBMCs, volunteers were injected subcutaneously with 240 μg/kg AMD3100 and PBMCs were collected 4 h later by apheresis. CD34$^+$ PBMCs were isolated using two rounds of CD34$^+$ magnetic cell selection (auto MACS; Miltenyi, Auburn, CA, USA) in accordance with the manufacturer's instructions [6].

Animal procedures were approved by the University of Iowa Animal Care and Use Committee and the Principles of Laboratory Animal Care (National Institutes of Health) were followed. Anaesthesia was induced with 4% isoflurane and maintained with 0.8 to 1.2% isoflurane. Death was induced by injection of 150 mg/kg sodium pentobarbital. Diabetes was induced with streptozotocin in 8- to 12-week-old $HFh11^{nu}$ athymic male mice (Jackson Laboratories, Bar Harbor, ME, USA), and 3 to 4 weeks after inducing diabetes, the left proximal femoral artery was ligated as described [6].

Mice were injected intraperitoneally with 5 mg/kg of a 1.25 mg/ml solution of AMD3100 ($n=8$) or an equal volume of normal saline ($n=12$) on the day of surgery and 2 days afterwards. Additional mice were injected intramuscularly into the ischaemic hindlimb with 1×10^6 unCD34$^+$ ($n=11$) or AMD3400 mCD34$^+$ ($n=7$) human PBMCs, 2 to 4 h after surgery. Untreated mice served as controls ($n=10$).

For analysis of leucocytosis, 200 μl of blood was collected into 20 μl 2.5% Na citrate by retro-orbital bleed 1 h after the first injection of AMD3100. Blood was subjected to density centrifugation on Histopaque 1.083 (Sigma, St Louis, MO, USA) according to manufacturer's instructions, and cell numbers determined using a haemocytometer.

Restoration of blood flow in the limb was analysed immediately before and after surgery and at various times thereafter in the entire limb distal to the ligation using laser Doppler analysis [6]. Resulting data were analysed by repeated measures ANOVA followed by Tukey's honestly significant difference test using SPSS software (SPSS Science, Chicago, IL, USA). $p<0.05$ was considered statistically significant. Data are presented as percent mean blood flux in the operated ischaemic limb relative to the unoperated control limb. Mice whose post-surgery blood flow was >12% were excluded from the analysis [6].

Results and discussion

We first verified that AMD3100 causes leucocytosis in diabetic nude mice. The drug mobilised total PBMCs 2.6± 0.2-fold in non-diabetic and 3.1±0.5-fold in diabetic mice 1 h after dosing, resulting in 37% more mobilised cells in diabetic than in non-diabetic mice (p=0.04). On the day of surgical induction of hindlimb ischaemia and 2 days afterwards, diabetic mice were injected with AMD3100 or vehicle. Blood flow restoration was monitored in the limbs by laser Doppler scanning until flow values reached a plateau. Drug-induced improvement was observed 2 days after surgery, reaching its maximum in AMD3100-treated mice (Fig. 1a, p<0.05) at 7 days. Thereafter, blood flow remained at a mean of 74.6±2.8% that of the initial flow. Not until 16 days after surgery did blood flow in the control mice reach that of AMD3100-treated mice.

To determine whether AMD3100 mCD34$^+$ PBMC therapy improves limb revascularisation and whether the cells are as potent as their unmobilised counterparts, mCD34$^+$ or unCD34$^+$ PBMCs were injected into ischaemic limb muscle [6]. Laser Doppler measurements showed that both cell types significantly accelerated blood flow restoration relative to untreated controls by 6 days after injection (Fig. 1b, p<0.05). By 8 days after surgery, blood flow values plateaued in limbs injected with unCD34$^+$ PBMC, maintaining a mean of 70.7±3.9% of initial blood flow thereafter. Blood flow in limbs of control mice plateaued at a similar level (64.7±4.6%), but did not reach this level until 15 days. In contrast, blood flow in limbs treated with mCD34$^+$ PBMC continued to improve until day 12, after which it plateaued at 89.5±6.6% of initial flow, a significantly greater value than that of either control or limbs treated with unCD34$^+$ PBMC (p<0.05).

Our data demonstrate that systemic treatment with AMD3100 and local injection of AMD3100 mCD34$^+$ PBMCs accelerate blood flow restoration to ischaemic tissue in a diabetic environment with similar kinetics. Although AMD3100 systemic therapy accelerated blood flow restoration, the drug did not increase it. In contrast, mCD34$^+$ PBMC therapy both accelerated and increased blood flow restoration. Moreover, whereas AMD3100-mobilised cells and unCD34$^+$ cells are equally potent in accelerating blood flow restoration, only mCD34$^+$ PBMCs increased the amount of blood flow recovered in the ischaemic limbs relative to untreated controls.

Mobilisation therapy may be less effective in maximising the amount of flow restored than therapy employing mCD34$^+$ PBMCs (Fig. 1), but its simplicity makes it an attractive potential treatment. Moreover, two facts suggest that the vessels will be stable in the long term. First, the flow remained stable long after (i.e. at least 22 days) the drug was last administered. Second, blood flow, not capillary density
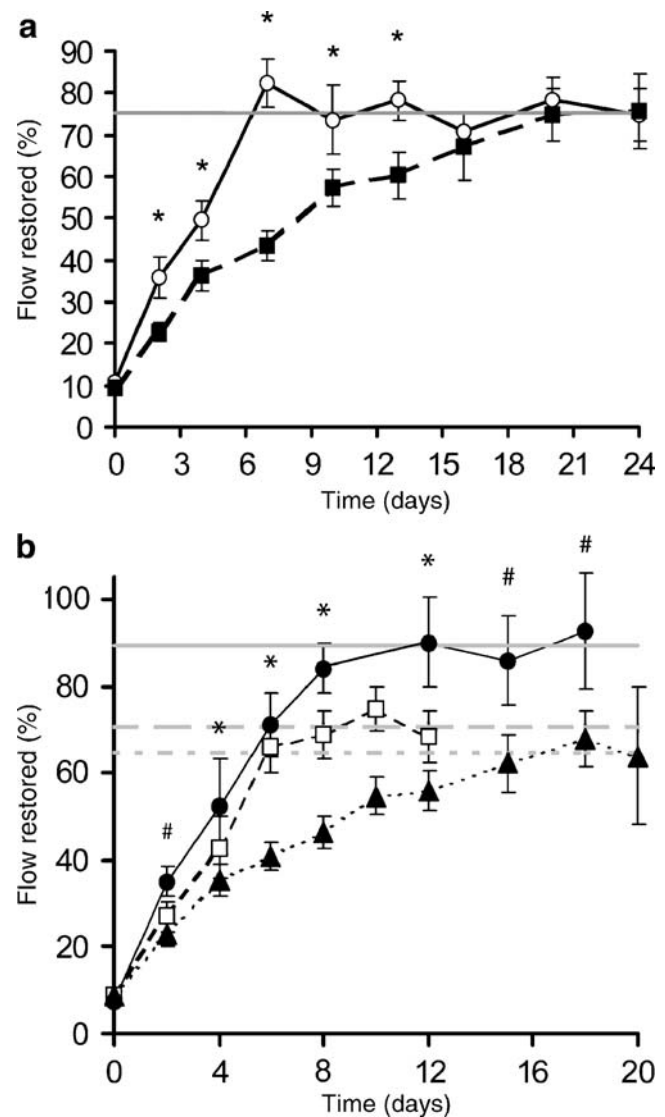

Fig. 1 Percent of blood flow restored over time in ischaemic limbs of diabetic mice assessed by laser Doppler scanning. Ischaemia was induced by femoral artery ligation. **a** Mice injected intraperitoneally with AMD3100 (n=8) (circles) or vehicle (control) (n=12) (squares) on days 0 and 2. Grey line, plateau of flow restoration (mean flow days 8–24) in AMD3100-treated mice. *p<0.05 AMD3100 vs control. **b** Mice injected with AMD3100-mobilised CD34$^+$ (n=7) (circles) or unmobilised CD34$^+$ (n=11) (squares) PBMCs on the day of ligation. Cells were injected into the ischaemic muscle. Controls (triangles) received no treatment. Grey lines, plateau of flow restoration using mean flow for days 15–20 in untreated mice (dotted), days 8–12 in mice treated with unmobilised CD34$^+$ PBMC (dashed), and days 12–18 in mice treated with mobilised CD34$^+$ cells (solid). #p<0.05 mobilised CD34$^+$ vs control. *p<0.05 mobilised and unmobilised cells vs control

was measured. Flow measurement assesses large blood vessel formation, and these tend to be stable in the long term. Nevertheless, although AMD3100 was beneficial in the diabetic mice, it remains to be seen whether it will be valuable in settings with multi-factorial cardiovascular disease. If not, the use of locally injected allogenic

AMD3100 mCD34$^+$ may be an alternative. Not only are the cells potent stimulators of neovascularisation, but the use of AMD3100 to mobilise cells could facilitate this therapeutic alternative by allowing PBMC donors to be mobilised and undergo pheresis on the same day. Thus, AMD3100 may be a powerful therapeutic tool to ameliorate tissue ischaemia in the diabetic environment.

References

1. Takahashi T, Kalka C, Masuda H et al (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 5:434–438
2. Natori T, Sata M, Washida M, Hirata Y, Nagai R, Makuuchi M (2002) G-CSF stimulates angiogenesis and promotes tumor growth: potential contribution of bone marrow-derived endothelial progenitor cells. Biochem Biophys Res Commun 297:1058–1061
3. Kang HJ, Kim HS, Zhang SY et al (2004) Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 363:751–756
4. Liles WC, Broxmeyer HE, Rodger E et al (2003) Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood 102:2728–2730
5. Broxmeyer HE, Orschell CM, Clapp DW et al (2005) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J Exp Med 201:1307–1318
6. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA (2000) Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 106:571–578
7. Stepanovic V, Awad O, Jiao C, Dunnwald M, Schatteman G (2003) Leprdb diabetic mouse bone marrow cells inhibit skin wound vascularization but promote wound healing. Circ Res 92:1247–1253
8. Heeschen C, Lehmann R, Honold J et al (2004) Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation, 109:1615–1622
9. Awad O, Jiao C, Ma N, Dunnwald M, Schatteman GC (2005) Obese diabetic mouse environment differentially affects primitive and monocytic endothelial cell progenitors. Stem Cells 23:575–583
10. Harraz M, Jiao C, Hanlon HD, Hartley RS, Schatteman GC (2001) Cd34(−) blood-derived human endothelial cell progenitors. Stem Cells 19:304–312
11. Hamano K, Li TS, Kobayashi T et al (2001) The induction of angiogenesis by the implantation of autologous bone marrow cells: a novel and simple therapeutic method. Surgery 130: 44–54
12. Basu S, Broxmeyer HE (2005) Transforming growth factor-{beta} 1 modulates responses of CD34+ cord blood cells to stromal cell-derived factor-1/CXCL12. Blood 106:485–493