Feasibility of intravitreal erythropoietin injections in humans

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Abstract

Background/aims: Preclinical data suggest that intravitreally administered erythropoietin (EPO) is both neuroprotective and safe. In a small pilot series, we intended to assess the feasibility of intravitreal EPO injections in humans.

Methods: Three patients with acute vascular occlusion of the posterior pole received a single intravitreal EPO injection of 2000 U. Immediately before the injection and over the ensuing three months, these patient were closely monitored by measuring visual acuity, visual fields, intraocular pressure, the electroretinogram, the hematocrit and serum EPO levels.

Results: Over the observational period, most parameters remained unchanged except for a short-term rise of serum EPO levels, which, however, did not exceeded normal serum levels. We did not observe injection-related toxicity.

Conclusion: Based on this limited set of data, a single EPO injection of 2000 U, a dose adapted from previous *in vivo* studies, is feasible and seems to induce no obvious damage. Hence, further investigations of this therapeutic approach appear justified.

Introduction

Optic atrophy is the second most common condition leading to legal blindness. It is the final common pathway of inflammatory, ischemic, and other optic neuropathies, however most often of primary open angle glaucoma. These diseases cause irreversible loss of neurons and axons leading to a permanent functional impairment, which may impose a serious burden on the affected individuals and on health care systems in general. So far, no evidence based, causative therapy exists to decelerate axonal loss in the optic nerve except for lowering intraocular pressure in glaucoma, which is, however, sometimes insufficient.

Neuroprotection is a paradigm aiming at the reduction or even prevention of neuronal damage by pharmaceutical interventions or molecular genetic techniques.[1] While being well established *in vitro* and *in vivo*, many clinical trials have failed for various reasons. It took until recently that randomized clinical trials yielded positive outcomes, i.e. with erythropoietin (EPO) in stroke, schizophrenia and multiple sclerosis[2], with riluzole in amyotrophic lateral sclerosis[3], and with memantine in Alzheimers disease[4] and vascular dementia.[5]

The hematopoietic growth factor EPO has been shown to have potent neuroprotective and neuroregenerative properties in the nervous system. We consider EPO an interesting neuroprotective candidate drug, because it is clinically available and its neuroprotective action has been well characterized. Over the last years, several groups have shown that EPO suppresses neuronal apoptosis and reduces inflammatory responses in different models of brain ischaemia[6] and inflammation.[7] The action of EPO is conveyed by interference with intracellular Jak2/STAT3- and PI3K/Akt-pathways[8] mediated by EPO-receptor activation.[9] EPO crosses the blood brain barrier[7] and non-hematopoietic mutants of EPO proved to be neuroprotective as well.[10]

With regard to protection of retinal ganglion cells (RGC) it has been shown that EPO alleviates apoptosis in vitro[11]. Subsequent *in vivo* experiments in rats supported this finding. RGC survival was promoted by EPO after orbital transection[11], experimental

autoimmune encephalomyelitis[12], chronically elevated intraocular pressure[13], and diabetic retinopathy.[14] In these studies EPO was administered either systemically[13] or by injection into the vitreous.[11, 14, 15]

Over the last years, intravitreal injections became a new treatment regimen in retinal diseases such as age related macular degeneration, mostly, due to the success of VEGF-inhibitors. Although not being without risks such as hemorrhages or endophthalmitis, they offer the advantage to deliver drugs locally in high concentrations. Hence, the question arises whether intravitreal injections of EPO may be possible in humans and have a neuroprotective potential on RGC and their axons in retinal and optic nerve diseases. Therefore, we conceived a small, prospective pilot trial encompassing three patients with vascular occlusions of the posterior pole in order to assess the feasibility of intravitreal EPO injections in humans.

Patients and methods

The investigation adhered to the declaration of Helsinki. Ethical and safety aspects were reviewed with the Institutional Review Board of the University Hospital Freiburg, which approved the limited patient number of three in this pilot case series. The inclusion criterion was an acute arterial occlusion of the retina or optic nerve head less than three day ago in patients between 50 and 80 years of age. Exclusion criteria comprised any kind of neoplasia, temporal arteritis, glaucoma, diabetic retinopathy, previous vascular occlusions of the posterior pole, and the eligibility for other interventions such intraarterial thrombolysis. Accordingly, three patients with three different types of vascular occlusions of the posterior pole were prospectively included and gave fully informed, written consent. The first patient was male, 76 years old and had central retinal artery occlusion (CRAO) with intact cilioretinal perfusion 20 hours before presentation. The second patient was female, 79 years old and had complete CRAO 22 hours before presentation. The third patient was male, 65 years old and presented 30 hours after non-arteritic anterior ischemic optic neuropathy (NAION). Each patient was subjected to the following diagnostic procedures: Best corrected visual acuity

measurement (Snellen chart), Goldmann applanation tonometry, Goldmann kinetic perimetry (stimulus III4e and I4e), indirect funduscopy, full field photopic electroretinography (ERG) according to ISCEV standards (Toennies Physiologischer Verstärker, Hoechberg, Germany) and measurement of serum EPO levels (IMMULITE 2000 EPO assay, Siemens, Germany; range of normal values 3.7 – 31.5 mU / ml) and hematocrit. The two patients with retinal vessel occlusion additionally underwent fluorescence angiography.

On the day of presentation, each of the three patients received one single injection of 0.05 ml Epoetin alfa containing 2000 I.U. (Erypo FS 40 000 I.E./ml, Janssen-Cilag, Germany) via a 30 gauge needle 4 mm posterior to the limbus into the temporal superior quadrant after local desinfection and topical anaesthesia. All diagnostic procedures listed above, except fluorescence angiography, were performed directly before, and one day, one week, and one and three months after the injection.

Results

Figures 1 to 3 summarize the clinical data of the three patients over the observational period. The patient with CRAO and intact cilioretinal perfusion showed a small increase of visual acuity the day after injection. It remained stable over the ensuing three months. So did his visual field. The intraocular pressure did not change after the injection when measured one day thereafter. The ERG peak times remained constant. The ERG amplitudes rose during the week after the injection and declined thereafter. The serum EPO levels doubled, but did not exceed the upper normal limit.

- figure 1 -

The patient with complete CRAO had a visual acuity in a very low range. It did not improve after the injection. Her visual fields were markedly depressed and showed central scotomata except for day seven. The intraocular pressure did not change. Her ERG parameters remained rather stable over the three months. After the injection, serum levels tripled within normal limits, and decreased again after one month.

- figure 2 -

The patients with NAION had variable visual acuities with a slight tendency towards improvement. A similar trend was seen in his visual fields. His intraocular pressure rose towards the end of the observational period, but remained with in normal limits. The ERG peak time was not changed, the amplitude decreased over three months. As seen in the other patients, his hematocrit did not change. Again, systemic serum EPO levels rose on the day after injection, but did not exceed normal limits.

- figure 3 -

Discussion

This is the first report about intravitreal injections of EPO into the human vitreous body, a potentially neuroprotective treatment regimen. In three patients with vascular occlusions of the posterior pole, we did not observe functional deterioration over three months of observation. Two of them showed a slight visual improvement. Hence, we conclude from this small pilot study that intravitreal EPO injections are feasible and probably safe and that this approach disserves further attention and clinical research.

Since we could not exclude a damaging effect, we only included patients with neuroretinal diseases having a rather unfavorable visual acuity and prognosis, i.e. arterial occlusions in the optic nerve head leading to NAION or CRAO. NAION has an incidence of 1/10.000 with resulting a mean visual acuity of 1.31 logMAR. After 2 years, 31% show an improvement in visual acuity of three lines or more.[16] CRAO has an incidence of 0.1/10.000 and a poor prognosis with a resulting average acuity of hand motion. Only 3% show a visual recovery of three lines or more after 6 weeks.[17] In both entities, no evidence-based therapy has so far been established as efficacious in randomized clinical trials.[18, 19] Because of this constellation and in the light of the hampered prognosis of these diseases, we consider our intervention as ethically justified.

In the human vitreous body, Müller cells are the mainly source of endogenous EPO[20]. Its vitreal levels have been determined in three clinical investigations: Inomata et al.[21] found a mean EPO concentration of 34 (range 20 – 64) mU/ml in subjects with macular holes and 881 (157 – 1850) mU/ml in cases of proliferative diabetic retinopathy. They interpreted the ischaemia-induced, elevated EPO level as a possible endogenous neuroprotective reaction. Garci-Arumi et al.[22] found a mean concentration of 25 (range 10 – 75) mU/ml in patients with epiretinal membranes and macular holes and 430 (range 41 – 3000) mU /ml in diabetic macular edema. Finally, Asensio-Sanchez et al.[23] measured 25 (range 5-201) mU/ml in non-ischaemic patients compared to 512 (120 – 880) mU/ml in cases of proliferative diabetic retinopathy. Taken together, these data are rather consistent and indicate a 20fold increase in retinal ischaemia compared to non-ischaemic conditions. These increased EPO levels in retinal ischaemia are about 800 times lower than the concentration applied in our case series, in which we chose a single, intravitreal dose of 2000 IU. This dose was adapted from previous *in vivo* experiments. With regard to neuroprotection, intravitreal doses so far most effectively applied in rats were 2 IU[11], 7.65 IU[14] or 10 IU.[15] Rats have an estimated vitreous volume of 0.013 ml[24], while humans have a vitreous volume of about 4 ml, thus being about 300 times higher. In a recent *in vivo* toxicity study in rabbits, intravitreal injections of up to 1000 U were considered safe by electroretinography, fluorescein angiography and histology. Vitreal half-life times were found to be 2 – 3 days.[25]

With regard to potential systemic side effects, we measured EPO levels in the blood serum of our patients and determined their hematocrit. The systemic EPO levels remained within normal clinical limits at all measurements. However, the dose applied in our patients was obviously sufficient to increase systemic EPO levels shortly after the injection. The hematocrit did not change. We therefore are not concerned that the EPO dose applied in this case series lead to unwanted systemic levels.

The potential use of intravitreal EPO is manifold, as several diseases affect the inner retina and the subsequent anterior visual pathway, but also the outer retina, causing neuronal loss which cannot be decelerated in many instances such as AION, CRAO, optic neuritis,

photoreceptor dystrophies and sometimes glaucoma. However, one has to keep in mind that the potential benefit should outweigh the potential harm of such a treatment. Further, it is yet unknown, which EPO dose is most useful and whether repeated injections are feasible or necessary.

It is well known that intravitreal injections cause a transient rise of intraocular pressure, which may compromise retinal circulation, especially in the diseases chosen herein. Hollands et al. measured intraocular pressure before and 2, 5, and 30 minutes after injection of an identical volume of bevacizumab.[26] The mean intraocular pressures were 14, 36, 26, and 16 mm Hg, respectively. Accordingly, one has to assume that the injection of 0.05 ml significantly raises the pressure for about half an hour, which may affect the perfusion pressure. In order to avoid this, a paracentesis may be performed. The patient with the NAION indeed noted a short term, reversible deterioration of vision for one hour after the injection. Intravitreal injections have been performed in NAION before. The clinicaltrials.gov data base lists a current trial in the recruiting phase, in which patients with NAION receive intravitreal injections of ranibizumab (NCT00561834).

In conclusion, this small pilot study with a single injection of 2000 U EPO and its limited set of data may stimulate further clinical research regarding intravitreal injections aiming at neuroprotection. The dosage, which was adapted from preclinical *in vivo* studies, exceeded endogenous intravitreal EPO levels by far, but induced no obvious damage. The intervention seemed feasible, and did not reveal, as expected, any positive effect for the three patients.

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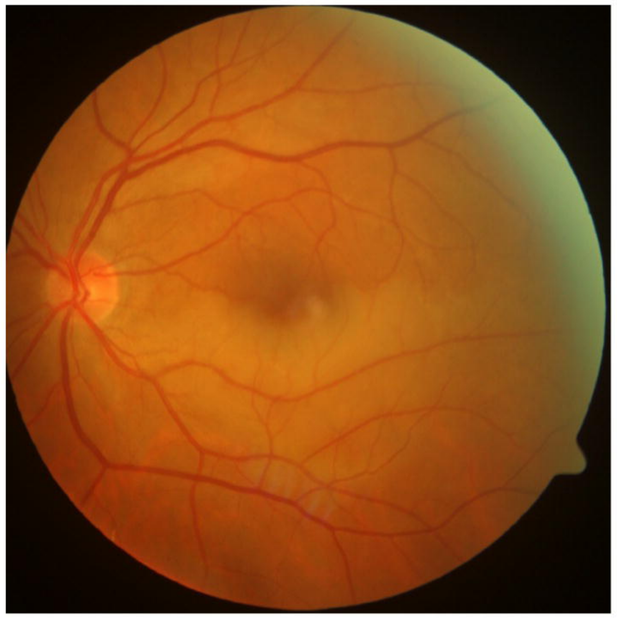
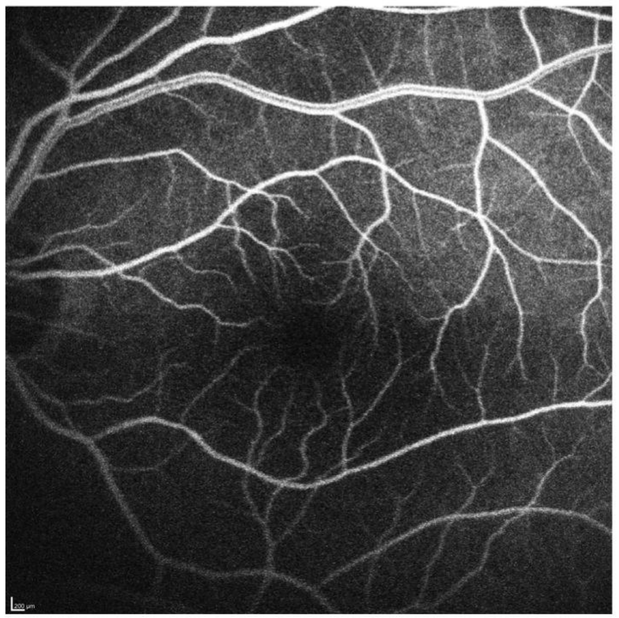
Figure legends

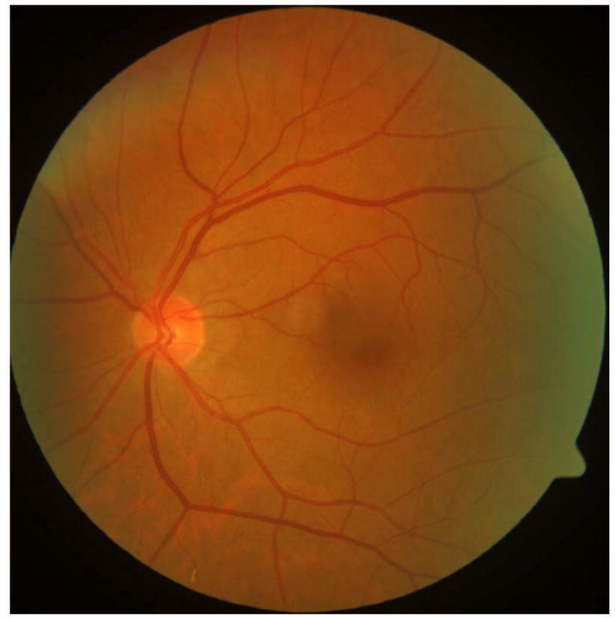
Figure 1: Top left: Fundus image of a patient with central retinal artery occlusion and intact cilioretinal perfusion showing retinal oedema sparing the papillomacular bundle. Top middle: Fluorescence angiogram showing attenuated and delayed venous filling 38 sec after dye injection. Top right: Fundus image 90 days after treatment. Middle: Table indicating clinical and laboratory parameters (abbreviations: IOP = intraocular pressure, ERG = electroretinogram, Hkt = hematocrit, EPO = erythropoietin). Bottom: Goldmann kinetic visual field on days 0, 1, 7, 30, and 90 (Outside border isopter Goldmann III4e, inside isopter I4e, dashed area scotoma for stimulus III4e).

Figure 2: Top left: Fundus image of a patient with central retinal artery occlusion retinal oedema and a foveal red spot. Top middle: Fluorescence angiogram showing massively reduced circulation 40 sec after dye injection Top right: Fundus image 90 days after treatment showing cessation of oedema and optic atrophy. Middle: Table indicating clinical and laboratory parameters (abbreviations: IOP = intraocular pressure, ERG = electroretinogram, Hkt = hematocrit, EPO = erythropoietin). Bottom: Goldmann kinetic visual field on days 0, 1, 7, 30, and 90 (Outside border isopter Goldmann III4e, inside isopter I4e).

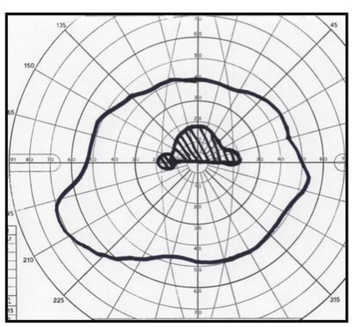
Figure 3: Top left: Fundus image of a patient with anterior ischaemic optic neuropathy showing optic disc swelling. Top right: Fundus image 90 days after treatment showing cessation of papilloedema and optic atrophy. Middle: Table indicating clinical and laboratory parameters (abbreviations: IOP = intraocular pressure, ERG = electroretinogram, Hkt = hematocrit, EPO = erythropoietin). Bottom: Goldmann kinetic visual field on days 0, 1, 7, 30, and 90 (Outside border isopter Goldmann III4e, inside isopter I4e, dashed area scotoma for stimulus III4e).

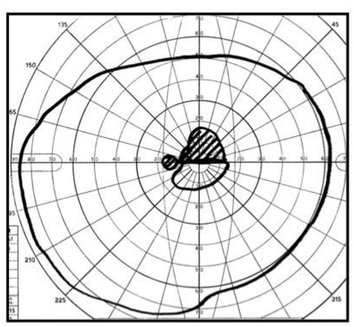


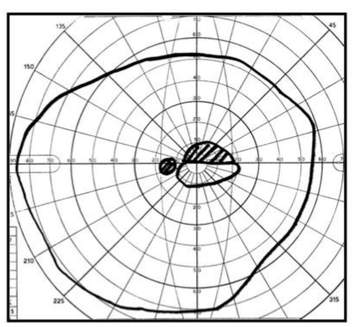


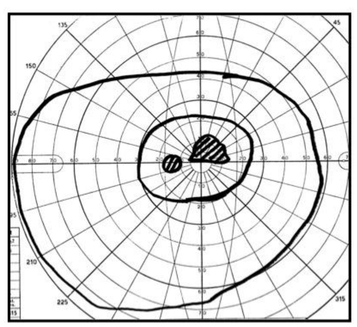


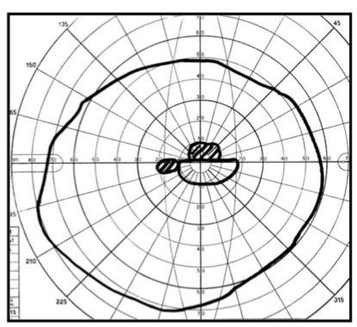
Parameter	Day 0	Day 1	Day 7	Day 30	Day 90
Visual acuity	20/40	20/25	20/30	20/25	20/20
IOP [mm Hg]	12	14	19	12	14
ERG-Amplitude [µV]	45	58	60	32	32
ERG peak time [msec]	35	32	32	29	30
Hkt [%]	49	43	45	43	47
Serum level EPO [mU/ml]	10	25	18	13	8

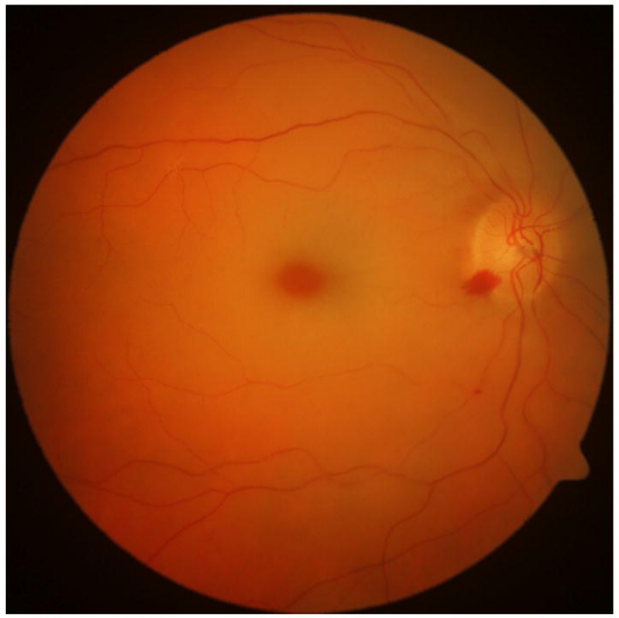


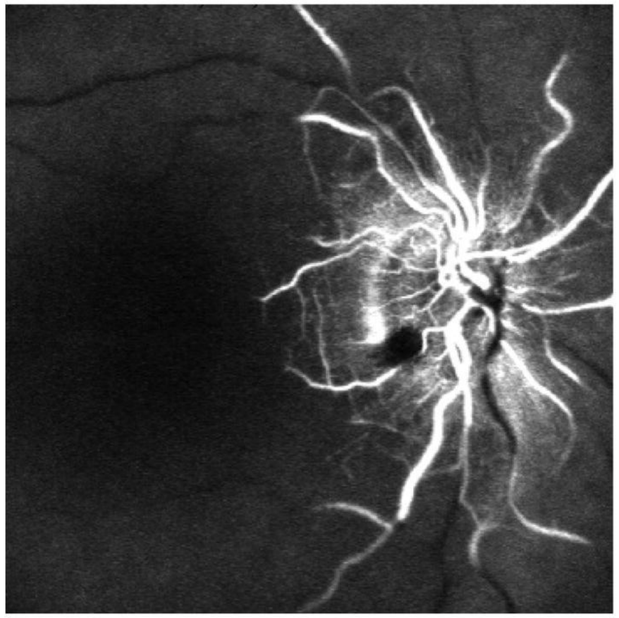


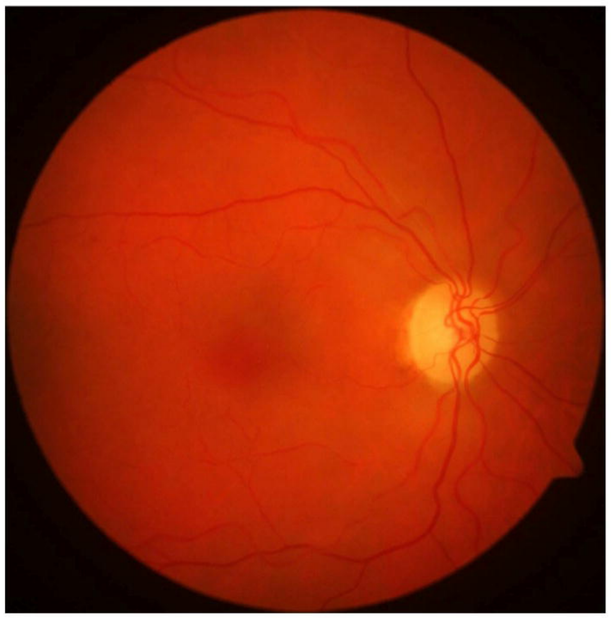




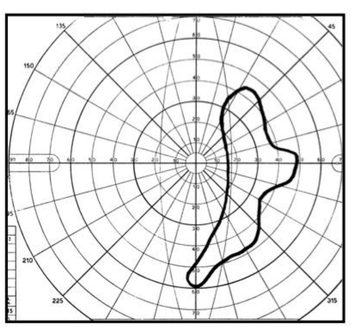


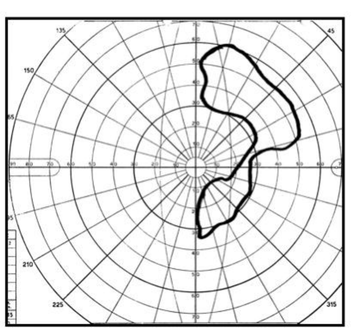


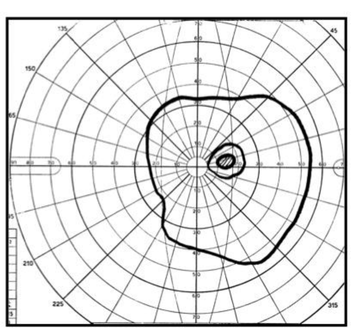


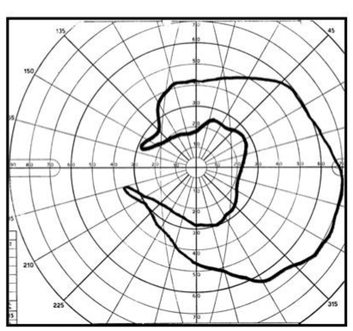


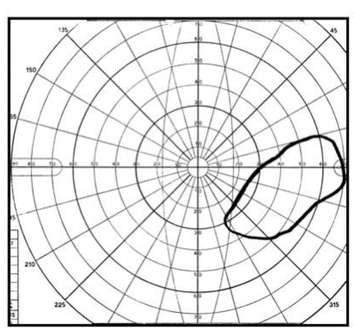
Parameter	Day 0	Day 1	Day 7	Day 30	Day 90
Visual acuity	Light perception	20/4000	20/4000	20/4000	Light perception
IOP [mm Hg]	15	17	16	14	17
ERG-Amplitude [µV]	19	10	24	18	16
ERG peak time [msec]	37	37	34	34	33
Hkt [%]	41	41	41	41	41
Serum level EPO [mU/ml]	8	26	26	9	8

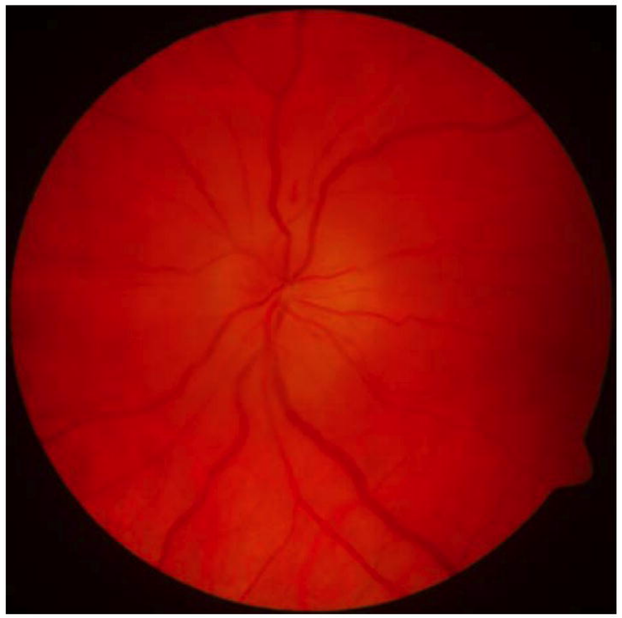


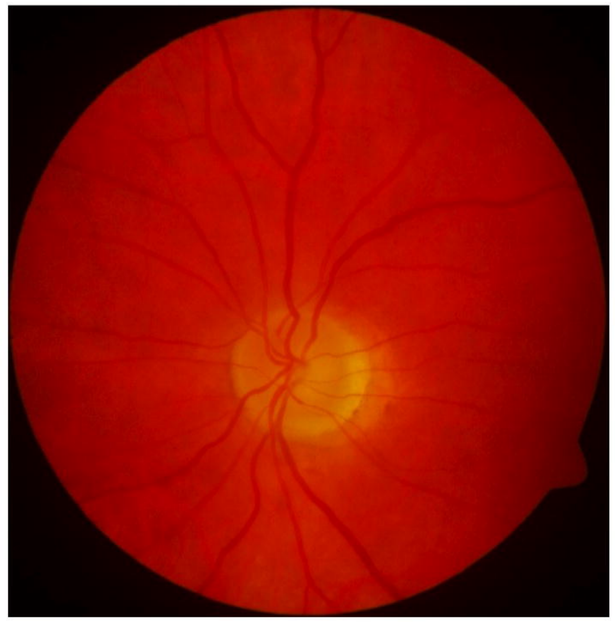












Parameter	Day 0	Day 1	Day 7	Day 30	Day 90
Visual acuity	20/700	20/300	20/400	20/500	20/200
IOP [mm Hg]	12	13	13	18	20
ERG-Amplitude [µV]	54	49	38	37	32
ERG peak time [msec]	30	29	30	29	31
Hkt [%]	46	44	45	44	44
Serum level EPO [mU/ml]	15	27	17	16	15