Cholesterol homeostasis in the retina: seeing is believing

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The means by which various tissues throughout the body maintain a particular steady-state level of cholesterol—balancing de novo synthesis, uptake of exogenous cholesterol from circulating lipoproteins, and export of cholesterol from the tissue to the circulation—is a complex process known as cholesterol homeostasis [reviewed in (1, 2)]. The set point for cholesterol varies from one tissue to the next and may be influenced significantly by numerous factors, including species, age, sex, genetics, nutritional status, diurnal and circadian rhythms, and epigenetic factors [reviewed in (1–6)]. Whereas oxidized derivatives of cholesterol and biogenically related sterols (oxysterols) historically have been considered to play a major role in cholesterol homeostasis in vivo (5), the results of more recent studies have challenged this concept [see (7)]. In the brain, cholesterol homeostasis is complicated further by the fact that, unlike most extrahepatic tissues, its ability to take up cholesterol from circulating lipoproteins is stringently curtailed by the blood-brain barrier [reviewed in (8)]. Also, unlike most other tissues, the lifetime of cholesterol in the brain is extraordinarily long: some cholesterol molecules in the brain can persist for years instead of hours or days. Despite the fact that brain cholesterol has been characterized as having “a long secret life” (8), the life of cholesterol in the retina is even more secretive. Although the retina is a bona fide part of the central nervous system and an extension of the brain, comparatively little is known about the synthesis and turnover of cholesterol in the retina or about the cellular and molecular regulatory mechanisms that govern cholesterol homeostasis in the retina, compared with the brain [reviewed in (9–11)].

In this issue of the Journal of Lipid Research, Zheng et al. (12) provide fundamental information that sheds new light on cholesterol homeostasis in the retina, with insights that have potentially important clinical implications for understanding and possibly treating certain progressive blinding diseases such as age-related macular degeneration. In particular, they focused on transcriptional regulatory mechanisms involved in SREBP [reviewed in (2, 13)] and LXR/FXR [reviewed in (14)] target gene expression in the mouse, using standard approaches of both dietary (high-fat/high-cholesterol) and pharmacological (simvastatin) manipulation of cholesterol pathway-related genes, as well as treatment with the LXR/FXR agonist TO901317 (15). Hence, these manipulations targeted the input side of cholesterol homeostasis, i.e., de novo synthesis and cholesterol uptake. Also, these experiments were performed using both wild-type and Cyp27a1-null mice, the latter in order to disrupt the output side of cholesterol homeostasis, because CYPT27A1 (sterol 27-hydroxylase, a cytochrome P450 enzyme) has been shown by this group previously to be present in the retina and to be critical for oxidation/metabolism and export of cholesterol from the retina (16, 17). This paper contains an extensive amount of both primary and supplementary data and complements and extends a recently published parallel study by these authors and their collaborators, which employed human donor eyes and tissues dissected therefrom (18). This commentary will address a few of the highlights in the context of current knowledge in the field.

Neither a high-cholesterol diet nor orally administered simvastatin had more than a modest impact on the steady-state levels of cholesterol in the retina [combined neural retina and retinal pigment epithelium (RPE), in this study], nor did these treatments have any substantive effect on the expression of cholesterol pathway-related genes or on visual pathway-related genes in the retina under conditions where serum cholesterol levels and liver cholesterol pathway gene expression were markedly affected,

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as expected (positive control). Some of the genes [see Fig. 2 in (12)] exhibited substantial sexual dimorphism (>10-fold higher expression in retinas from male vs. female mice), but none of these were involved in sterol biosynthesis. Taken together, these results suggest that, contrary to what might be expected on the basis of "conventional wisdom" and findings obtained with other bodily tissues (particularly liver) under comparable treatment conditions, the SREBP-SCAP-INSIG system appears to be, at best, a minimal player in the transcriptional regulation of cholesterol homeostasis in the mouse retina. This finding is consistent with those reported in the parallel study of human retina (18). As the correlative immunohistochemistry, Western blot, and PCR array data clearly show, this was not due to lack of expression of SREBP-related gene products in the retina. Rather, posttranscriptional mechanisms, particularly as involve HMG-CoA reductase (HMGR), the major rate-limiting enzyme in cholesterol biosynthesis [reviewed in (19)], appear to be dominant in regulating the cholesterol content of the mouse retina.

With regard to the effects of oral statins such as simvastatin on retinal cholesterol content, the present study's results are consistent with, but do not definitively prove, that pharmacologically significant levels of the drug actually were achieved in the retina under the conditions employed. A more compelling demonstration of such would be to perform intravitreal injection of a suitably labeled de novo precursor of cholesterol (e.g., mevalonate, acetate) in simvastatin-treated versus untreated control mice and then compare the specific activities of cholesterol isolated from the retinas. Intravitreal injection of [3H]acetate in rats, recovering the majority of the incorporated radiolabel in cholesterol isolated from the neural retina, has been used to demonstrate the capacity of the neural retina to generate its own cholesterol de novo (20). However, due to the very small size (< 2 mm in diameter) and anatomical features (>75% of the vitreal volume is taken up by the lens) of the mouse eye, such an experiment is not only technically challenging but also limited by the amount of precursor (and volume) one can inject with retention in the eye. Also, the fact that systemically administered simvastatin was not effective in altering the steady-state cholesterol content of the retina is not all that surprising considering: a) orally administered statins largely are taken up and sequestered by the liver; b) the residual statin that escapes the liver undergoes marked dilution in the circulation; and c) the retina would compete with all other bodily tissues for uptake of circulating statin (21). In addition, even direct intravitreal injection of cholesterol pathway inhibitors (e.g., lovastatin, NB-598) in rat eyes has been shown to have no effect on the steady-state levels of cholesterol in the retina despite marked inhibition of local (retinal) de novo cholesterol synthesis (22). [Interestingly, intravitreally injected lovastatin can provoke massive retinal degeneration in adult rats, a fact that has been attributed to inhibition of protein prenylation rather than disruption of cholesterol synthesis (22).]

One very interesting and novel finding in this study was that orally administered TO901317 (single treatment, 50 mg/kg dose) caused statistically significant upregulation of several genes in the retina, both in wild-type and Cyp27a1-null mice, notably apoD [encoding a lipoprotein implicated in lipid and other hydrophobic molecule transport (22)], Idol [encoding inducible degrader of the LDL receptor (23)], and Rpe65 [encoding the rhodopsin isomerase resident in the RPE and requisite for generating the chromophore (11-retinaldehyde) of the visual pigment, rhodopsin (24)]. Heretofore, the only other report extant pertaining to the effects of oral TO901317 on the retina is a very recent study (25) showing that this pharmacological treatment [same dosage as used in (12)] caused activation of LRXα and dramatic reduction of ocular inflammation in a mouse model of experimental autoimmune uveitis. That study examined the expression of various LRX target genes (notably, pro-inflammatory genes) but not those involved in cholesterol homeostasis. Unlike the case for apoD and Idol, which are known target genes of LRXs, this is the first report implicating RPE65 as a target gene modulated by LRXs. That said, it’s not altogether clear that this is a direct drug effect, as noted by the authors. One way to resolve this might be to assess effects of LRX agonists on Rpe65 expression in vitro in cultured primary or transformed RPE cells. It also would be of interest to see whether or not such orally administered synthetic LRX agonists can perturb the steady-state level or the cellular distribution (among the various, distinct histological layers of the retina) of cholesterol in the retina. As an aside, it’s tempting to speculate that driving Rpe65 expression with a suitable LRX agonist might provide a new means of therapeutic intervention for retinal degenerations involving loss-of-function mutations in the Rpe65 gene or other visual cycle defects involving perturbed retinoid metabolism (26). The operative word here is “suitable”, as TO901317 and related compounds are known to have some undesirable off-target effects (27).

The information afforded by PCR arrays and correlative Western blots of whole tissue, especially considering the cellular complexity, heterogeneity, and architectural stratification of the retina, has obvious limitations. While the aforementioned data afford an understanding of what genes are expressed and how their expression responds to various treatments as employed in the present study, they are not informative with respect to what cell types express which genes or how the expression of those genes differs among the various cell types and cellular layers of the retina. To partially get at that issue, the authors have performed immunohistochemistry [see Fig. 3 (12)] using established, characterized antibodies raised against some of the key players in the cholesterol homeostasis machinery. Some general patterns have emerged. For one, the spatial localization of the cholesterol pathway tends to favor the inner retina (which contains the second- and third-order neurons of the visual pathway, as well as the cell bodies of the Müller glia and the astrocytes), rather than the outer retina (which contains the rod and cone photoreceptor cells, the first-order neurons in the visual pathway) [see Fig. 1B (12) for an instructive schematic of retinal architecture]. Notably, HMGR is immunolocalized predominantly to the inner nuclear layer, as well as more diffusely in a compartment closer to the vitreoretinal
interface [nerve fiber layer (NFL) and ganglion cell layer (GCL)]. This would suggest that most of the de novo synthesis of cholesterol in the retina takes place concomitantly in the inner retinal layers. On first principles, this may be surprising, considering the fact that photoreceptor cells constitute the overwhelming majority of cells in the retina and they undergo a prodigious amount of membrane biogenesis (with cholesterol as a component) on a daily basis to support renewal of the photoreceptor outer segment [reviewed in (28)]. However, it's also known that the majority of the sterol content of the vertebrate retina resides in the plexiform layers (particularly the inner plexiform layer), where synaptic connections are made, and that the photoreceptor outer segments are comparably cholesterol-deficient relative to typical plasma membranes [see (29)]. While the HMGCR immunostaining likely represents inner retinal neurons, it's also possible that at least some of this labeling is due to Müller glia; this could be either confirmed or ruled out by showing concordance (or lack thereof) of immunolabeling with a Müller cell marker such as glutamine synthetase as was done for ApoD [see Fig. 9D (12)] in this study. The relevance of this resides in the fact that glial-derived cholesterol has been implicated as an important source of neuronal cholesterol via glia-neuron lipoprotein transport, particularly for making new synaptic membranes [reviewed in (30)]. Correlative high-resolution in situ hybridization and electron microscopic immunogold analyses would be desirable adjuncts to further define the cell types and cellular compartments where cholesterol pathway genes are expressed and their gene products are localized in the retina.

While the retina is often thought of as “a simplified version of the brain”, there are some key differences that may significantly impact a comparison of cholesterol homeostasis in the two tissues. Importantly, the blood-retina barrier is distinct from the blood-brain barrier in that the capillary network (i.e., the choriocapillaris) of the choroidal blood supply that nourishes the outer retina in all vertebrates including humans is fenestrated. Hence, it is permeable (unlike the vasculature of the brain) to blood-borne lipoproteins that not only transport cholesterol, cholesteryl esters, and other lipids and lipophilic substances to the retina (the input side of homeostasis), but also to those that help to carry such molecules away once they are exported from the retina (the output side). Second, the retinal photoreceptor cell is arguably the most highly differentiated and specialized cell in the entire body and utilizes a disproportionate percentage of its total cellular energy, biosynthetic capacity, and reducing equivalents to support the daily renewal (both synthesis and turnover) of its outer segment, the membranous compartment of the cell that contains the phototransduction machinery requisite for vision. This process is aided and abetted by the photoreceptor’s intimate relationship with its neighbor, the RPE. Outer segment membrane turnover represents a sizeable portion of the retina’s overall turnover of cholesterol. There are no equivalent cell types or relationships in the brain.

The present study now affords a detailed comparison of mouse versus human retinas with regard to cholesterol homeostasis (12, 18), something that undoubtedly is underway in the Pikuleva lab currently. However, one must keep in mind some of the very distinct differences between these two species with regard to retinal architecture and physiology [reviewed in (31)], which may affect some of the details regarding cholesterol synthesis, metabolism, utilization, and export. First, unlike the human retina, which has a distinct, highly cone-enriched geographic zone known as the macula (with a pure cone fovea at its center), the retina of rodents such as mice and rats has no macula or fovea. Second, the photoreceptor population in a mouse or rat retina is comprised by about 97% rod cells; the remaining cone photoreceptors are not concentrated in any particular zone but do exhibit dorsal-ventral distribution differences as a function of the cone subtype. Third, humans live many decades, while the mouse lives perhaps a year or two. These features may limit the extent to which one can credibly model human retinal physiology in a mouse, especially pathobiological conditions such as age-related macular degeneration. Fourth, due to the marked difference in eye size between humans and mice, the human retina is far more expansive in area than is the mouse retina. Finally, humans and mice differ in their serum lipoprotein composition and class distribution, with mouse being far more HDL-dominated compared with humans (32); this may affect both the input and output sides of cholesterol homeostasis in the two species. These considerations aside, the fundamental architectural, molecular, and cell biological features, as well as the tissue biochemistry of the human and mouse retina are very similar. The mouse also is a convenient, affordable, and tractable laboratory animal amenable to both in vivo and in vitro experimental manipulation, including genetic modification, making it an attractive model system to aid our further understanding of cholesterol homeostasis in the retina. With the present publication by Zheng et al. (12), we are further along in this quest than ever before, and as the related recent review by Pikuleva and Curcio promises, “the best is yet to come.”

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