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Pharmacologic targeting of the diabetic stem cell mobilopathy

M. Albiero[a,b], G.P. Fadini[a,b,*]

[a] Venetian Institute of Molecular Medicine, Laboratory of Experimental Diabetology, 35100 Padova, Italy
[b] Department of Medicine, Metabolic Division, University of Padova, 35100 Padova, Italy

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ABSTRACT

Diabetes is a chronic metabolic disease characterized by hyperglycemia and several associated biochemical abnormalities. Diabetes leads to multiorgan complications that collectively reduce life expectancy. Hematopoietic stem cells (HSCs) are nested within bone marrow (BM) niches whence they can be mobilized to the peripheral circulation. Clinically, this is done for HSC collection and autologous or allogenic transplantation. A great amount of data from basic and clinical studies support that diabetic patients are poor HSC mobilizers owing to BM remodeling. Dysfunction of the BM shares pathophysiological features and pathways with typical chronic diabetic complications that affect other issues (e.g. the retina and the kidney). From a clinical perspective, impaired HSC mobilization translates into the failure to collect a minimum number of $CD34^+$ cells to achieve a safe engraftment after transplantation. Furthermore, blunted mobilization is associated with reduced steady-state levels of circulating HSCs, which have been consistently described in diabetic patients and associated with increased risk of adverse outcomes, including cardiovascular events and death. In this review, we discuss the most clinically relevant pharmacological options to overcome impaired HSC mobilization in diabetes. These therapeutic strategies may result in an improved outcome of diabetic patients undergoing HSC transplantation and restore circulating HSC levels, thereby protecting from adverse cardiovascular outcomes.

1. Introduction

Diabetes mellitus is a chronic disease characterized by biochemical abnormalities in carbohydrate, protein, and lipid metabolism. Owing to gluco- and lipotoxicity, diabetes leads to multiorgan chronic complications affecting the retina, nerves, kidneys and cardiovascular system that altogether reduce life expectancy. In 2017 an estimated 425 million people had diabetes worldwide, with a dramatic increase especially in low-income countries [1].

The pathophysiological mechanisms involved in the development of vascular and cardiovascular complications in both type 1 and type 2 diabetes have been the objective of a frantic amount of research, yet the impact of diabetic macrovascular and microvascular complications on morbidity and mortality is still an unmet clinical need [2–4].

Macrovascular complications are the result of an atherosclerotic process that in diabetic patients is characterized by early onset and rapid progression, causing coronary artery disease, stroke and peripheral artery disease [5]. Endothelial dysfunction, driven by hyperglycemia and the inflammatory milieu, is considered a key pathogenic trigger in prompting atherosclerosis in diabetic patients [6]. Hyperglycemia, on the other hand, strikes the small vessels of the eye, the kidney and the nerves (vasa nervorum) and causes microvascular complications known as diabetic retinopathy, nephropathy and neuropathy [7,8].

The devastating impact of diabetic complications on patients' quality of life and life expectancy has diverted the focus of clinician and researchers from relatively neglected organs that could also be hit by diabetes but have apparently no relevant pathophysiological or clinical impact on patients' life. The bone marrow (BM) as a target of diabetic complications has been almost ignored by diabetologists for decades, but researchers investigating bone marrow-derived progenitors as a cellular source for regenerative purposes have more recently highlighted that diabetic progenitors were endowed with less regenerative potential and were intrinsically defective [9–11]. This prompted to investigate whether diabetes could impact BM structure and function. Our group was among the first to show that in type 1 diabetic rats the BM was unable to release hematopoietic stem cells (HSCs), a process defined mobilization, after a direct stimulation with granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF) suggesting that the diabetic BM might be intrinsically compromised [12]. Such a defect in HSC mobilization has been termed "bone marrow stem cell mobilopathy" [13].

In this review, we will discuss the current knowledge about diabetic BM dysfunction and the possible pharmacological strategies to

* Corresponding author at: Department of Medicine, University of Padova, Via Giustiniani 2, 35100 Padova, Italy.
E-mail address: gianpaolo.fadini@unipd.it (G.P. Fadini).

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overcome it.

2. Bone marrow alteration and dysfunction in diabetes

The architecture of the BM is devoted to preserving the HSC pool throughout adult life while maintaining hematopoiesis. To fulfill this commitment, HSCs are nested in niches, which are a framework of cellular and structural components that regulates HSCs fate and homeostasis. The concept of "stem cell niche" was introduced by Raymond Schofield in 1978, who proposed that such microenvironment might be instructive for stem cells in determining their fate [14]. The hypothesis of Schofield has proved true with the most robust evidences and clarifying the general concept that a stem cell to maintain its status and properties (that is to be a stem cell) must resides within a niche [15,16].

To achieve a task as regulating HSCs fate, it is not surprising that the BM niche is an extremely complex, yet tightly regulated, microenvironment, which encompasses osteoblasts, stromal cells, resident macrophages, adipocytes, blood vessels, megakaryocytes, and nerve terminals from the sympathetic nervous system [17].

It is easy to understand how such a delicate microenvironment can be disentangled by the harsh diabetic milieu. As an example, hyperglycemia per se is sufficient in reprogramming steady state hematopoiesis driving myelopoiesis that in turn can fuel atherosclerosis [18].

The first evidence linking diabetes with marked alterations in BM described a severe form of microangiopathy in the BM of type 1 diabetic mice, with capillary rarefaction and a reduced amount of Lineage$^-$ c-Kit$^+$ Sca-1$^+$ hematopoietic progenitor cells (LKS cells) in the low-perfused part of the BM [19]. Diabetes, probably through oxidative stress and hyperglycemia, impinged upon the vasculature and this could have a profound impact on the physiology of the BM. The BM is a rather hypoxic tissue where vascularity, cellularity, and oxygen tension are driving forces in the spatial and functional organization of HSC niches [20]. Furthermore, vascular and peri-vascular stromal cells form an extremely complex niche secreting major niche-regulating factors such as CXCL12 and SCF, which also regulate retention and mobilization of HSCs [21].

The existence of a form of BM microangiopathy was also validated in BM samples from human patients with type 2 diabetic, which showed signs of vascular rarefaction and fat deposition, in turn being associated with reduced abundance of CD34$^+$ progenitor cells [22].

Fat accumulation in the BM, conceived as physiological phenomenon due to aging in humans, is exacerbated in patients and animal models of type 2 diabetes, but is reported to have a detrimental role on the bone marrow microenvironment by reducing HSCs and progenitors number and their regenerative potential after transplantation [23]. Indeed, when the stromal compartment of the HSCs niche is pushed toward adipogenic differentiation, as in type 2 diabetes, the niche support function is compromised [24]. The stromal compartment of the niche could be severely affected by diabetes, since specific subpopulation of mesenchymal stem cells (MSCs) can be irreversibly depleted in models of both type 1 and type 2 diabetes, regardless of restoration of normal glucose levels [25].

To the same extent, MSCs from nonobese diabetic mice (NOD) showed a distinct gene expression profile compared to BALB/c control mice. In particular NOD-MSC expressed more proinflammatory genes and had a reduced expression of PD-L1, which collectively dampened their immunomodulatory potential but could also affect the physiology of the HSCs niche [26]. PD-L1 is the ligand for the inhibitory programmed death 1 (PD-1) receptor, which is expressed primarily on activated T cells and might serve as mechanism by which MSC exert immunosuppressive actions on autoreactive T cells. Indeed, exploiting this mechanism, MSCs have been used as immunomodulatory tool to revert a model newly onset type 1 diabetes but could also be couple to islet transplantation to induce immune tolerance and enhance graft survival [26].

BM macrophages have been recently recognized as important regulators of HSC niche homeostasis [27,21]. We have shown that BM macrophages secrete Oncostatin M (OSM), that signals to the stromal cells to express CXCL12, thereby retaining HSCs within the bone marrow. Diabetes caused an expansion of pro-inflammatory BM macrophages, resulting in excess OSM signaling, local CXCL12 production by niche cells and blunting stem cell mobilization [28]. This represents an important pathophysiological link between hyperglycemia and inflammation in causing diabetic stem cell mobilopathy. Hyperglycemia is believed to drive the myeloid bias that characterizes diabetes [18], but the same mechanism could cause the expansion of BM macrophages, leading to mobilopathy.

Further studies expanded the knowledge of the impact of diabetes on BM, showing that sympathetic innervation of the BM was dysfunctional in diabetic animals [29–31], which might explain the refractory response to G-CSF. The concept that G-CSF might "trans-activate" mobilization of HSCs through sympathetic innervation is based on two evidences: i) G-CSF receptor (G-CSFR) is expressed on both HSCs and nerves in the bone marrow but G-*CSFR*$^{-/-}$ mice show no mobilization response to G-CSF, while G-*CSFR*$^{-/-}$ HSCs can be mobilized from chimeric animals [32]; ii) sympathetic innervation is required for stem cell mobilization and circadian oscillations of CXCL12 [33]. From a clinical standpoint, it was reported that patients with type 2 diabetes featured a form of sensory and autonomic neuropathy in the BM, because of the pauperization of BM nerves [34]. A defective nociceptive response after G-CSF stimulation, measured as an impaired release of Substance P was correlated with poor CD34$^+$ cells mobilization in the peripheral blood [34]. It is important to underline that, in diabetic patients, the coexistence of pathological cardiovascular autonomic tests, used for the diagnosis of diabetic neuropathy, is associated with a reduction of circulating CD34$^+$ cells [31].

3. Diabetes impairs stem cells mobilization

One may question why impaired HSC mobilization is relevant in the contest of diabetes. The first reason is strictly hematological: G-CSF with or without chemotherapy, is used to mobilize HSCs from the BM to peripheral blood (PB) and allows HSC collection by apheresis for transplantation. PB CD34$^+$ cells count has been proposed as a successful predictor of apheresis yield and an absolute count of 20 cells/microL has been used as a threshold to obtain successful HSC collection [35]. Impaired mobilization translates into the failure to collect a minimum number of CD34$^+$ HSC (2×10^6/kg) to achieve a safe engraftment. The definition of any potential risk factor of failing mobilization might improve clinical success of transplantation procedures.

A second, less acknowledged, consequence of the diabetic stem cell mobilopathy derived from the concept that blunted mobilization is responsible of the reduced steady-state level of circulating HSCs, due to impaired bone marrow function [36]. A reduced level of circulating HSCs has been consistently described in diabetic patients On the other side, in patients with type 1 diabetes for more than 50 years, the level of circulating progenitors was higher amongst those without cardiovascular diseases, strengthening their protective role for the cardiovascular system [37].

Finally, dysfunctional diabetic bone marrow could be also the culprit for poor immunotolerance involved in the onset of autoimmune type 1 diabetes. Indeed, preclinical studies showed that HSCs can exert potent immunomodulatory activity on T cells that could potentially revert the onset of diabetes [38]. While allogenic HSCs therapy has been explored, rescuing autologous HSCs mobilization could potentially avoid all major adverse effects associated with allogenic HSCs therapies [39]. Furthermore, empowering immunotolerance with HSCs could be relevant for islet transplantation, which is a therapeutic option that hold great hopes for diabetic patients albeit long-term survival of islet graft remain problematic [40]. Mobilization of HSCs showed promising preclinical results in improving islet allograft survival, via a PD-L1-