

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

We appreciate the response by Filippi et al¹ concerning our recent study.² We share a common concern for the safety of premature infants in any trial who might be exposed unnecessarily to systemic treatment with propranolol, a nonselective beta-adrenergic receptor blocker that has not yet been evaluated thoroughly in preterm infants.³ In older infants propranolol often is well tolerated but may be associated with severe side effects.^{4,5} We felt it was important to have more than one preclinical animal study to lay the foundation for (or against) any clinical trial testing propranolol as a potential treatment for retinopathy of prematurity (ROP) in preterm infants, particularly for stage 2 ROP,⁶ because at stage 2, most ROP regresses spontaneously⁷ and does not require ablative treatment.⁸

Using the same oxygen-induced mouse model of ROP, we found that propranolol did not inhibit retinal neovascularization,² while Ristori et al. reported a positive response.⁹ We used a different, more commonly applied method with a vascular endothelial cell specific stain isolectin B₄ to visualize and measure vasculature quantitatively after dissection of retina, which yields more complete staining of all parts of retinal vasculature, including pathologic neovascularization. In addition, we used a different mouse strain 129S6 (129S6/SvEvTac) known to have a more robust neovascular response compared to C57BL/6J strain to highlight any potential treatment effects of propranolol. When neovascular responses are stronger, treatment effects are detected and quantified more easily. These also were chosen because 129S6 mice have a higher beta-adrenergic receptor activity,¹⁰ and thereby are more rather than less responsive in the central nervous system to beta-adrenergic receptor inhibition by propranolol compared to C57BL/6J mice.¹⁰ In these 129S6 mice subjected to induction of retinopathy in the ROP model, we tried to optimize any possible propranolol suppression of neovascularization with several different delivery routes and a wide range of doses (from standard human dose to up to 30 times greater). We found that propranolol is not effective in suppressing ROP, although we cannot rule out the possibility that unanticipated genetic differences in 129S6 versus C57BL/6J mice might contribute to the different results in the two studies. We suggest that propranolol should be tested in C57BL/6 mice (and other strains) using dosing comparable to the human trial, and standard evaluation of neovascularization and vaso-obliteration in retinal flat mounts with induced retinopathy,^{11,12} to provide the strongest basis possible for a clinical evaluation.

Additional studies by Bagnoli et al.^{13,14} have examined different experimental beta-adrenergic receptor inhibitors and agonists, such as ICI 118,551 and isoproterenol, in oxygen-induced retinopathy using the more standard method of angiogenesis staining and quantification. These studies certainly are helpful and necessary for identifying any specific beta-adrenergic receptors that might regulate retinal angiogenesis. However, since these drugs (ICI 118,551 and isoproterenol) are not in use in human ROP trials, and the doses needed in these studies are very high to see an inhibitory effect on retinal neovascularization, we did not consider them in our study. ICI

118,551 is not used in the clinic, and in laboratory animal studies it usually is used at 1 to 2 mg/kg body weight (up to 30 mg/kg),¹⁴ while in the published study 30 mg/kg was required to see an effect in retinopathy.¹⁴ Isoproterenol, if used as treatment for atrioventricular heart block, is administered at 0.2 mg subcutaneously as an initial dose for human adults (~50–70 kg), and in the retinopathy study it was used at 0.5 mg/kg subcutaneously for a mouse,¹³ which is more than a 100-fold higher dose. Unfortunately, the study on isoproterenol¹³ was published after our report was accepted and went to press, and therefore could not be cited when we prepared our manuscript. This is an interesting study. However, together these studies show seemingly paradoxical results that beta-adrenergic receptor-2 antagonist (ICI 118,551) and beta-adrenergic receptor-2 agonist (isoproterenol) lead to similar effects on dampening retinal hypoxia response and inhibition of neovascularization in oxygen-induced retinopathy. This work may uncover some interesting biology of sympathetic control of retinal angiogenesis beyond the suggested beta-adrenergic receptor-2 desensitization effect by isoproterenol, that needs further investigation.

In light of the different efficacy of propranolol reported in mouse oxygen-induced retinopathy, we suggest that further studies are needed to provide the strongest foundation possible for a clinical trial. If propranolol is ineffective in mice in OIR or is effective in only some mouse strains, then that variability in treatment response should be taken into account in planning clinical trials. We hope that future work will be able to elucidate the differences reported here further, and more importantly, address the safety and efficacy concerns of propranolol use in ROP patients.

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References

1. Filippi L, Dal Monte M, Bagnoli P. Different efficacy of propranolol in mice with oxygen-induced retinopathy: could differential effects of propranolol be related to differences in mouse strains? *Invest Ophthalmol Vis Sci.* 2012;53:7421–7423.
2. Chen J, Joyal JS, Hatton CJ, et al. Propranolol inhibition of beta-adrenergic receptor does not suppress pathologic neovascularization in oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci.* 2012;53:2968–2977.
3. Hard AL, Hellstrom A. On the use of antiangiogenic medications for retinopathy of prematurity. *Acta Paediatr.* 2011;100:1063–1065.
4. Lawley LP, Siegfried E, Todd JL. Propranolol treatment for hemangioma of infancy: risks and recommendations. *Pediatr Dermatol.* 2009;26:610–614.
5. Bonifazi E, Acquafredda A, Milano A, Montagna O, Laforgia N. Severe hypoglycemia during successful treatment of diffuse hemangiomatosis with propranolol. *Pediatr Dermatol.* 2010; 27:195–196.

6. Filippi L, Cavallero G, Fiorini P, et al. Study protocol: safety and efficacy of propranolol in newborns with Retinopathy of Prematurity (PROP-ROP): ISRCTN18523491. *BMC Pediatr.* 2010;10:83.
 7. Fledelius HC. Retinopathy of prematurity in Frederiksborg County 1988-1990. A prospective investigation, an update. *Acta Ophthalmol Suppl.* 1993;210:59-62.
 8. Good WV. Final results of the Early Treatment for Retinopathy of Prematurity (ETROP) randomized trial. *Trans Am Ophthalmol Soc.* 2004;102:233-248; discussion 248-250.
 9. Ristori C, Filippi L, Dal Monte M, et al. Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: antiangiogenic effects of beta-adrenoreceptor blockade. *Invest Ophthalmol Vis Sci.* 2011;52:155-170.
 10. Schimanski LA, Ali DW, Baker GB, Nguyen PV. Impaired hippocampal LTP in inbred mouse strains can be rescued by beta-adrenergic receptor activation. *Eur J Neurosci.* 2007;25:1589-1598.
 11. Connor KM, Krah NM, Dennison RJ, et al. Quantification of oxygen-induced retinopathy in the mouse: a model of vessel loss, vessel regrowth and pathological angiogenesis. *Nat Protoc.* 2009;4:1565-1573.
 12. Stahl A, Connor KM, Sapiha P, et al. Computer-aided quantification of retinal neovascularization. *Angiogenesis.* 2009;12:297-301.
 13. Dal Monte M, Martini D, Latina V, Pavan B, Filippi L, Bagnoli P. Beta-adrenoreceptor agonism influences retinal responses to hypoxia in a model of retinopathy of prematurity. *Invest Ophthalmol Vis Sci.* 2012;53:2181-2192.
 14. Martini D, Monte MD, Ristori C, et al. Antiangiogenic effects of beta(2) -adrenergic receptor blockade in a mouse model of oxygen-induced retinopathy. *J Neurochem.* 2011;119:1317-1329.
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