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EXPERT OPINION

Possibility of enhanced risk of retinal neovascularization in repeated blood donors: blood donation and retinal alteration

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Abstract: Repeated blood donors manifest clinical, subclinical, and biochemical signs of iron deficiency anemia, have significantly higher erythropoietin and vascular endothelial growth factor (VEGF) concentrations, and decreased tissue oxygen saturation, oxygenated tissue hemoglobin, and regional cerebral oxygen saturation. Erythropoietin and VEGF are potent retinal angiogenic factors which may initiate and promote the retinal angiogenesis process independently or simultaneously. Increases in circulating levels of erythropoietin and VEGF are proportionate to the levels of hematocrit, hypoxemia, and tissue hypoxia. It is suggested that higher erythropoietin production following iron deficiency anemia-induced chronic hypoxemia/hypoxia may, hypothetically, enhance the risk of retinal angiogenesis and/or neovascularization, possibly by inducing hypoxia inducible factor-1 alpha, which consequently upregulates genes stimulating angiogenesis, resulting in formation of a new vasculature, possibly by modulation of signal transducer and activator of transcription 3 signaling in the retina. Implications of this hypothesis cover erythropoietin doping, chronic hypoxia, and hypoxemic situations, such as angiogenesis-related cardiac and pulmonary diseases.

Keywords: repeated blood donation, erythropoietin, retinal neovascularization, vascular endothelial growth factor, hypoxia

Introduction

The need for blood products is constant and unremitting.[1] Only a small percentage of eligible individuals answer the appeal to donate. However, there is a subgroup of people who donate blood repeatedly. High demand for blood may have led to a bias towards investigations analyzing and reporting beneficial effects of blood donation, such as reduced risk of myocardial infarction,[2,3] improved rheological properties,[4,5] and blood lipid-lowering effects.[6] Overstressing any beneficial health effect might trigger the eagerness of blood donors, especially considering the fact that in some countries these people are appreciated in many ways (for example, being shown on television). This may spark an extreme desire to donate blood repeatedly among multitime donors. Also, blood donors might be categorized to include healthy people and ill people who, for whatever reason, think they should or have been advised to donate blood. For example, there is a widespread belief that blood donation improves blood coagulation in diabetics and in smokers. While these ideas might be true, this should not lead to a situation where the decision to donate blood becomes obsessive.

It would not be surprising if a disease with low prevalence but high severity, such as retinal angiogenesis and/or retinal neovascularization, was underdiagnosed in a very small group of repeated blood donors if there were no structured items included

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in a donor medical questionnaire, or if ophthalmological observations are not considered when providing preliminary data to be analyzed prospectively or retrospectively. Importantly, this is likely to happen because there is currently no hypothesis proposing a link between them.

To the best of the author's knowledge, any detrimental effects of repeated blood donation on erythropoiesis counterbalance the risk of induced retinal hypoxia, and its subsequent possible effects on retinal angiogenesis and/or retinal neovascularization in humans have been overlooked to date. This review suggests that repeated blood donors might be physiologically at increased risk of ocular alterations. This personal view may have relevant applications in other angiogenesis-related disorders. If this theory is proven, a simple and inexpensive prophylactic measure can be provided for multitime blood donors. Also, eligibility and deferral criteria for this special group should be revisited if proven. An extensive search for evidence concerning a possible relationship between repeated blood donation and retinal alteration included the CENTRAL, MEDLINE, EMBASE, CINAHL, ERIC, Informit, and JST databases, as well as gray literature and trial registries from inception to May 2011. Key search terms included "carcinogenesis", "angiogenesis", "vascularization", "retina", "blood donation", "erythropoietin", "vascular endothelial growth factor", and "hypoxia inducible factor-1 alpha".

Erythropoietin, blood donation, and the retina

The discovery that erythropoietin and its receptor play a significant biological role in tissues outside of the hematopoietic system has fueled significant interest in erythropoietin as a novel cytoprotective agent in both neuronal and vascular systems.[7] In addition to the kidney, which is the main site of production in the adult, additional organs and cells, including the liver, uterus, endothelial cells, vascular smooth muscle cells, and insulin-producing cells have been found to secrete erythropoietin.[7,8] The erythropoietin receptor (EPOR) is expressed by a variety of cells, including neurons, microglia, astrocytes, and cerebral endothelial cells, myelin sheaths on human peripheral nerves, and the neural retina of the eye.[9–11]

EPOR is a member of the type 1 cytokine receptor family which modulates cell growth, apoptosis, and differentiation.[12,13] Erythropoietin shows angiogenic activity in EPOR-expressing vascular endothelial cells, which stimulate proliferation, migration, and angiogenesis.[14] Furthermore, EPOR is upregulated in a variety of solid malignant neoplasms.[13,15] In the pterygium stroma, a variety of endothelial cells forming vascular cavities has shown cytoplasmic immunoreactivity for EPOR. In normal conjunctival epithelium, a few basal cells showed weak homogeneous immunoreactivity for EPOR in the cytoplasm. The number of EPOR-expressing epithelial cells was much higher in the pterygium compared with the normal conjunctiva. EPOR expression was marginally detected in stromal microvessels of the normal conjunctiva. However, immunoreactivity for erythropoietin was not noted in the pterygium epithelium and stroma, or in normal conjunctiva, suggesting that the erythropoietin-independent EPOR-signaling pathway plays a potential role in cell proliferation and angiogenesis in the human pterygium.[16]

In healthy humans, erythropoiesis counterbalances the continuous removal of aged red blood cells. Erythropoietin is the main growth factor responsible for regulation of red blood cell production in steady-state conditions and for enhancing the rate of production of red blood cells whenever blood loss or hemolysis occurs, and is considered to be the main hormone that controls erythropoiesis.[17,18] For example, small decreases in hematocrit as would be typical after a situation such as presurgical autologous blood donation often do not result in increased erythropoietin levels or in compensatory erythropoiesis.[19] However, this might be different in the case of repeated blood donation and/or phlebotomy.

The effect of repeated phlebotomy on serum immunoreactive erythropoietin levels studied prospectively in autologous blood donors has shown an increase in the level of serum immunoreactive erythropoietin with successive phlebotomies.[20] Maeda et al evaluated endogenous serum erythropoietin levels in normal subjects after a single 400 mL phlebotomy. The subjects were followed up for 56 days. The hemoglobin values of both males and females decreased to a nadir on days 3–7 post-phlebotomy. Hemoglobin values gradually increased, but did not completely recover to pre-phlebotomy levels by day 56. Serum erythropoietin levels increased at six hours post-phlebotomy to 20.1 ± 5.4 mU/mL in males and to 20.7 ± 7.0 mU/mL in females from pre-phlebotomy levels of 14.6 ± 4.0 mU/mL in males and 13.4 ± 4.1 mU/mL in females, respectively.[21]

Duda et al studied the effect of exercise performed before and 24 hours after withdrawal of 450 mL of blood on serum erythropoietin levels in 12 male subjects aged 23.2 ± 2.6 years with a body mass index of 23.6 ± 2.1 kg/m^2 and $VO_{2\ max}$ of 2937 ± 324 mL/min. Withdrawal of 450 mL of blood within 24 hours significantly increased the serum erythropoietin concentration. The subjects performed an

incremental exercise test until exhaustion twice, separated by a period of about 7–10 days. The second test was performed 24 hours after withdrawal of 450 mL of blood. In the control study, no effect of incremental exercise on serum erythropoietin concentration was seen, which amounted to 14.24 ± 7.66 mU/mL at rest and 14.97 ± 6.07 mU/mL at the end of the incremental test. During the experiment performed 24 hours after withdrawal of 450 mL of blood, the serum erythropoietin concentration at rest was significantly elevated ($P < 0.01$) in relation to the control measurement (amounting to 24.85 ± 13.60 mU/mL) and, at the end of incremental exercise, a tendency towards further elevation ($P = 0.09$) in erythropoietin concentration up to 28.32 ± 14.51 mU/mL was observed.[22]

In another study, Bofill et al investigated whether the presence of type 2 diabetes mellitus influences the erythropoietin response to repeated phlebotomies in normal subjects. Subjects were aged 43–79 years, with a mean age of 64.8 ± 8.8 years in diabetic patients and 65.0 ± 6.1 years in controls. As a group, the diabetic patients donated a mean of 2.71 ± 0.70 (range 1–4) blood units and control patients donated a mean of 3.14 ± 0.65 (range 2–5) blood units. These differences were statistically significant ($P < 0.05$), representing 12.1% less blood being predonated in diabetic patients. Serum erythropoietin levels in controls increased uniformly between 1.5-fold and 3.2-fold from initial values, but changes in diabetic patients ranged from no increase in four patients to a 12-fold increase in one patient.[23]

Higher whole blood and plasma viscosity has been correlated with several ocular disorders, including age-related macular degeneration,[24,25] retinal vein thrombosis,[26] and retinal neovascularization.[24,26,27] In a study by Meada et al,[21] erythropoietin levels after a single 400 mL phlebotomy continued to increase to peak levels of 25.5 ± 6.3 mU/mL in males and 28.7 ± 11.5 mU/mL in females on days 7–14 and thereafter decreased until day 56.

What does this observation suggest? Although blood donation has been shown to reduce viscosity,[28] it is possible that successive blood donations chronically increase erythropoietin levels and upregulate EPOR. Although it is usually considered that blood donation improves blood viscosity, this might be an immediate effect, and longitudinal data are needed to compare single versus repeated blood donation. However, considering the enhancing effect of blood donation on serum erythropoietin until day 56, it is possible that blood viscosity might actually be increased in the long term, and thus interactions with different severity in different people in different physiologic or pathologic situations would be likely. This view by no means negates the possibility that elevated erythropoietin might trigger retinal angiogenesis without modulating blood viscosity. Trials are warranted to compare erythropoietin levels in first-time blood donation versus repeated blood donation.

The author knows of no direct evidence of higher EPOR expression in repeated blood donors. However, a study in children with acute lymphoblastic leukemia showed that ectopic expression of ETV6/RUNX1 induced upregulation of EPOR. However, anemia did not appear to influence EPOR expression on leukemic cells, although children with ETV6/RUNX1-positive leukemias had a lower median hemoglobin than controls.[29] It remains to be elucidated whether repeated blood donation upregulates EPOR expression in the human retina.

VEGF, blood donation, and the retina

VEGF is expressed in human retina and choroid[30] as well as sickle cell and choroid,[31] and its expression precedes retinal neovascularization in the retina and optic nerve.[32] VEGF mediates tissue hypoxia-induced vasculoangiogenesis, hematopoiesis,[33] and erythropoiesis.[34] Low hemoglobin is associated with increased serum levels of VEGF in cancer patients,[35] and it has been suggested that anemia might increase the progression of angiogenesis in malignant and benign tumors.[35,36]

Kawamura et al examined the effect of controlled phlebotomy on blood flow in an ischemic mouse leg model, in which 200 μL of blood were drawn from the tail vein once a week. After four weeks, blood flow in the ischemic leg was significantly better in the phlebotomy group, and capillary density was significantly higher. Repeated phlebotomies increased serum erythropoietin levels, as well as expression of hypoxia inducible factor-1 alpha (HIF-1α) and VEGF, and both expression and activity of Akt and endothelial nitric oxide synthase in ischemic legs. Repeated phlebotomies resulted in increased blood flow in ischemic legs via an angiogenic action that involved the Akt/endothelial nitric oxide synthase pathway, endothelial progenitor cell mobilization, and their complicated cross-talk.[37]

Hypoxia/hypoxemia, blood donation, and the retina

Iron homeostasis alterations leading to hypoxia has been implicated in the regulation of VEGF transcription.[38] Production of VEGF and basic fibroblast growth factor is stimulated in hypoxic patients with exacerbated chronic

obstructive pulmonary disease, and elevated levels of VEGF and basic fibroblast growth factor activate the process of neoangiogenesis.[39] VEGF and basic fibroblast growth factor augment proliferation of retinal pigment epithelium (REP) and pericytes, especially under hypoxia, and it has been proposed that these two cytokines have a synergistic effect at several stages of angiogenesis in the retina.[40]

Systemic hypoxemia (lung or heart disease) or a vascular disease of the retina can cause retinal hypoxia. Oxygen plays the key role in stabilizing HIF-1α and its function. When the oxygen tension is normal, HIF-1α is rapidly oxidized by hydroxylase enzymes, but when cells become hypoxic, HIF-1α escapes degradation and starts to accumulate, triggering activation of a large number of genes, including VEGF and erythropoietin. HIF-1α has been shown both clinically and experimentally to have a mediating or contributing role in several oxygen-dependent retinal diseases, and this subject has been reviewed elsewhere.[41]

Jeger et al investigated whether clinically relevant blood loss of 500 mL from healthy volunteers can be detected by changes in tissue oxygen saturation after a standardized ischemic event. They performed occlusion of the brachial artery for three minutes in 20 healthy female blood donors before and after blood donation. Tissue oxygen saturation and total oxygenated tissue hemoglobin were measured continuously at the thenar eminence. Ten healthy volunteers were assessed in the same way to examine whether repeated vascular occlusion without blood donation exhibits time-dependent effects. Blood donors had a median age of 30.5 (range 19–62) years. Their median body mass index was 21 (range 18–23.5) kg/m^2. Median blood volume was estimated to be 70 mL/kg body weight or 4060 (range 3500–4550) mL. Median capillary hemoglobin was 139 (range 127–157) g/L. This was measured once immediately after arrival at the blood donation service. In the control group, the median age was 23 (range 22–25) years. Median body mass index was 21 (range 19–23) kg/m^2. Median blood volume was 4165 (range 3500–4480) mL. Blood donation caused a substantial decrease in systolic blood pressure, but did not affect resting tissue oxygen saturation and oxygenated tissue hemoglobin values. No changes were seen in the blood donor group with regard to the vascular occlusion test, but in the control group there was an increase in the oxygenated tissue hemoglobin rate of recovery during the reperfusion phase. The authors concluded that tissue oxygen saturation measured at the thenar eminence is insensitive to blood loss of 500 mL, but blood loss greater than this would probably lead to detectable changes.[42]

Because the retina is a part of the neural system, it would be interesting to consider the effect of blood donation on regional cerebral oxygenation and cerebral blood volume. In 50 healthy blood donors who donated 450 mL of whole blood within 4–9 minutes, changes in regional cerebral oxygen saturation and cerebral tissue hemoglobin concentration were measured. Within the study group, regional cerebral oxygen saturation decreased by 0.44% ($P < 0.01$) on average during blood donation, which is still within the range of individual physiologic baseline variation. The average venous hemoglobin concentration decreased significantly by 4.6%, whereas cerebral tissue hemoglobin concentration increased significantly by 2.5% and cerebral blood volume by 7.%. An increase in cerebral blood volume indicates cerebral vasodilation, which seems to be the major compensatory mechanism during acute blood loss. The decrease in regional cerebral oxygen saturation was relatively small, indicating that cerebral oxygenation was maintained within the physiologic range.[43] However, it is noteworthy that a state of anemia and iron deficiency exists in repeated blood donors.[44,45] Iron deficiency may thus enhance expression of HIF-1α,[46–49] which consequently upregulates genes stimulating angiogenesis, resulting in the formation of a new vasculature in the retina.[50,51]

With regard to how hypoxia may be causally related to iron deficiency, Smith et al investigated whether increasing or decreasing iron availability modifies altitude-induced hypoxic pulmonary hypertension. They conducted two randomized, double-blind, placebo-controlled trials. In the first one, 22 healthy men aged 19–60 years resident at sea level were studied over one week of hypoxia at an altitude of 4340 m. In the second study, 11 men aged 30–59 years resident at high altitude and diagnosed with chronic mountain sickness were studied over one month of hypoxia at the same altitude. In the first protocol, participants received intravenous infusions of Fe(III)-hydroxide sucrose 200 mg or placebo on the third day of hypoxia. In the second protocol, patients underwent staged isovolemic venesection of 2 L of blood. Two weeks later, the patients received intravenous infusions of Fe(III)-hydroxide sucrose 400 mg or placebo, which were subsequently crossed over. In the sea-level residents, approximately 40% of the pulmonary hypertensive response to hypoxia was reversed by infusion of iron, which reduced pulmonary artery systolic pressure by 6 mmHg (95% confidence interval [CI] 4–8 mmHg), from 37 mmHg (95% CI 34–40 mmHg) to 31 mmHg (95% CI 29–33 mmHg; $P = 0.01$). In the chronic mountain sickness group, progressive iron deficiency induced by venesection was associated

with an approximately 25% increase in pulmonary artery systolic pressure of 9 mmHg (95% CI 4–14 mmHg), from 37 mmHg (95% CI 30–44 mmHg) to 46 mmHg (95% CI 40–52 mmHg; $P = 0.003$). It was concluded that hypoxic pulmonary hypertension may be attenuated by iron supplementation and exacerbated by iron depletion.[52]

The amount of blood donated by multitime donors is higher than by first-time blood donors, and both direct and indirect evidence[42,43,52] suggests that there might be a level of tissue hypoxia in multitime donors because of a chronic state of anemia.

Anemia, erythropoietin, and VEGF

Increases in circulating levels of erythropoietin are proportional to the levels of tissue hypoxia, which are influenced by hematocrit,[53] and there are several papers reporting that anemic patients have elevated levels of VEGF, which is a marker of tissue hypoxia.[36,54] In a prospective study, plasma VEGF levels were measured prospectively in three groups of infants suspected of requiring red blood cell transfusions to find a vascular endothelial growth factor cutoff value indicative of tissue hypoxia. The three groups were acutely anemic (an episode of acute bleeding [hematocrit drop $>$ 5%] per day), chronically anemic (hematocrit drop $<$ 5% per day), and nontransfused (hematocrit drop $<$ 5% per day) but not meeting the clinical criteria for a transfusion. VEGF concentrations were lower in the acutely anemic infants than in the chronically anemic infants, but erythropoietin levels did not differ between these groups. The VEGF concentration was $<$140 pg/mL in all acutely anemic infants, and this was deemed to be the threshold level indicating sufficient tissue oxygenation in subsequent analysis. Interestingly, 30% of the chronically anemic infants had VEGF levels $>$ 140 pg/mL.[54]

In patients with untreated locoregionally confined solid cancers of the head and neck, cervix, rectum, and lung, and 59 additional patients without malignant disease (36 nonanemic patients without serious disease and 23 patients with renal anemia) it was shown that plasma levels of VEGF were 16.2 ± 12.7 pg/mL in 36 nonanemic patients without malignant disease, 49.2 ± 34.5 pg/mL in 49 patients with cancer ($P < 0.001$), and 89.9 ± 67.8 pg/mL in 23 patients with renal anemia ($P = 0.001$). VEGF levels in cancer patients were correlated significantly with hemoglobin levels. Patients with cancer had higher plasma levels of VEGF than patients without malignant disease if hemoglobin was ≥12 g/dL (33.1 ± 17.5 pg/mL versus 16.6 ± 13.0 pg/mL, $P < 0.001$) than if hemoglobin was 11.0–11.9 g/dL (56.1 ± 26.4 pg/mL versus 18.5 ± 14.5 pg/mL, $P = 0.038$). If hemoglobin was $<$11 g/dL, plasma VEGF levels were significantly elevated in patients with and without cancer (67.0 ± 47.5 pg/mL versus 88.9 ± 68.8 pg/mL). A significant association between low hemoglobin levels and increased plasma levels of VEGF was confirmed. In patients with renal anemia, changes in hemoglobin under erythropoietin treatment were inversely correlated with changes in plasma VEGF levels, with decreasing VEGF after an increase in hemoglobin ($P = 0.01$).[36]

Erythropoietin and VEGF interplay with retinal angiogenesis and retinopathy

It was recently shown that anemia of the newborn induces erythropoietin expression in the developing mouse retina.[55] Erythropoietin mRNA expression levels in the retina are greatly elevated during the hypoxia-induced proliferation phase of retinopathy in the mouse.[56] A paper by Sato et al[57] confirms that the expression of ocular VEGF and erythropoietin are temporally linked in humans. In fact, expression of erythropoietin and VEGF mRNA is regulated by the same transcription factor, ie, HIF-1α, that binds to the *cis*-acting hypoxia-response element located in the 3′-flanking region of the human erythropoietin and VEGF gene.[58] Like the proteins regulated by HIF-1α, HIF-1α activity can be protective at the right time and destructive at the wrong time. The right time of expression is when capillaries are present that are healthy enough to be protected by VEGF and erythropoietin. The wrong time is when ischemia is permanent, because the ischemic tissue is already expressing too much of these proteins.[59]

Takagi et al investigated the potential role of erythropoietin during retinal angiogenesis in proliferative diabetic retinopathy. The vitreous EPO level in patients with proliferative diabetic retinopathy was significantly higher than that in nondiabetic patients. Erythropoietin and VEGF were both independently associated with proliferative diabetic retinopathy, and erythropoietin was more strongly associated with proliferative diabetic retinopathy than VEGF. Blockade of erythropoietin inhibited retinal neovascularization in vivo, and inhibited endothelial cell proliferation response to proliferative diabetic retinopathy vitreous in vitro. Their data provide strong evidence that erythropoietin is a potent retinal angiogenic factor independent of VEGF, and is capable of stimulating ischemia-induced retinal angiogenesis in proliferative diabetic retinopathy.[60]

Measurement of both erythropoietin and VEGF levels in the vitreous fluid of diabetic patients with proliferative

diabetic retinopathy has been shown to be much higher than that in patients without diabetes (464.0 mI/mL versus 36.5 mI/mL, $P < 0.001$). The median VEGF level in patients with retinopathy was also significantly higher than that in patients without diabetes (345.0 pg/mL versus 3.9 pg/mL, $P < 0.001$). Erythropoietin and VEGF were independently associated with proliferative diabetic retinopathy, and erythropoietin was more strongly associated with the presence of proliferative diabetic retinopathy than was VEGF. Erythropoietin and VEGF gene expression levels are upregulated in the murine ischemic retina, and blockade of erythropoietin inhibits retinal neovascularization in vivo and endothelial cell proliferation in the vitreous of patients with diabetic retinopathy in vitro.[61] Erythropoietin is a potent ischemia-induced angiogenic factor that acts independently of VEGF during retinal angiogenesis in proliferative diabetic retinopathy.

Chen et al investigated the inhibition of retinal erythropoietin mRNA expression with RNA interference as a potential strategy to suppress retinal neovascularization and to prevent proliferative retinopathy. They used a mouse model of oxygen-induced retinopathy. A small interference RNA (siRNA) targeting erythropoietin or control negative siRNA was injected intravitreally at postnatal days 12, 14, and 15 during the hypoxic phase, and the effect on neovascularization was evaluated in retinal flat mounts at postnatal day 17. Retinal erythropoietin mRNA expression in the total retina was suppressed during the initial phase of vessel loss in retinopathy and was significantly elevated during the hypoxia-induced proliferative phase in all three neuronal layers in the retina, corresponding to an increased level of retinal hypoxia. EPOR mRNA expression levels also increased during the second neovascular phase, specifically in hypoxia-induced neovascular vessels. Intravitreous injection of erythropoietin siRNA effectively inhibited approximately 60% of retinal erythropoietin mRNA expression and considerably suppressed retinal neovascularization by approximately 40%.[62]

Evidence of retinal findings in anemic and hypoxic situations

In two prospective case series, it has been shown that retinal vascularization is affected by maternal anemia,[63] neonatal anemia,[63] and the need for oxygen for more than 48 hours.[63,64] There are mounting case reports of ocular alterations in anemic or hypoxemia situations, such as retinal vasculopathy,[65] peripheral retinal neovascularization in Fanconi anemia,[66] sickle cell hemoglobin C retinopathy,[67] or idiopathic polypoidal choroidal vasculopathy and sickle cell retinopathy,[68] branch retinal artery occlusion,[69] retinal cotton wool spots and preretinal hemorrhages,[70] unusual morphological features of intraretinal and preretinal neovascularization and of chorioretinal lesions in sickle cell retinopathy,[71] peripheral retinal neovascularization in sarcoidosis and sickle cell anemia,[72] and central retinal vein occlusion and nonarteritic ischemic optic neuropathy.[70] Blood et al described a retinopathy secondary to anemia from myeloid metaplasia in polycythemia vera.[73] It is noteworthy that Fanconi anemia is characterized by high levels of serum erythropoietin as well as serum ferritin.[74,75] A full review of numerous case reports of retinal alterations in anemia would be beyond the scope of this paper, and interested readers may refer to medical sources.

Relationship between erythropoietin and retinopathies

Diabetic retinopathy

The retinal angiogenic potential of erythropoietin in humans exacerbates proliferative diabetic retinopathy.[76,77] Diskin et al examined whether severity and progression of diabetic retinopathy could be accelerated by administration of rhEPO to patients with chronic renal failure. There was significantly greater deterioration of retinopathy after one year in the patients who had received erythropoietin ($P = 0.004$). The prevalence and severity of proliferative retinopathy appeared to have increased and was most closely associated with erythropoietin dosing.[76] The erythropoietin vitreous concentration in the patients with proliferative diabetic retinopathy (512 [range 120–880] mU/mL) was significantly higher than in the patients with retinal detachment, preretinal macular membranes, and macular holes (25.1 [range 5.2–201] mU/mL, $P < 0.001$).[78] Also, the vitreous erythropoietin level was upregulated in eyes with proliferative vitreoretinopathy.[79]

Retinopathy of prematurity

Both early erythropoietin[80] and late erythropoietin[81,82] treatment increases the risk of retinopathy of prematurity. There is a consensus that rhEPO is a significant independent risk factor for the development of retinopathy of prematurity.[57,80–87] It has been suggested that the timing of rhEPO treatment during different stages of retinopathy is likely critical for determining its beneficial or destructive role in retinopathy of prematurity,[88] and may explain the discrepancies seen in some studies.

Oxygen-induced retinopathy and neovascularization

Chen et al studied the role of erythropoietin in a mouse model of retinopathy characterized by oxygen-induced vascular loss followed by hypoxia-induced pathological neovascularization for the first time. Without treatment, local retinal erythropoietin levels were suppressed during the vessel loss phase. Administration of exogenous erythropoietin prevented both vessel dropout and subsequent hypoxia-induced neovascularization. Early use of erythropoietin also protected against hypoxia-induced retinal neuron apoptosis. In contrast, retinal erythropoietin mRNA levels were highly elevated during the retinopathy neovascular phase. Exogenous late erythropoietin treatment did not protect the retina, but rather enhanced pathological neovascularization. The early protective effect of erythropoietin occurred through both systemic retinal recruitment of proangiogenic bone marrow-derived progenitor cells and activation of prosurvival NF-κB via erythropoietin receptor activation on retinal vessels and neurons. Early retinal erythropoietin suppression contributed to retinal vascular instability, and elevated erythropoietin levels during the proliferation stage contributed to neovascularization and disease.[89]

It has been shown that the vitreous erythropoietin level is upregulated in eyes with rhegmatogenous retinal detachment.[77–79,90] Anterior segment ischemia is a dreaded complication of retinal detachment. Conditions of relative hypoxia, such as sickle cell anemia[91] and sickle cell hemoglobinopathy anemia,[92] may precipitate vaso-occlusive phenomena in retinal detachment. While tissue hypoxia secondary to the anemia in these patients with sickle cell anemia may play a role, it is also likely that direct vascular occlusion and endothelial damage from interaction with abnormal hemoglobin tactoids also play a major role in retinopathy.

Hypothesis linking repeated blood donation to retinal angiogenesis

Previous studies have shown that maternal anemia in early pregnancy influences the pattern of placental vascularization,[93] and that maternal iron deficiency anemia may trigger a cascade of pathophysiological processes, involving alterations in placental angiogenesis,[94] chronic placental hypoxemia, and oxidative stress apoptosis.[95] In adults with cyanotic congenital heart disease (n = 4, 27–47 years of age, mean systemic arterial oxygen saturation 77% [range 71%–81%] and mean hematocrit 64.5% [range 53.7%–69.5%]), retinal vascular tortuosity was increased, but no patient had ocular symptoms, and all eyes had good visual acuity. Increased retinal vascular tortuosity, which appears to be prevalent in adults with cyanotic congenital heart disease, is likely to be in response to hypoxemia and erythrocytosis because normalization of the retinal vascularity patterns after surgical relief of cyanosis resulted in resolution of hypoxemia and erythrocytosis.[96]

Shortt et al tested the hypothesis that chronic systemic hypoxia leads to angiogenesis in the adult retinal circulation in the absence of pre-existing vascular disease. Adult male Sprague-Dawley rats (n = 9) were exposed to a fraction of inspired oxygen of 0.10 for two weeks while control animals (n = 10) were exposed to room air. Chronic systemic hypoxia, in the absence of other pathological processes, caused angiogenesis in the adult rat retina and provided an in vivo model for investigating this important process in the adult retina, in particular pathways specific to this tissue.[97]

To provide a possible mechanism by which repeated blood donation might enhance the risk of retinal neoangiogenesis and/or neovascularization, it might be helpful to mention the results of a study that was carried out to investigate the mechanisms of adaptive response of the heart to long-term anemia induced by iron deficiency. Weanling Sprague-Dawley rats were fed an iron-deficient diet for 20 weeks to induce iron deficiency anemia. The iron-deficient diet initially induced severe anemia, which resulted in left ventricular hypertrophy and dilation with preserved systolic function associated with increased serum erythropoietin concentration. Cardiac signal transducer and activator of transcription 3 (STAT3) phosphorylation and VEGF gene expression increased by 12 weeks of iron deficiency anemia, causing angiogenesis in the heart. Thereafter, sustained iron deficiency anemia induced upregulation of cardiac HIF-1α gene expression and maintained upregulation of cardiac VEGF gene expression and cardiac angiogenesis. However, sustained iron deficiency anemia promoted cardiac fibrosis and lung congestion, with decreased serum erythropoietin concentration and cardiac STAT3 phosphorylation after 20 weeks of iron deficiency anemia compared with 12 weeks.[47]

STAT3 is an angiogenic factor and is expressed in the human retina. It has been shown that erythropoietin treatment may offer a unique dual-function strategy for neuroprotection and regeneration of retinal ganglion cells. Erythropoietin induced STAT3 phosphorylation in retinal ganglion cells, and inhibition of Jak2/STAT3 abolished erythropoietin-induced growth. Erythropoietin-facilitated neuritogenesis was paralleled by upregulation of Bcl-X, a Bcl-2 homolog capable of promoting retinal ganglion cell regeneration. The PI3 K/Akt pathway was also involved in antiapoptotic

and regeneration-enhancing erythropoietin actions.[98] Also, VEGF rapidly induces STAT3 tyrosine phosphorylation and nuclear translocation in retinal microvascular endothelial cells.[99] STAT3 expression has been shown to be enhanced in hypoxia.[100] Thus, it is possible that erythropoietin-induced and/or VEGF-induced STAT3 tyrosine phosphorylation is partly responsible for the retinal alterations in iron deficiency anemia and possibly in repeated blood donors.

Conclusion

Indirect evidence of enhanced erythropoietin production following repeated phlebotomy,[20,23] single 400 mL phlebotomy,[21] and single 450 mL blood donation,[22] together with findings of decreased hepcidin levels in superdonors,[101] suggests decreased tissue oxygen saturation and oxygenated tissue hemoglobin values in repeated blood donors,[42] decreased regional cerebral oxygen saturation (almost by half) after a single 450 mL blood donation,[43] and enhanced VEGF production in hypoxic situations,[39] suggesting the possibility of existence of a chronic hypoxia and or hypoxemia in repeated blood donors, especially considering the fact that the primary stimulus for increasing erythropoietin synthesis is tissue hypoxia resulting from reduced blood oxygen availability.[8,102] Such a chronic hypoxia and/or hypoxemia status in repeated blood donors may induce HIF-1α expression, which in turn, might upregulate genes stimulating angiogenesis, resulting in the formation of a new vasculature.[50,51]

A linkage between repeat blood donation and retinal proliferation is possible, and further studies should be undertaken to see if changes in guidelines are required. This personal view does not negate the health effects of blood donation. Rather, it raises a question about ocular safety in multitime blood donors. Gathering and analyzing data on retinal findings from these people, either retrospectively or prospectively, might yield preliminary safety information, as well as those who donate blood for humanitarian reasons.

Disclosure

The author reports no conflicts of interest in this work.

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