Competing Effects of Renin Angiotensin System Blockade and Sodium-Glucose Cotransporter-2 Inhibitors on Erythropoietin Secretion in Diabetes

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Keywords

Angiotensin converting enzyme inhibitors · Angiotensin receptor blockers · Sodium-glucose cotransporter-2 inhibitors · Hematocrit · Haemoglobin

Abstract

Background: Anaemia is a common finding in diabetes, particularly in those patients with albuminuria or renal dysfunction and is associated with impaired erythropoietin (EPO) secretion. This review focuses on mechanisms involved in the regulation of erythropoiesis in diabetic patients in an effort to elucidate the competing effects of the renin angiotensin system (RAS) blockade and sodium-glucose cotransporter-2 (SGLT2) inhibitors on haemoglobin concentration and hematocrit values. **Summary:** The RAS shows significant activation in diabetic subjects. Angiotensin II, its active octapeptide, causes renal tubulointerstitial hypoxia, which stimulates hypoxia-inducible factors (HIF) and increases EPO secretion and erythropoiesis. As expected, drugs that inactivate RAS, such as angiotensin converting enzyme inhibitors or angiotensin receptor blockers (ACEi/ARB) are associated with a significant hematocrit-lowering effect and/or anaemia in various clinical conditions, including diabetes. Dual

blockade by a combination of ACEi and ARB in diabetic patients achieves a better RAS inhibition, but at the same time a worse drop of haemoglobin concentration. Increased glucose reabsorption by SGLTs in diabetic subjects generates a high-glucose environment in renal tubulointerstitium, which may impair HIF-1, damage renal erythropoietin-producing cells (REPs) and decrease EPO secretion and erythropoiesis. SGLT2 inhibitors, which inhibit glucose reabsorption, may attenuate glucotoxicity in renal tubulointerstitium, allowing REPs to resume their function and increase EPO secretion. Indeed, EPO levels increase within a few weeks after initiation of therapy with all known SGLT2 inhibitors, followed by increased reticulocyte count and a gradual elevation of haemoglobin concentration and hematocrit level, which reach zenith values after 2–3 months. **Key Messages:** The competing effects of RAS blockade and SGLT2 inhibitors on erythropoiesis may have important clinical implications. The rise of hematocrit values by SGLT2 inhibitors given on top of RAS blockade in recent outcome trials may significantly contribute to the cardiorenal protection attained. The relative contribution of each system to erythropoiesis and outcome remains to be revealed in future studies.

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Introduction

Obesity and type 2 diabetes mellitus (T2DM) are increasing steadily worldwide and have reached epidemic proportions [1, 2]. Diabetic kidney disease (DKD) is considered to be the leading cause of end-stage renal disease in the industrialized societies [3]. Anaemia is a common finding in patients with diabetes, particularly in those with albuminuria or renal impairment. At least 1 in 5 diabetic patients followed in tertiary outpatient clinics have anaemia. This association is most pronounced in patients with DKD, where anaemia affects nearly half of them. Of note, anaemia is found earlier and is more severe in patients with DKD than in those with non-DKD, and its presence markedly increases the risk for microvascular and macrovascular complications [4–7]. Pathogenetic mechanisms thought to be responsible for the increased prevalence and severity of anaemia in diabetes have included the widespread use of renin-angiotensin system (RAS) inhibitors, hyporeninemia, chronic inflammation, hypogonadotrophic hypogonadism with low serum testosterone levels in male diabetic patients and above all impaired erythropoietin (EPO) secretion attributed to tubulointerstitial damage and/or autonomic neuropathy. Interestingly, 70% of anaemic patients with diabetes without renal impairment also have inappropriately low EPO levels [8–14].

EPO is an indispensable erythropoietic hormone primarily produced from peritubular fibroblasts in the renal cortex, the so-called renal erythropoietin-producing cells (REPs) and is tightly regulated in a hypoxia-inducible manner to maintain tissue oxygen homeostasis. Its production is controlled at the transcriptional level. Hypoxia increases EPO production and secretion by attenuating the inhibition of the EPO promoter by GATA2 and by supporting the availability of heterodimeric (α/β) hypoxia-inducible transcription factors (HIFs). Circulating EPO acts in the bone marrow, as an anti-apoptotic factor and allows survival, proliferation and differentiation of erythrocytic progenitors. Insufficient EPO production by REPs causes renal anaemia and anaemia associated with chronic disorders [15]. Recent studies have broadened our understanding of REPs from prototypic hypoxia-responsive cells to dynamic fibrogenic cells, which in chronic kidney disease (CKD), are the major source of scar-forming myofibroblasts and actively produce fibrogenic molecules, including inflammatory cytokines, rather than EPO [16].

The RAS and Erythropoiesis

The Activation of RAS Is Associated with Enhanced Erythropoiesis

In most hypertensive subjects with T2DM, the RAS shows significant activation, and it is poorly suppressed, even in high salt diets [17]. There are many possible mechanisms underlying RAS activation in diabetes. First, the avid glucose reabsorption in the proximal tubule of the subjects with diabetes is coupled with avid sodium reabsorption by sodium-glucose co-transporters (SGLTs), the so-called SGLTs. This results in decreased distal sodium delivery to the macula densa and increased renin release [18]. Second, a growing body of experimental evidence suggests that insulin itself can cause RAS activation in subjects with metabolic syndrome or diabetes that have insulin resistance and hyperinsulinemia. Even in healthy volunteers, acute hyperinsulinemia increased renin and circulating angiotensin II (Ang II) levels [19, 20]. Finally, GPR91 (G protein-coupled receptor 2)-mediated paracrine signaling pathway in the juxtaglomerular apparatus may serve as a link between diabetes, RAS activation and systemic hypertension [21].

Ang II is a relatively selective vasoconstrictor of the efferent arterioles. Therefore, Ang II is expected to increase intraglomerular pressure and filtration fraction, while at the same time to decrease oxygen delivery to the tubulointerstitium via the postglomerular peritubular capillary bed. Additionally, Ang II exerts a dose-dependent direct stimulatory effect on proximal sodium reabsorption, thus enhancing the oxygen demand of tubular cells. Therefore, after RAS activation, the amalgamation of decreased oxygen supply and increased oxygen demand in the tubulointerstitium causes parenchymal hypoxia, which triggers EPO secretion (Fig. 1) [22]. Ang II can also stimulate EPO secretion by directly affecting HIFs. In this regard, HIF-1 expression, at both the messenger RNA and protein levels, is stimulated by adding Ang II in human placental explant cultures [23]. Moreover, Ang II is a growth factor that can directly stimulate the erythroid progenitors in the bone marrow to produce erythrocytes [24], while at the same time alters the expression of hepcidin and iron transporters to facilitate iron absorption and utilization [25].

RAS Blockade Is Associated with Lowering of Hematocrit Values and/or Anaemia

Pharmacological interruption of RAS by angiotensin converting enzyme inhibitors or angiotensin receptor

Fig. 1. Competing effects of Ang II and avid glucose reabsorption by SGLTs on EPO secretion in diabetic patients. **a** Ang II selectively constricts the efferent arterioles, increases intraglomerular pressure and glomerular filtration rate, while at the same time decreases oxygen delivery to the tubulointerstitium. Additionally, Ang II exerts a direct stimulatory effect on proximal sodium reabsorption, thus enhancing the oxygen demand of tubular cells. The amalgamation of decreased oxygen supply and increased oxygen demand causes tubulointerstitial hypoxia, HIF-1 activation and

increased EPO secretion by the peritubular fibroblasts (REPs). **b** Glomerular hyperfiltration in diabetic patients increases glucose load that is filtered and reabsorbed daily in the proximal tubules by SGLTs. This creates a high-glucose tubulointerstitial environment, which decreases HIF-1, damages and transforms REPs into myofibroblasts and causes reduced EPO secretion. Ang II, Angiotensin II; GFR, glomerular filtration rate; EPO, erythropoietin; HIF, hypoxia-inducible factors; SGLTs, sodium-glucose co-transporters.

blockers (ACEi/ARB), as part of the treatment algorithms in hypertensive, cardiac and renal patients with T2DM has been proven beneficial by several landmark studies. If RAS up-regulates erythropoiesis, as discussed above, it should be expected that drugs or modalities inactivating RAS may induce a hematocrit-lowering effect and/or anaemia. For instance, administration of renin or Ang II in experimental animals causes increased EPO secretion. Captopril blocks the renin- but not the Ang II-induced EPO secretion in rats, while losartan blocks the Ang II-induced EPO secretion in normal volunteers [26–29]. Knockout mice for the ACE or angiotensinogen genes develop substantial anaemia, which is corrected by infusion of Ang II for 2 weeks [30, 31].

RAS contribution to erythropoiesis may be imperceptible in normal or near normal conditions, such as in uncomplicated hypertensive subjects with T2DM [32], but it is exaggerated when bone marrow requires all available stimuli to augment erythropoiesis, such as in patients with severe renal insufficiency, congestive heart failure or immunosuppression (Table 1). Administration of ACEi/ARB is capable to normalize hematocrit values in individuals with altitude polycythemia, post-transplant erythrocytosis or polycythemia associated with COPD [33–35]. In the SOLVD study including patients with heart

failure randomized to enalapril or placebo, the odds of incident anaemia due to enalapril therapy increased by approximately 50% [36]. In a community study on anaemia and heart failure, the prevalence of anaemia increased by an estimated 16% between 1979 before the discovery of RAS inhibitors and 2002, when RAS inhibition was a cornerstone treatment modality ($p = 0.008$) [37]. In patients with CKD, RAS inhibition decreased haemoglobin levels on the order of 0.6–0.9 g/dL. In contrast, no changes in haemoglobin concentrations occurred in patients with CKD receiving conventional anti-hypertensive drugs [38].

Similar results have been reported in subjects with diabetes treated with ACEi or ARBs. A post hoc analysis of the RENAAL study, which included patients with T2DM and DKD for an average follow-up period of 3.4 years showed that, compared with placebo, losartan treatment was associated with a significant decrease of haemoglobin concentration [39]. A subanalysis of the PAERI study comparing 3,361 patients with DKD with 1,861 patients with non-diabetic CKD found that the diabetic patients were treated more often with ACEi (73.9 vs. 55.4%, $p < 0.0001$) and had a higher prevalence of anaemia (52.7 vs. 39.4%, $p < 0.0001$) [40]. Dual blockade by ACEi and ARB may achieve better RAS blockade, while

Table 1. Hematocrit-lowering effect of RAS blockade may be imperceptible in normal or uncomplicated subjects [32], but it is exaggerated when bone marrow requires all available stimuli to augment erythropoiesis, such as in patients with CKD with or without diabetes, heart failure or immunosuppression

Reference, year	Number of patients	Hct pre, %	Hct post, %	Clinical condition	Drug	Duration, months
Kamper and Nielsen [38], 1990	27	36.7	32.4	Adults with CKD	Enalapril	3
Vlahakos et al. [62], 1991	10	42.0	33.0	Renal transplant recipients	Enalapril	3
Gaston et al. [63], 1991	12	57.0	47.0	Post-transplant erythrocytosis	Enalapril	9
Herrlin et al. [64], 1991	8	42.6	39.0	Stable moderate heart failure	Enalapril	3
Barr et al. [65], 1997	41	41.4	37.7	Early heart failure	Enalapril	12
Mazzali and Filho [66], 1998	27	56.0	46.0	Post-transplant erythrocytosis	Enalapril	3
Vlahakos et al. [35], 2001	9	56.0	46.0	COPD with polycythemia	Losartan	1
Plata et al. [33], 2002	13	63.5	56.8	Altitude polycythemia	Enalapril	12
Iodice et al. [67], 2003	10	43.8	41.4	Nonnephrotic proteinuria; normal renal function	Ramipril	4
Jacobsen et al. [42], 2003	20	41.1	37.7	Adults with T1DM	Benazepril and valsartan	8
Wuhl et al. [68], 2004	352	36.6	34.8	Children with CKD	Ramipril	6
Ogawa et al. [41], 2007	72	35.7	30.0	DKD	Temocapril and candesartan	12

RAS, renin angiotensin system; CKD, chronic kidney disease; T1DM, type 1 diabetes mellitus; DKD, diabetic kidney disease; Hct, hematocrit; COPD, chronic obstructive pulmonary disease.

at the same time may be associated with a worse deterioration of haemoglobin concentration. A 24-week study in patients with DKD, revealed that the group receiving a combination of temocapril with candesartan had a more intense anti-proteinuric effect and at the same time a more profound drop in hematocrit values from $35 \pm 4.7\%$ at baseline to $30 \pm 4.6\%$ at the end of the study, compared to the group receiving a combination of temocapril with amlodipine, whose hematocrit values dropped from $36.7 \pm 3.6\%$ at baseline to $34.3 \pm 4.5\%$ at the end of the study [41]. Similar observations were reported by Jacobsen et al. [42] in patients with T1DM treated with benazepril, valsartan or their combination. Benazepril and valsartan were equally effective in blocking RAS (PRA increased from $30 \mu\text{U/L}$ during placebo to 161 and $163 \mu\text{U/L}$ respectively) and in decreasing haemoglobin concentrations (from 8.5 mmol/L with placebo to 8.2 and 8.1 mmol/L respectively), whereas dual blockade by benazepril and valsartan achieved a better RAS blockade (PRA increased to $331 \mu\text{U/L}$), but a worse drop of haemoglobin to 7.8 mmol/L .

The SGLTs and Erythropoiesis

The Role of SGLTs in EPO Secretion

In normal physiology, the kidneys play an important role in the regulation of glucose homeostasis via glucose utilization, glucose production and glucose reabsorption. The kidneys take $\sim 10\%$ of all glucose utilized by the body and along with the liver are the only organs that are able to release glucose through gluconeogenesis [43]. The contribution of kidneys to overall gluconeogenesis accounts for 20–25% [44]. Regarding glucose reabsorption, glucose is freely filtered in the Bowman's space and is returned to the circulation via 2 SGLTs, specifically SGLT-2 (which contributes to 90% of all glucose reabsorbed) and SGLT-1 (10% contribution) both located in the brush border of the proximal convoluted tubules. Renal tubular reabsorption of glucose is an active process, and if their capacity is exceeded, the transporters are unable to reabsorb the excess filtered glucose and glucosuria occurs.

In a healthy person, with plasma glucose levels $\sim 100 \text{ mg/dL}$ and a glomerular filtration rate of 125 mL/min ,

Table 2. A modest increase in hematocrit values by 2–4% points is consistently observed following administration of all 6 gliflozins (empagliflozin, canagliflozin, dapagliflozin, ipragliflozin, ertugliflozin and sotagliflozin)

Reference, year	Number of patients	Hct pre, %	Hct post, %	Clinical condition	Drug	Duration, months
Zinman et al. [69], 2015	7,020	41.20	46.00	T2DM at high CV risk	Empagliflozin	30
Januzzi et al. [70], 2017	450	41.00	43.00	Older subjects with T2DM	Canagliflozin	25
Kimura et al. [52] 2018	31	39.10	41.70	T2DM and hypertension	Ipragliflozin	3
Maruyama et al. [56], 2019	9	37.10	40.40	T2DM with anemia	Canagliflozin	3
van Raalte et al. [71], 2019	1,049	42.00	44.00	T1DM	Sotagliflozin	13
Gallo et al. [72], 2019	199	42.12	45.13	T2DM	Ertugliflozin	3
van Bommel et al. [73], 2020	24	40.70	42.50	T2DM	Dapagliflozin	3

T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; Hct, hematocrit; CV, cardiovascular.

180 g of glucose are filtered and reabsorbed daily. In T1DM and T2DM individuals, hyperglycaemia increases filtered glucose load. For example, in a subject with diabetes and average plasma glucose level of 200 mg/dL and hyperfiltration (glomerular filtration rate 160 mL/min), a huge amount of glucose (~460 g) is filtered and reabsorbed daily [45]. The molecular mechanism responsible for increased renal glucose reabsorption during hyperglycaemia involves an increase in the expression of glucose transporter genes in the proximal tubule. Renal tubular cells isolated from the urine of subjects with T2DM have an increase in the expression of SGLT2 mRNA and protein and demonstrate enhanced capability of glucose transport [46]. The local high-glucose environment in renal tubulointerstitium due to the increased glucose reabsorption by SGLTs in diabetes may directly and indirectly damage REPs and diminish EPO production (Fig. 1). First, high-glucose concentration can impair hypoxia-dependent protection of HIF-1 against proteasomal degradation and interferes with cellular responses to hypoxia in diabetic patients [47]. Second, hyperglycaemia can stimulate renal tubular epithelial cells to produce pro-inflammatory molecules that could negatively affect the neighbouring peritubular REPs. Incubation of human renal proximal tubular epithelial cells (HK-2) in high glucose concentration activates the p38 mitogen-activated protein kinases pathway and the nuclear factor- κ B, and increases the production of pro-inflammatory cytokines, such as tumour necrosis factor- α , interleukin-1 (IL-1) and IL-6 [48]. Both IL-1 and tumour necrosis factor- α were found capable to suppress EPO gene expression in isolated perfused rat kidneys and in human hepatoma cell

cultures, whereas IL-6 can inhibit EPO synthesis in the kidney [49].

SGLT2 Inhibitors Are Associated with Increased EPO Secretion and Hematocrit Values

As shown in Table 2, a significant rise of haemoglobin concentration and hematocrit values has been consistently noted in patients treated with various SGLT inhibitors, even in patients without diabetes. In the beginning, these phenomena were thought to be initiated by the diuretic action of SGLT-2 inhibitors causing hemoconcentration, but this does not seem to be the only explanation. First, in a pioneer trial with T2DM, treatment with dapagliflozin was associated with a reduction in plasma volume of approximately 7% along with an increase in plasma renin activity and serum aldosterone levels. Of particular interest, however, in the same trial, a true augmentation of erythropoiesis was revealed in the dapagliflozin group, as evidenced by transient elevations in serum EPO levels and reticulocyte counts that preceded the rise of hematocrit by 2.2% points, haemoglobin by 0.6 g/dL and red blood cell mass by 6% [50]. In another study, urine volume increased within 24 h after the initiation of treatment with SGLT-2 inhibitors, but returned to baseline within a week. The body fluid status, as measured by total and extracellular water and reflected in renin and aldosterone levels remained stable thereafter [51]. Second, more potent diuretics, such as thiazide and loop diuretics are not associated with sustained increase in hematocrit values despite decreasing body fluid volume. In fact, switching patients from thiazide diuretics to the SGLT-2 inhibitor ipragliflozin was accompanied by an

increase in haemoglobin concentration from 12.9 ± 1.6 to 13.6 ± 1.8 g/dL and hematocrit values from 39.1 ± 4.3 to $41.7 \pm 4.9\%$ points after 3 months [52]. Finally, diuretics of loop of Henle, distal tubule and collecting duct (furosemide, hydrochlorothiazide, and amiloride, respectively) do not seem to affect EPO production, whereas diuretics acting predominantly at the proximal tubular site, such as acetazolamide, significantly reduce the EPO production in response to hypoxia in mice [53].

In comparison, SGLT2 inhibitors seem to actively stimulate the erythropoietic process. For example, in a randomized controlled trial comparing the effects of dapagliflozin and DPP-4 inhibitors in T2DM, a significant elevation of haemoglobin concentration and hematocrit values was noted with dapagliflozin treatment. EPO levels increased after initiation of dapagliflozin treatment and reached a plateau in 2–4 weeks. The reticulocyte count increased simultaneously, followed later on by the elevation of haemoglobin and hematocrit levels [54]. In a sub-study of the EMPA-HEART CardioLink-6 randomized clinical trial individuals with T2DM and stable coronary artery disease were randomized to either empagliflozin 10 mg daily or placebo for 6 months. EPO levels increased significantly after 1 month of empagliflozin treatment. Hematocrit increased after 6 months by 2.34% points, associated with reduced ferritin levels and mean cell haemoglobin concentration [55]. Maruyama et al. [56] studied 9 subjects with diabetes administered canagliflozin 100 mg qd for 12 weeks on top of standard treatment with conventional anti-diabetic drugs and RAS inhibitors. Serum EPO concentration increased by 38% between baseline and 2–4 weeks. Reticulocyte count transiently increased at 2 weeks followed by increased haemoglobin concentration (from 11.8 ± 0.6 g/dL at baseline to 12.9 ± 1.1 g/dL, at study completion, $p = 0.0049$), hematocrit values (from 37.1 ± 2.3 at baseline to $40.4 \pm 3.2\%$ points, at study completion, $p = 0.002$) and decreased serum ferritin levels ($p = 0.003$).

The mechanism(s) by which SGLT2 inhibitors augment erythropoiesis have not yet been fully elucidated. It is possible that the reduced glucose reabsorption in the proximal tubules by the action of SGLT2 inhibitors decreases high-glucose accumulation in the renal tubulointerstitial micro-environment and attenuates glucotoxicity on various cells including REPs. Terami et al. [57] showed that dapagliflozin decreased macrophage infiltration and oxidative stress into the kidney of db/db mice in a dose-dependent manner. Shin et al. [58] showed that dapagliflozin decreased the expression of tissue oxidative stress markers, inflammatory cell infiltration, mesangial widening, inter-

stitial fibrosis and total collagen content in diabetic rats. In vitro work in human proximal tubular cells suggests that the SGLT-2 inhibitor, empagliflozin, reduces inflammatory and fibrotic markers induced by hyperglycaemia and may limit glucose-induced damage of the proximal tubule. The specific mode of action is thought to be blockage of glucose entry into the cell [59]. Finally, SGLT2 inhibitors could increase erythropoiesis by suppressing hepcidin production and modulating other iron regulatory proteins to facilitate iron absorption and utilization [60].

Synthesis and Conclusions

The competing nature of erythropoiesis in subjects with diabetes treated with RAS blockade and SGLT2 inhibitors emanates from the fact that RAS activation and avid glucose reabsorption by SGLTs exert opposing effects on renal tubulointerstitium. RAS activation that accompanies diabetes and in particular, Ang II augments erythropoiesis by causing tubulointerstitial ischemia, increasing EPO secretion, acting directly on erythroid progenitors, as growth factor and reducing hepcidin to facilitate iron absorption and utilization. On the other hand, activated SGLTs during hyperglycaemia in subjects with diabetes increase renal glucose reabsorption resulting in a local high-glucose tubulointerstitial environment, which directly and indirectly damage REPs and decrease EPO secretion. The opposite sequence of events is expected when inhibitors of RAS or SGLT2 are administered. RAS inhibitors are associated with a drop in EPO levels and a hematocrit-lowering effect or even anaemia, which actually carries an ominous and independent prognostic role in cardiac patients. For instance, in the Val-HeFT trial, an increased risk of death was found in the lower 2 compared to the upper 2 haemoglobin quartiles [61]. By contrast, SGLT2 inhibitors are associated with increased EPO secretion and a consistent elevation in hematocrit values and haemoglobin concentrations in patients already treated with RAS inhibitors. This beneficial effect might have important clinical implications, as revealed in a recent post hoc analysis of the EMPA-REG OUTCOME study, where the elevation of hematocrit values following empagliflozin treatment in patients with T2DM and coronary artery disease seemed to account for over half of the mortality benefit in comparison to placebo [55]. The relative contribution of each system to erythropoiesis remains to be revealed in future studies, in whom ACEi/ARB will be added in patients on SGLT2 inhibitors, or SGLT2 inhibitors will be administered on top of RAS blockade in a random or-

der. It is worth mentioning, however, that in all major outcome studies with SGLT2 inhibitors, beneficial cardiorenal effects were observed in the study group, even if >80% of participants were already treated with ACEi/ARB.

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