Oxygen-Induced Retinopathy in Newborn Kittens

A Model For Ischemic Vasoproliferative Retinopathy

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Fourteen kittens were reared in 80% oxygen for 65–72 hr, after which they were returned to room air. At that time, funduscopic examination of all the kittens showed complete obliteration of the retinal vasculature and even optic disc vessels. In the following 18–21 days, florid preretinal new vessels were found growing into the vitreous from the revascularized retina and from the optic disc itself. Subsequent histologic examinations confirmed the above clinical findings and reinforced the authors to suggest this animal model as a future research tool for ischemic vasoproliferative retinopathy, rather than a model for retinopathy of prematurity (ROP). The similarities and dissimilarities between this animal model and the human ischemic retinal disease are discussed. Invest Ophthalmol Vis Sci 28:126–130, 1987

Ashton et al1 and Patz et al,2 working independently, produced vasoproliferative changes in the retinas of newborn animals of several species, including kittens. By exposure to hyperoxic conditions, they found that the developing retinal vasculature of the kitten responds in a biphasic way: (a) initial vasoconstriction of major vessels and subsequent vaso-obliteration of the capillary bed, and (b) on return to room air, by preretinal neovascular proliferations due to peripheral retinal ischemia.1–3 Evidence that a vasoproliferative factor is released under these conditions has recently been demonstrated.4 As regards the histologic changes seen in the immature retina after oxygen exposure and transfer to air, Ashton et al1,3 showed, in kittens, that an intense angioblastic activity developed in the inner layers of the retina. The above mentioned findings were considered by Ashton and Patz1,2 to reflect the early stages of retinopathy of prematurity. Nevertheless, Ashton3 mentioned the differences between the experimental and human disease, and stated that, while the preretinal vasoproliferations in premature infants may progress to cicatrization and traction retinal detachment, in the animal eye they regress and traction retinal detachment never occurs in the kitten. Ashton tried to explain this difference by the fact that the neovascular growths of the kitten’s retina are lacking a fibroblastic element.5 Gole et al6 re-examined the kitten model by a technique of corrosion vascular casting, and compared it to the human disease. They found that the predominant morphological features of the kitten model were preretinal and intraretinal neovascularization being more numerous posteriorly than anteriorly, and preferred the term “oxygen-induced retinopathy” to describe the latter pathology.

By employing more modern laboratory methods, we could carry out dynamic follow-up of the different stages of revascularization and preretinal neovascularization in the kitten retina after oxygen exposure. We are of the opinion that oxygen-induced retinopathy in the kitten is basically an acute vasoproliferative retinopathy, induced by total retinal ischemia, which has some similarities to the human ischemic vasoproliferative retinopathies. It still is not understood why the neovascular growths in the kitten spontaneously regress, without leaving any signs of proliferative vitreoretinopathy (PVR) with subsequent traction retinal detachment, which are the usual end result of human vasoproliferative retinopathies. The funduscopic follow-up findings of oxygen-induced retinopathy in kittens, together with a clinicopathological correlation, are presented, and the similarities and dissimilarities between the two entities are discussed.

Materials and Methods

Fourteen kittens, aged between 3 and 10 days, were reared with their mothers in 80% oxygen, within a large incubator especially designed for this experiment in which CO₂ concentration, humidity, and temperature were fully controlled. The kittens were kept in these conditions for a period of 65–72 hr, after which they were removed from the incubator and returned with their mothers to normal oxygen concentration in air.

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Between several hours and 3 days after being returned to air, the kittens were anaesthetized with intraperitoneally administered pentobarbitone sodium (6 mg per 200 g body weight), and mydriasis was obtained with cyclopentolate hydrochloride 1.0% and phenylephrine hydrochloride 5% drops. Fundus examination of both eyes was done with an indirect ophthalmoscope, in order to confirm the constrictive and obliterative effect of the oxygen therapy on the retinal vasculature.

The kittens subsequently underwent funduscopic examination of both eyes, under general anaesthesia, every 2 days for the first 10 days, and every 4 days thereafter. During the follow-up period, fundus photography with a coaxial fundus camera was done in three animals. After a final funduscopic examination, they were killed between 18 and 21 days following oxygen exposure. India ink injection was performed in one of the kittens. The eyes were enucleated, fixed in buffered formaldehyde, and processed in the conventional way for hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining, except for the two eyes pretreated with India ink, which were fixed in buffered formaldehyde only.

These experiments adhered to the ARVO Resolution on the Use of Animals in Research.

Results

All 14 kittens showed complete obliteration of the retinal vasculature and even optic disc vessels after 3 days of oxygen therapy, a finding which was identical in both eyes of each kitten. The kittens subsequently developed preretinal new vessels in both eyes as early as 8 days after being returned from oxygen to air breathing. The new vessel formation started on the optic disc itself, and spread circumferentially as small tufts growing into the vitreous from the retina. In the same period, although starting a few days earlier, apparently normal intraretinal vessels were seen growing from the center of the optic nerve head centrifugally. Two weeks after oxygen therapy, the posterior poles of both eyes were completely vascularized with slightly dilated and engorged intraretinal vessels. The border between the avascular and vascular retina, in all cases, was located quite posteriorly, within a radius of about 5 disc diameters (D.D.) away from the optic disc in both eyes, and was characterized by dilated brush-like vascular endings (Fig. 1). The arborizing glomerular tufts of new vessels were actually seen growing forwards at random in both eyes from the whole area of the revascularized retina, but were clinically most conspicuous in the posterior retina (Fig. 2). These preretinal new vessels were sometimes associated with small to medium vitreous hemorrhages. Additionally, dilated and irregular numerous iris vessels were seen in all the kittens' eyes on the 14th day after oxygen treatment, the latter being interpreted by us as iris new vessels (rubeosis iridis). The histological sections stained with HE and PAS confirmed the above clinical findings, and showed well-developed preretinal new vessels, growing in a diffuse pattern into the vitreous from the retina of the posterior
Fig. 3. Numerous delicate new vessels are seen growing into the vitreous from the retina, and forming a confluent preretinal vascular membrane (Hematoxylin-eosin ×200).

Fig. 4. A carpet of confluent small preretinal new vessels covering the posterior retina including the optic disc (Hematoxylin-eosin ×80).

Fig. 5. Delicate new vessels coming out from the optic nerve head and growing into the vitreous. A small hemorrhage (arrow) is seen on the disc (Hematoxylin-eosin ×120).

Fig. 6. The anterior border of the growing preretinal new vessels, consisting of proliferating endothelial cells (arrows). No spindle cells are seen in the retina adjacent to the advancing preretinal new vessels (Hematoxylin-eosin ×200).

pole and from the optic disc itself (Figs. 3–5). It is important to note that no spindle cells were seen near the anterior border of the growing preretinal vessels (Fig. 6). These delicate preretinal new vessels were actually found to grow by budding from preexisting intraretinal vessels, and spread into the vitreous without any accompanying glial or fibrous tissue (Fig. 7). The eyes pretreated with India ink injection also showed the extensive preretinal neovascular growths coming out from the optic disc itself and from the intraretinal vessels of the revascularized retina. These specimens demonstrated much better the dense network of intraretinal capillaries and the other branches of the vascular tree of the kitten’s retina, which developed in the process of revascularization (Fig. 8).

Discussion

Our experiments confirm the observations of Ashton and Patz et al., Gole et al., and Yoneya and Tso, and demonstrate the extensive changes occurring in the kitten’s retinal vasculature following exposure to high concentration of oxygen. These changes consisted of intraretinal revascularization, associated with a network of intraretinal capillaries, and a carpet of neovascular tufts growing into the vitreous from both the retina and optic disc, between 2–3 weeks following oxygen exposure. Of equal importance is the fact that all our kittens showed complete obliteration of the retinal vasculature and even optic disc vessels after 3 days of oxygen therapy, which means that the neovascular growths developing during air breathing were preceded by total retinal ischemia. An additional significant finding was the dilated tortuous and irregular vessels seen on the irides of all the kittens, 14 days following oxygen exposure. All the above findings, together with the recently demonstrated evidence that a vasoprolif-
ferative factor is released under these conditions, point towards the ischemic origin of this florid vasoproliferative retinopathy, which reminds us of several ischemic vasoproliferative retinopathies, like major branch retinal vein occlusion,8 Eales' disease,9 postradiation retinopathy,10,11 “pulseless” disease,12 “Rush-type” ROP,13 proliferative diabetic retinopathy,14-16 and the ischemic type of central retinal vein occlusion.17 The common denominator of all the above conditions is retinal capillary obliteration, and areas of non-perfusion, which are a hallmark of retinal ischemia.18-22 Experimental support for this idea is provided by the neovascularization that follows the occlusion of branch arterioles18 or branch veins.19 The extent of the areas of capillary nonperfusion found by fluorescein angiography10,14,15 in each of the above mentioned retinopathies is in accord with the severity and location of the vasoproliferative disease. New vessels in the region of the optic disc usually develop following diffuse reduction of retinal blood flow, whereas in other parts of the retina tend to arise from the edges of circumscribed foci of non-perfusion.14,16,20-22 Shimizu et al,14 by performing fluorescein angiographic studies in diabetic patients, have related the degree of capillary non-perfusion in the entire fundus to peripheral retinal and disc new vessels, and also anterior segment neovascularization. They found that the areas of capillary occlusion were limited to the periphery when there were only peripheral new vessels, and extended to the midperiphery until the posterior pole, when disc new vessels were found. Massive or generalized nonperfused areas were seen whenever there was anterior segment neovascularization, in addition to disc and retinal new vessels. The latter situation, together with the category called “diffuse capillary retinopathy,”16 are similar to the above described findings of oxygen-induced retinopathy in the kitten by the way in which total retinal ischemia is the inducing factor of disc, retinal, and iris new vessels.

Another correlation can be made between the intraretinal revascularization found in the kittens after the total obliteration of the retinal vasculature by oxygen, and the recent clinical and experimental studies which have shown that nonperfused retinal areas may be revascularized.19-23 This finding has been mainly demonstrated by using branch retinal vein occlusion as clinical or experimental models.19,21 Recently, recanalization and, mainly, intraretinal neovascularization of non-perfusion areas have been demonstrated by Muraoka et al23 in preproliferative diabetic retinopathy. Typically, the intraretinal new vessels originated from and drained into a retinal venule. These new vessels were frequently associated by time and incidence with preretinal neovascularization of the proliferative phase of diabetic retinopathy.

It should be noted that, although most of the human ischemic retinopathies demonstrate the following retinal findings: “cotton wool” patches, dot and blot hemorrhages, “hard” exudates, and intraretinal microangiopathy (IRMA), including microaneurisms, before the appearance of preretinal new vessels,8-23 they are never seen in oxygen-induced retinopathy of the kitten, except for intraretinal revascularization, which, in a way, resembles the IRMA changes found in the human ischemic retinal diseases. Interestingly enough, the abovementioned classic retinal changes are also never seen in retinopathy of prematurity.13 We assume that “cotton wool” patches, being microinfarcts of the nerve fiber layer, do not develop in the kitten's disease, nor in human ROP, because the hyperoxia attributable to choroidal blood flow maintains neuronal viability.
despite retinal ischemia. Additionally, "hard" exudates do not develop, because the retinal vessels are closed in the oxygen-treated kitten, and, therefore, no blood is passing through them. There is still a lot to learn about this interesting animal model, and we hope that this work will stimulate further research connected with the kitten model and advance the understanding of ocular neovascularization.

Key words: oxygen-induced retinopathy, vasoproliferative retinopathy, intraretinal revascularization, preretal neo-
vascularization

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