Retinal and choroidal vascular drop out in a case of severe phenotype of Flammer Syndrome. Rescue of the ischemic-preconditioning mimicking action of endogenous Erythropoietin (EPO) by off-label intra vitreal injection of recombinant human EPO (rhEPO)

Claude Boscher¹, Dominique Jordan², Anne Sophie Alonso³, Ozlem Erol⁴, Francois Tarragano², Richard Luscan⁴

¹Fondazione Retina 3000, Milano, Italy
²Department of Pharmacy, American Hospital of Paris, Neuilly, France
³Optometrists, Center for Investigation and Clinical Research, Adolphe de Rothschild Ophthalmological Foundation, Paris, France
⁴Ophthalmologist, currently retired.

Abstract

Background: Erythropoietin (EPO) is a pleiotropic anti-apoptotic, neurotrophic, anti-inflammatory, and pro-angiogenic endogenous agent, in addition to its effect on erythropoiesis. Exogenous EPO is currently used notably in human spinal cord trauma, and pilot studies in ocular diseases have been reported. Its action has been shown in all (neurons, glia, retinal pigment epithelium, and endothelial) retinal cells. Patients affected by the Flammer Syndrome (FS) (secondary to Endothelin (ET)-related endothelial dysfunction) are exposed to ischemic accidents in the microcirculation, notably the retina and optic nerve.

Case Presentation: A 54 years old female patient with a diagnosis of venous occlusion OR since three weeks presented on March 3, 2019. A severe Flammer phenotype and underlying non arteritic ischemic optic neuropathy; retinal and choroidal drop-out were obviated. Investigation and follow-up were performed for 36 months with Retinal Multimodal Imaging (Visual field, SD-OCT, OCT-Angiography, Indo Cyanin Green Cine-Video Angiography). Recombinant human EPO (rhEPO)(EPREX®)(2000 units, 0.05 cc) off-label intravitreal injection was performed twice at one month interval. Visual acuity rapidly improved from 20/200 to 20/63 with disparition of the initial altitudinal scotoma after the first rhEPO injection, to 20/40 after the second injection, and gradually up to 20/32, by month 5 to month 36. Secondary cystoid macular edema developed ten days after the first injection, that was not treated via anti-VEGF therapy, and resolved after the second rhEPO injection. PR1 layer integrity, as well as protective macular gliosis were fully restored. Some level of ischemia persisted in the deep capillary plexus and at the optic disc.

Conclusion: Patients with FS are submitted to chronic ischemia and paroxystic ischemia/reperfusion injury that drive survival physiological adaptations via the hypoxic-preconditioning mimicking effect of endogenous EPO, that becomes overwhelmed in case of acute hypoxic stress threshold above resilience limits. Intra vitreal exogenous rhEPO injection restores retinal hypoxic-preconditioning adaptation capacity, provided it is timely administrated. Intra vitreal rhEPO might be beneficial in other retinal diseases of ischemic and inflammatory nature.
Key words: Erythropoietin, Retinal vein occlusion, Anterior ischemic optic neuropathy, Flammer syndrome, Primary Vascular Dysfunction, Anti-VEGF therapy, Endothelin, microcirculation, Off-label therapy.

Introduction

Retinal Venous Occlusion (RVO) treatment still carries insufficiencies and contradictions (1) due to the incomplete deciphering of the pathophysiology and of its complex multifactorial nature, with overlooking of factors other than VEGF up-regulation, notably the roles of retinal venous tone and Endothelin-1 (ET) (2-5), and of endothelial caspase-9 activation (6). Flammer Syndrome (FS) (Primary Vascular Dysfunction) is related to a non atherosclerotic ET-related endothelial dysfunction in a context of frequent hypotension and increased oxidative stress (OS), that alienates organs perfusion, with notably changeable functional altered regulation of blood flow (7-9), but the pathophysiology remains uncompletely elucidated (8). FS is more frequent in females, and does not seem to be expressed among outdoors workers, implying an influence of sex hormons and light (7)(9). ET is the most potent pro-proliferative, profibrotic, pro-oxidative and pro-inflammatory vasoconstrictor, currently considered involved in many diseases other than cardio-vascular ones, and is notably an inducer of neuronal apoptosis (10). It is produced by endothelial (EC), smooth vascular muscles (SVMC) and kidney medullar cells, and binds the surface Receptors ET-A on SVMC and ET-B on EC, in an autocrine and paracrine fashion. Schematically, binding on SVMC Receptors (i.e. through local diffusion in fenestrated capillaries or dysfunctioning EC) and on EC ones (i.e. by circulating ET) induce respectively arterial and venous vasoconstriction, and vasodilatation, the latter via Nitrite oxide (NO) synthesis. ET production is stimulated notably by Angiotsen 2, insulin, cortisol, hypoxia, and antagonized by endothelial gaseous NO, itself induced by flow shear stress. Schematically but not exclusively, vascular tone is maintained by a complex regulation of ET-NO balance (8) (10-11). Both decrease of NO and increase of ET production are both a cause and consequence of inflammation, OS and endothelial dysfunction, that accordingly favour vasoconstriction; in addition ET competes for L-arginine substrate with NO synthase, thereby reducing NO bioavailability, a mechanism obviated notably in carotid plaques and amaurosis fugax (reviewed in 11).

Severe FS phenotypes are rare. Within the eye, circulating ET reaches retinal VSMC in case of Blood-Retinal-BARRIER (BRB) rupture and diffuses freely via the fenestrated choroidal circulation, notably around the optic nerve (ON) head behind the lamina cribrosa, and may induce all pathologies related to acute ocular blood flow decrease (2-3)(5)(7-9). We previously reported two severe cases with rapid onset of monocular cecity and low vision, of respectively RVO in altitude and non arteritic ischemic optic neuropathy (NAION) (Boscher et al, Société Francaise d’Ophtalmologie and Retina Society, 2015 annual meetings).

Exogenous Recombinant human EPO (rhEPO) has been shown effective in humans for spinal cord injury (12), neurodegenerative and chronic kidney disease (CKD) (reviewed in 13). Endogenous EPO is released physiologically in the circulation by the kidney and liver; it may be secreted in addition by all cells in response to hypoxic stress, and it is the prevailing pathway induced via genes up-regulation by the transcription factor Hypoxia Inducible Factor 1 alpha, among angiogenesis (VEGF pathway), vasomotor regulation (inducible NO synthase), antioxidation, and energy metabolism (14). EPO Receptor signaling induces cell proliferation, survival and differentiation (reviewed in 13), and targets multiple non hematopoietic pathways as well as the long-known effect on erythropoiesis (reviewed in 15). Of particular interest here, are its synergistic anti-inflammatory, neural antiapoptotic (16) pro-survival and pro-regenerative (17) actions upon hypoxic injury, that were long-suggested to be also indirect, via blockade of ET release by astrocytes, and assimilated to ET-A blockers action (18). Quite interestingly, endogenous EPO’s pleiotropic effects were long-summarized (back to 2002), as “mimicking hypoxic-preconditioning” by Dawson (19), a concept applied to the retina (20). EPO Receptors are present in all retinal cells and their rescue activation targets all retinal cells, i.e. retinal EC, neurons (photoreceptors PR), ganglion (RGG) and bipolar cells), retinal pigment epithelium (RPE) osmotic function through restoration of the BRB, and glial cells (reviewed in 21), and the optic nerve (reviewed in 22). RhEPO has been tested experimentally in animal models of glaucoma, retinal ischemia-reperfusion (I/R) and light phototoxicity, via multiple routes (systemic, subconjunctival, retrobulbar and intravitreal injection (IVI) (reviewed in 23), and used successfully via IVI in human pilot studies, notably first in diabetic macular edema (24) (reviewed in 25 and 26). It failed to improve neuroprotection in association to corticosteroids in optic neuritis, likely for bias reasons (reviewed in 22). Of specific relation to the current case, it has been reported in NAION (27) (reviewed in 28) and traumatic ON injury (29 Rashad), and in one case of acute severe central RVO (CRVO) (Luscan and Roche, Société Francaise d’Ophtalmologie 2017 annual meeting). In addition EPO RPE gene therapy was recently suggested to prevent retinal degeneration induced by OS in a rodent model of dry Age Macular Degeneration (AMD) (30).

Case Report Presentation

This 54 years female patient was first visited on March 2019 4th, seeking for second opinion for ongoing vision deterioration OR on a daily basis, since around 3
weeks. Sub-central RVO (CRVO) OR had been diagnosed on February 27th; available SD-OCT macular volume was increased with epiretinal marked hyperreflectivity, one available Fluorescein angiography picture showed a non-filled superior CRVO, and a vast central ischemia involving the macular and paraoptic territories. Of note there was ON edema with a para-papillary hemorrhage nasal to the disc on the available colour fundus picture.

At presentation on March 4, Best Corrected Visual Acuity (BCVA) was reduced at 20/100 OR (20/25 OS). The patient described periods of acutely excruciating retro-orbital pain in the OR. Intraocular pressure was normal, at 12 OR and 18 OS (pachymetry was at 490 microns in both eyes). The dilated fundus examination was similar to the previous color picture and did not disclose peripheral hemorrhages recalling extended peripheral retinal ischemia. Humphrey Visual Field disclosed an altitudinal inferior scotoma and a peripheral inferior scotoma OR and was in the normal range OS, i.e. did not recall normal tension glaucoma OS (Fig. 1). There were no papillary drusen on the autofluorescence picture, ON volume was increased (11.77 mm3 OR versus 5.75 OS) on SD-OCT (Heidelberg Engineering®) OR, Retinal Nerve Fiber (RNFL) and RGC layers thicknesses were normal (Fig. 2). Marked epimacular hypereflectivity OR with foveolar depression inversion, moderately increased total volume and central foveolar thickness (CFT) (428 microns versus 328 OS), and a whitish aspect of the supero-temporal internal retinal layers recalling ischemic edema, were present (video 1). EDI CFT was increased at 315 microns (versus 273 microns OS), with focal pachyvessels on the video mapping (video 1). OCT-Angiography disclosed focal perfusion defects in both the retinal and chorio-capillaris circulations (Fig. 3), and central alterations of the PR1 layer on en-face OCT (Fig. 4).

Altogether the clinical picture evoked a NAION with venous sub-occlusion, recalling Fraenkel’s et al early hypothesis of an ET interstitial diffusion-related venous vasoconstriction behind the lamina cribrosa (2), as much as a rupture of the BRB was present in the optic nerve area (hemorrhage along the optic disc). Choroidal vascular dropout was suggested by the severity and rapidity of the VF impairment (31). The extremely rapid development of a significant “epiretinal membrane”, that we interpreted as a reactive - and protective, in absence of cystoid macular edema (CME) - ET2-induced astrocytic proliferation (reviewed in 32), was an additional sign of severe ischemia.

The mention of the retro-orbital pain evoking a “ciliary angor”, the absence of any inflammatory syndrome and of the usual metabolic syndrome in the emergency blood test, oriented the etiology towards a FS. And indeed anamnthesis collected many features of the FS, i.e. hypotension (“non dipper” profile with one symptomatic nocturnal episode of hypotension on the MAPA), migrains, hypersensitivity to cold, stress, noise, smells, and medicines, history of a spontaneously resolutive hydroxy six months earlier, and of paroxystic episodes of vertigo (which had driven a prior negative brain RMI investigation for Multiple Sclerosis, a frequent record among FS patients (33) and of paroxystic visual field alterations (7)(9), that were actually recorded several times along the follow-up.

The diagnosis of FS was eventually confirmed in the Ophthalmology Department in Basel University on April 10th, with elevated retinal venous pressure (20 to 25 mmHg versus 10-15 OS) (4)(7)(9), reduced perfusion in the central retinal artery and veins on ocular Doppler (respectively 8.3 cm/second OR velocity versus 14.1 mmHg OS, and 3.1/ second OR versus 5.9 cm OS), and impaired vasodilation upon flicker light-dependant shear stress on the Dynamic Vessel Analyser testing (7-9). In addition atherosclerotic plaques were absent on carotid Doppler.

On March 4th, the patient was at length informed about the FS, a possible off label rhEPO IVI, and a related written informed consent on the ratio risk-benefits was delivered.

By March 7th, she returned on an emergency basis because of vision worsening OR. VA was unchanged, intraocular pressure was at 13, but Visual Field showed a worsening of the central and inferior scotomas with a decreased foveolar threshold, from 33 to 29 decibels. SD-OCT showed a 10% increase in the CFT volume.

On the very same day, an off label rhEPO IVI OR (EPREX® 2000 units, 0,05 cc in a pre-filled syringe) was performed in the operating theater, i.e. the dose reported by Moradres et al (27), and twenty times inferior to the usual weekly intravenous dose for treatment of chronic anemia secondary to CKD. Intra venous acetazolamide (500 milligrams) was performed prior to the injection, to prevent any increase in intra-ocular pressure. The patient was discharged with a prescription of chlorhydrate betaxolol (Betoptic® 0.5 %) two drops a day, and high dose daily magnesium supplementation (600 mgr).

Incidentally the patient developed bradycardia the day after, after altogether instillation of 4 drops of betaxolol only, that was replaced by acetazolamide drops, i.e. a typical hypersensitivity reaction to medications in the FS (7)(9).

Subjective vision improvement was recorded as early as D1 after injection. By March 18 th, eleven days post rhEPO IVI, BCVA was improved at 20/63, the altitudinal scotoma was discharged, IVI, BCVA was improved at 20/63, the altitudinal scotoma had resolved (Fig. 5), Posterior Vitreous Detachment had developed with a disturbing marked Weiss ring, optic disc swelling had decreased; vasculogenesis within the retinal...
Figure 1: Humphrey visual field at baseline on March 7th 2019, showing an altitudinal central scotoma, an inferior peripheral scotoma with a normal and symmetrical foveolar sensitivity threshold, and a normal visual field OS.
plexi and some regression of PR1 alterations were visible on OCT-en face. Indeed by 11 days post EPO significant functional, neuronal and vascular rescue were observed, while the natural evolution had been seriously vision threatening.

However cystoid ME (CME) had developed (video 2). Indo Cyanin Green-Cine Video Angiography (ICG-CVA) OR, performed on March 23, i.e. 16 days after the rhEPO IVI, showed a persistent drop in ocular perfusion: ciliary and central retinal artery perfusion timings were dramatically delayed at respectively 21 and 25 seconds, central retinal vein perfusion initiated by 35 seconds, was pulsatile, and completed by 50 seconds only (video 3). Choroidal pachyveins matching the ones on SD-OCT video mapping were present in the temporal superior and inferior fields, and crossed the macula; capillary exclusion territories were present in the macula and around the optic disc.

By April 1, 23 days after the rhEPO injection, VA was

---

Figure 2: Retinal Nerve Fiber (RNFL) evolution from normal at baseline on March 2019 7th, to development of a superior sequellar deficit that remained stable on last follow-up.
unchanged, but CME and perfusion voids in the superficial deep capillary plexi and choriocapillaris were worsened, and optic disc swelling had recurred back to baseline, in a context of repeated episodes of systemic hypotension; and actually Nifepidin-Ratiopharm® oral drops (34), that had been delivered via a Temporary Use Authorization from the central Pharmacology Department in Assistance Publique Hopitaux de Paris, had had to be stopped because of hypersensitivity.

A second off label rhEPO IVI was performed in the same conditions on April 3, i.e. approximately one month after the first one.

Evolution was favourable as early as the day after EPO injection 2: VA was improved at 20/40, CME was reduced, and perfusion improved in the superficial retinal plexus as well as in the choriocapillaris. By week 4 after EPO injection 2, CME was much decreased, i.e. without anti VEGF injection. On august 19th, by week 18 after EPO 2,
Figure 5: Humphrey visual field follow-ups: at follow-up 1 eleven days after rhEPO intra vitreal injection showing resolution of the altitudinal central scotoma and decrease of the inferior scotoma, and at last visual field follow-up on January 20th 2021, showing residual defects corresponding to the RNFL ones on Figure 2.
perfusion on ICG-CVA was greatly improved, with ciliary timing at 18 seconds, central retinal artery at 20 seconds and venous return from 23 to 36 seconds, still pulsatile. Capillary exclusion territories were visible in the macula and temporal to the macula after the capillary flood time that went on by 20.5 until 22.5 seconds (video 4); they were no longer persistent at intermediate and late timings.

Last complete follow-up was recorded on January 7, 2021, at 22 months from EPO injection 2. BCVA was at 20/40, ON volume had dropped at 7.46 mm3, a sequeleral superior deficit was present in the RNFL (Fig. 2) with some corresponding residual defects on the inferior para central Visual Field (Fig. 5), CFT was at 384 mm3 with an epimacular hyperreflectivity without ME, EDI CFT was dropped at 230 microns. Perfusion on ICG-CVA was not normalized, but even more improved, with ciliary timing at 15 seconds, central retinal artery at 16 seconds and venous return from 22 to 31 seconds, still pulsatile (video 5), indicating that VP was still above IOP. OCT-A showed persisting perfusion voids, especially at the optic disc and within the deep retinal capillary plexus. The latter were present at some degree in the OS as well (Fig. 6). Choriocapillaris and PR1 layer were dramatically improved.

Last recorded BCVA was at 20/32 by February 14, 2022, at 34 months from EPO 2. SD-OCT showed stable gliosis hypertrophy and mild alterations of the external layers (video 6).

**Discussion**

What was striking in the initial clinical phenotype of CRVO was the contrast between the moderate venous dilation, and the intensity of ischemia, that were illustrating the pioneer hypothesis of Professor Flammer’s team regarding
the pivotal role of ET in VO (2), recently confirmed (3)(35), i.e. the local venous constriction backwards the lamina cribrosa, induced by diffusion of ET-1 within the vascular interstitium, in reaction to hypoxia. NAION was actually the primary and prevailing alteration, and ocular hypoperfusion was confirmed via ICG-CVA, as well as by the ocular Doppler performed in Basel. ICG-CVA confirmed the choroidal drop-out suggested by the severity of the VF impairment (31) and by OCT-A in the choriocapillaris. Venous pressure measurement, which instrumentation is now available (8), should become part of routine eye examination in case of RVO, as it is key to guide cases analysis and personalized therapeutic options.

Indeed, the endogenous EPO pathway is the dominant one activated by hypoxia and is synergetic with the VEGF pathway, and coherently it is expressed along to VEGF in the vitreous in human RVO (36). Diseases develop when the individual limiting stress threshold for efficient adaptive reactive capacity gets overwhelmed. In this case by Week 3 after symptoms onset, neuronal and vascular resilience mechanisms were no longer operative, but the BRB, compromised at the ON, was still maintained in the retina.

As mentioned in the introduction, the scientific rationale for the use of EPO was well demonstrated by that time, as well as the capacities of exogenous EPO to mimic endogenous EPO vasculogenesis, neurogenesis and synaptogenesis, restoration of the balance between ET-1 and NO. Improvement of chorioretinal blood flow was actually illustrated by the evolution of the choriocapillaris perfusion on repeated OCT-A and ICG-CVA. The anti-apoptotic effect of EPO (16) seems as much appropriate in case of RVO as the caspase-9 activation is possibly another overlooked co-factor (6).

All the conditions for translation into off label clinical use were present: severe vision loss with daily worsening and unlikely spontaneous favourable evolution, absence of toxicity in the human pilot studies, of contradictory comorbidities and co-medications, and of context of intraocular neovascularization that might be exacerbated by EPO (37).

Why didn't we treat the onset of CME by March 18th, i.e. eleven days after EPO IVI 1, by anti-VEGF therapy, the "standard-of-care" in CME for RVO ?

In addition to the context of functional, neuronal and vascular improvements obviated by rhEPO IVI by that timing in the present case, actually anti VEGF therapy does not address the underlying causative pathology. Coherently, anti-VEGF IVI: 1) may not be efficient in improving vision in RVO, despite its efficiency in resolving/improving CME.
in FS; it allows real time evaluation of the ocular perfusion hypoxia.

It would suppress one of the mechanisms of resilience to hypoxia, that seemed no longer reached again during the relapse-free follow-up. Of note, this “epiretinal membrane hyperreflectivity without ME and ongoing chronic ischemia accounts for a persisting state of chronic ischemia.”

To our best knowledge, ICG-CVA was never reported in FS; it allows real time evaluation of the ocular perfusion and illustration of the universal rheological laws that control choroidal blood flow as well. Pachyveins recall a “reverse” veno-arteriolar reflex in the choroidal circulation, that is NO and autonomous nervous system-dependant, and that we suggested to be an adaptive choroidal microcirculation process to hypoxia (45). Their persistence during follow-up accounts for a persisting state of chronic ischemia.

The optimal timing for reperfusion via rhEPO in a non resolved issue: In the case reported by Luscan and Roche, rhEPO IVI was performed on the very same day of disease onset, where it induced complete recovery from VA reduced at counting fingers at 1 meter, within 48 hours. This clinical human finding is on line with a recent rodent stroke study that established the timings for non lethal versus lethal ischemia of the neural and vascular lineages, and the optimized ones for beneficial reperfusion: the acute phase - from Day 1 where endothelial and neural cells are still preserved, to Day 7 where proliferation of pericytes and Progenitor Stem Cells are obtainable - and the chronic stage, up to Day 56, where vasculogenesis, neurogenesis and functional recovery are still possible, but with uncertain efficiency (46). In our particular case, PR rescue after rhEPO IVI 1 indicated that Week 3 was still timely. RhEPO IVI efficacy was shown to last between one (restoration of the BRB) and four weeks (antiapoptotic effect) in diabetic rats (24). The relapse after Week 3 post IVI 1 might indicate that it might be approximately the interval to be followed, should repeated injections be necessary.

The bilateral chronic perfusion defects on OCT-A at last follow-up indicate that both eyes remain in a condition of chronic ischemia and I/R, where endogenous EPO provides efficient ischemic pre-conditioning, but is potentially susceptible to be challenged during episodes of acute hypoxia that overwhelm the resilience threshold.

Conclusion

The present case advocates for individualized medicine with careful recording of the medical history, investigation of the systemic context, and exploiting of the available retinal multimodal imaging for accurate analytical interpretation of retinal diseases and their complex pathophysiology. The Flammer Syndrome is unfortunately overlooked in case of RVO; it should be suspected clinically in case of absence of the usual vascular and metabolic context, and in case of elevated RVP. RhEPO therapy is able to restore the beneficial endogenous EPO ischemic pre-conditioning in eyes submitted to challenging acute hypoxia episodes in addition to chronic ischemic stress as in the Flammer Syndrome and fluctuating ocular blood flow, when it becomes compromised by the overwhelming of the hypoxic stress resilience threshold. The latter physiopathological explanation illuminates the cases of RVO where anti-VEGF...
therapy proved functionally inefficient, and/or worsened retinal ischemia. RhEPO therapy might be applied to other chronic ischemia and I/R conditions, as non neo-vascular Age Macular Degeneration (AMD), and actually EPO was listed in 2020 among the nineteen promising molecules in AMD in a pooling of four thousands (47).

References


47. Screening Medications for Association with Progression to Wet Age-Related Macular Degeneration. Wang SV, Kulldorff M, Poor S, et al . doi.org/10.1016/j.ophtha.2020.08.004.