Different Efficacy of Propranolol in Mice with Oxygen-Induced Retinopathy: Could Differential Effects of Propranolol Be Related to Differences in Mouse Strains?

We read with great interest the article "Propranolol inhibition of β-adrenergic receptors does not suppress pathologic neovascularization in oxygen-induced retinopathy" by Chen et al.[1] This article is part of a growing literature that explores the role of the adrenergic system in retinopathy. The interest on the adrenergic system originated from the observation that propranolol, a nonselective β-adrenergic receptor (β-AR) blocker, was recently reported to control the growth of human hemangiomas, the most common vascular tumor of newborns and infants, which is often associated with retinopathy of prematurity (ROP).[2]

Recent results from our research group demonstrated that propranolol (a β_1- and β_2-AR blocker) protects the retina against pathologic neovascularization in a mouse model of oxygen-induced retinopathy (OIR).[3] These results, however, were contradicted by Chen et al.,[1] who observed that propranolol fails to suppress retinopathy in OIR mice and brought into question whether propranolol and its inhibition of β_1- and β_2-ARs may be a reasonable therapeutic approach for treating ROP.

The discrepancy between the two groups of studies was discussed by Chen et al.,[1] by assuming that fluorescent angiography used by Ristori et al.[3] may fail to define all pathologic vessels compared with the standard method of staining retinal vessels with specific endothelial markers, such as isolectin or CD31. In fact, pathologic neovascular tufts usually do not have a fully formed lumen and are often inadequately perfused.

In this respect, the first question to be asked of Chen et al.[1] is why our additional results supporting that the β-AR blockade may be protective against retinal angiogenesis in OIR were not discussed in their work. In particular, the finding that the retinal norepinephrine is upregulated by hypoxia adds some evidence to the hypothesis that increased levels of norepinephrine may overstimulate β-ARs potentially acting as a proangiogenic switch.[4] In addition, Martini et al.[5] used CD31 immunohistochemistry to demonstrate that ICI 118551, a specific inhibitor of β_2-ARs, which are blocked by nonselective β-AR blockers, such as propranolol, reduces retinal neovascularization. Martini et al.[5] also observed that ICI 118551 restores electroretinographic responses impaired by hypoxia, suggesting an important role of β_2-ARs in regulating retinal function. Moreover, Dal Monte et al.[4] used appropriate immunohistochemical methods to demonstrate that β_2-AR desensitization after prolonged administration of the β-AR agonist isoproterenolol reduces retinal neovascularization, suggesting that hypoxia-induced retinal neovascularization depends at least in part on increased sympathetic transmission, as a reduction of sympathetic drive inhibits OIR.

In light of these results, the second question to be asked of Chen et al.[1] is why they ascribed us the opinion that propranolol suppresses VEGF production via activation of β_3-ARs. Our impression is that our results have been not correctly understood, probably because we were not sufficiently clear. We indeed demonstrated that β_3-ARs are localized to retinal vessels and that their expression is drastically upregulated by hypoxia.[3] Interestingly, Chen et al.[1] also found that β_3-ARs are highly upregulated in OIR; however, we agree with these authors that the functional role of this upregulation may not necessarily be related to pathologic angiogenesis. We also agree with Chen et al.[1] that retinal neovessels cannot be considered a significant source of VEGF in retinopathy and that VEGF is secreted mostly by neuroglial cells, which are localized in the neurosensory layers of the retina.[6] In this respect, Martini et al.[5] demonstrated that β_2-ARs are localized to Müller cells, which are critically involved in VEGF accumulation in response to hypoxia,[7,8] suggesting the possibility that β_2-ARs may play a role in regulating VEGF production by these cells.[3,5]

Assuming that the validity of the measurements performed by the two groups of studies is unquestionable, we are now tempting to reconcile the results of our study demonstrating beneficial effects of propranolol in OIR mice[3] with those of Chen et al.,[1] showing that propranolol fails to prevent the development of retinopathy.

An accurate analysis of the two groups of studies revealed that a main difference between them is the use of different mouse strains, that is, the C57BL/6J strain in Ristori et al.,[3] Martini et al.,[5] Dal Monte et al.,[4] and the 129S6 (129S6/SvEvTac) strain in Chen et al.[1]

Smith et al.[9] developed the oxygen-induced retinopathy model in C57BL/6J mice, defining for the first time a model with reproducible and quantifiable proliferative retinal neovascularization. This model has become the most used animal model of ROP. Most of the data obtained to date have been collected from different wild-type strains, among which C57BL/6 is the most widely used. The mouse strain used in the OIR model is not of secondary importance. For instance, the time course of vascular development in wild-type mice varies considerably among the strains.[10] In addition, as is also stated by the same authors involved in the study of Chen et al.,[1] "different severities of OIR have been found to develop in different wild-type strains."[11]

On the basis of the literature, our hypothesis is that the 129S6 strain used by Chen et al.[1] does not respond to propranolol because this strain develops an angiogenic response, which is resistant to β_1- and β_2-AR blockade. In this respect, the expression of pro- and anti-angiogenic growth factors has been shown to differ between mice of 129 and C57BL/6 background, which may determine heterogeneity in the angiogenic response and, potentially, susceptibility to angiogenesis-dependent diseases.[12] About that, it has been recently reported that rats of different genetic background may have different susceptibility to OIR with respect to pro-angiogenic factor expression during hyperoxic exposure.[13] In addition, not only different strains, but also the same strain may respond differently to hypoxia depending on the vendor. In particular, C57BL/6 mice from Jackson Laboratories are more susceptible to OIR than their C57BL/6 counterparts from Taconic Farms, probably because of differential accumulation of spontaneous mutations.[11] Moreover, in mice of 129 background, β-AR activity is higher than that in mice of C57BL/6 background[14] and this may explain the predisposition of 129S6 mice to develop a more aggressive neovascularization.

Interestingly, some phenotypic differences between C57BL/6 and 129/SV mice can be explained by the different genetic background between these two strains. For instance, p53 knock-out mice of C57BL/6 background have severe ocular abnormalities that are reminiscent of the histopathologic changes reported in human eyes with persistent hyperplastic primary vitreous, whereas p53 knock-out mice of 129/SV

background do not show this aberrant ocular phenotype.[15] This is probably because either 129/Sv mice must have alleles that can compensate for the loss of p53 function, whereas C57BL/6 mice do not, or that C57BL/6 mice, but not 129/SV mice, have susceptibility alleles for this phenotype.

Although these arguments are difficult to extrapolate to the human situation, a wide variability in clinical expression of ROP was demonstrated in human infants, in whom the incidence of progression to severe ROP occurs more commonly among non–African American infants than in African American infants.[16] Recently it has been hypothesized that this racial difference in ROP susceptibility may be attributable to β-blocker receptor polymorphisms, which exist in many African American people with the effect to render them "β blocked."[17] Therefore, it is likely that different animal strains as well as different human races are differentially sensitive to β-AR blockade depending on different genetic factors.

A main point raised by Chen et al.[1] is the importance of evaluating the efficacy of propranolol treatment in vulnerable premature infants with ROP who are still in a fragile state of incomplete development. Propranolol is commonly used to treat congestive heart failure in newborns and infants with congenital heart disease[18] or hypertrophic obstructive cardiomyopathy.[19] Propranolol is also used in newborns as an antiarrhythmic drug to treat supraventricular tachycardia,[20] in infants either in term or preterm to treat neonatal thyrotoxicosis,[21] and in newborns with Fallot's tetralogy to treat hypercyanotic spells.[22] In addition, propranolol is used in newborns with hypertension.[23] Propranolol is generally well tolerated in newborns and infants; however, we completely agree with Chen et al.[1] that the high incidence of complications associated with prematurity could make this treatment unsafe, especially if propranolol is systemically administered. Thus, any study related to the effects of propranolol therapy must address possible adverse effects of this drug. In the PROP-ROP study protocol,[24] a strict monitoring of metabolic, respiratory, hemodynamic, and neurodevelopmental outcome was established, together with an evaluation of possible efficacy of propranolol on the stage 2 ROP. Although the stage 2 ROP is a mild stage with high rates of spontaneous regression,[25] only the demonstration of the efficacy of a treatment in a precocious phase of a disease might open the way to its prevention.[17]

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