Delivery of Retinoid-Based Therapies To Target Tissues†

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Abstract

Through its various metabolites, vitamin A controls essential physiological functions. Both naturally occurring metabolites and novel retinoid analogues have shown effectiveness in many clinical settings that include skin diseases and cancer, and in animal models of human conditions affecting vision. In this review, we analyze several potential retinoid-based therapies from the point of view of drug metabolism and transport to target tissues. We focus on the endogenous factors that affect the absorption, transport, and metabolism of retinoids by taking into account data obtained from the analysis of animal models that lack the enzymes or proteins involved in the storage and absorption of retinoids. We also discuss findings of toxicity associated with retinoids in an effort to improve the outcome of retinoid-based therapies. In this context, we review evidence that esterification of retinol and retinol-based drugs within target tissues provides one of the most efficient means to improve the absorption and to reduce the toxicity associated with pharmacological doses of retinoids. Future retinoid-based therapeutic strategies could involve targeted delivery mechanisms leading to lower toxicity and improved effectiveness of retinoids.

Vitamin A, retinol, plays essential roles in many biological processes including vision, immunity, growth, development, and cellular differentiation. The various functions of vitamin A are carried out by several known physiologically active metabolites including 11-*cis*-retinal, the visual chromophore (1), and all-*trans*-retinoic acid (RA[1]), which controls gene expression through the RA receptor (RAR) (2,3). Other retinol metabolites that have shown biological activities *in vitro* are the 9-*cis*-isomer of RA, which activates both retinoid X receptors (RXRs) and RARs (4), and the *retro*-retinoids, anhydroretinol (AR), and 14-hydroxy-4,14-*retro*-retinol (14-HRR), which are involved in the regulation of lymphocyte proliferation (5–7). All-*trans*-13,14-dihydroxy-retinol was shown to have activity comparable to that of 14-HRR in supporting the proliferation of promyelocytic HL-60 cells and in the activation of T cells (8). The conversion of all-*trans*-retinol to all-*trans*-13,14-dihydroretinol by the recently described

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[1]Abbreviations: A2E, pyridinium bis-retinoid; ADH, medium-chain alcohol dehydrogenases; ADRP, autosomal dominant retinitis pigmentosa; apoE, apolipoprotein E; AR, anhydroretinol; ARAT, acyl-CoA–retinol acyltransferase; CRBP, cellular retinol-binding protein; DGAT1, diacylglycerol acyltransferase 1; 4-HPR, *N*-(4-hydroxyphenyl)retinamide (or fenretinide); 14-HRR, 14-hydroxy-4,14-*retro*-retinol; LCA, Leber congenital amaurosis; LPL, lipoprotein lipase; LRAT, lecithin–retinol acyltransferase; RA, retinoic acid; 13-*cis*-RA, 13-*cis*-retinoic acid (or isotretinoin, Accutane); RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RBP, retinol-binding protein; RetSat, all-*trans*-retinol–all-*trans*-13,14-dihydroretinol saturase (or retinol saturase); RPE65, retinal pigmented epithelial-specific 65 kDa protein; RXR, retinoid-X receptor; SDR, short-chain dehydrogenase/reductase; STRA6, stimulated by retinoic acid 6; TTR, transthyretin; UGT1, UDP-glucuronosyltransferase 1; vitamin A, all-*trans*-retinol; VAD, vitamin A deficiency; VLDL, very low-density lipoprotein.

enzyme all-*trans*-retinol–all-*trans*-13,14-dihydroretinol saturase (retinol saturase or RetSat) enzyme leads to the formation of all-*trans*-13,14-dihydroretinol (9), which can be further oxidized to all-*trans*-13,14-dihydrore-tinoic acid (10). The metabolism of vitamin A in relation to the formation of known or potential endogenous bioactive retinoids is shown in Figure 1.

Therapeutic applications for retinoids have made use of both naturally occurring retinoids and synthetic agonists and antagonists of RAR and RXR. Supplementation with all-*trans*-retinol is used in developing countries to correct vitamin A deficiency (VAD). This treatment is one of the safest and most efficacious therapeutic uses of retinoids and has enormous worldwide impact, saving millions of lives every year at a cost of pennies per treated individual (11). All-*trans*-RA (tretininoin, Retin-A) and 13-*cis*-RA (isotretinoin, Accutane) are clinically used in the treatment of severe acne and other dermatological conditions (12). Retinoids like all-*trans*-RA, 13-*cis*-RA, *N*-(4-hydroxyphenyl)retinamide (4-HPR or fenretinide), and non-retinoid synthetic subtype specific RAR and RXR-agonists and modulators are used in the treatment and chemoprevention of various forms of cancer (13–16). With respect to vision, there are several proposed retinoid-based therapies with potential in the treatment of autosomal dominant retinitis pigmentosa (ADRP), Leber congenital amaurosis (LCA), macular degeneration, or Stargardt disease (17). Supplementation of the substitute chromophore 9-*cis*-retinal can reconstitute the visual pigment in animal models of LCA (18–21). Inhibitors of the visual cycle, such as retinylamine (22) and 13-*cis*-RA (23), and reduction of serum retinol using 4-HPR (24) have shown effectiveness in animal models of Stargardt disease and macular degeneration by slowing the rate of the visual cycle and preventing the accumulation of all-*trans*-retinal and the production of phototoxic byproducts such as pyridinium bis-retinoid (A2E) (17). Some of the currently investigated retinoid-based drugs and therapeutic targets are depicted in Figure 2.

Retinoid therapies have demonstrated clinical efficacy as treatments for many debilitating diseases; however, one must overcome significant obstacles associated with the well-established teratogenic and toxic effects of retinoids. Many of these obstacles could be overcome if the delivery mechanism is more specific. In this review, we focus on factors affecting the delivery of retinol and other retinoids to specific target tissues.

ABSORPTION OF RETINOIDS

Retinoids delivered orally are efficiently absorbed by the intestine. Retinyl esters are hydrolyzed to retinol within the intestinal lumen by the actions of pancreatic triglyceride lipase (25) or at the intestinal brush border by the membrane-bound enzyme phospholipase B (26, 27). The newly generated retinol as well as the retinol obtained from the diet or supplements is taken into the enterocyte as retinol. Because of the lipophilic character of retinol, absorption is enhanced by a fatty meal (28). Within the enterocyte, newly absorbed retinol is re-esterfied through the actions of the enzyme lecithin retinal–acyl transferase (LRAT), which catalyzes the transacylation of a fatty acyl group from lecithin to retinol (29–33). Cellular retinol-binding protein II (CRBP-II), a member of the family of intracellular lipid-binding proteins, which binds retinol with high affinity, helps facilitate retinol uptake into the body providing the retinol as a substrate for LRAT (34,35). Although LRAT is the predominant enzyme for catalyzing retinyl ester formation within the intestine, a study of $Lrat^{-/-}$ mice indicates that an acyl-CoA-dependent enzyme, referred to as acyl-CoA–retinol acyltransferase (ARAT), may also be involved in retinyl ester formation in the liver, mammary gland, or adipose tissue (32,36). Ultimately, the retinyl ester newly synthesized by LRAT and/or ARAT is incorporated along with other dietary lipids into nascent chylomicrons that are then secreted from the enterocyte into the lymphatic system (37).

TRANSPORT OF RETINOIDS TO TARGET TISSUES

Role of Chylomicrons and Their Remnants

Nascent chylomicrons in the lymphatic system enter the general circulation through the thoracic duct where they undergo metabolism to form chylomicron remnants. The conversion of chylomicrons to chylomicron remnants involves the actions of lipoprotein lipase (LPL), which hydrolyzes chylomicron triglycerides to free fatty acids, resulting in a smaller particle and facilitating the recruitment to the lipolyzed particle of several apolipoproteins, especially apolipoprotein E (apoE), that are needed for receptor-mediated uptake of the remnants by the liver (38,39). About 66–75% of retinyl esters contained in chylomicrons are taken up by the liver and resecreted into the circulation as retinol bound to serum retinol-binding protein (RBP) or stored as esters in hepatic stellate cells (also called Ito cells, lipocytes, or fat-storing cells) (40,41). The remaining 25–33% of the retinyl esters from chylomicrons are taken up by target tissues that include adipose tissue, heart, muscle, lungs, reproductive organs, and bone marrow. The clearance of chylomicron retinoids appears to involve the actions of LPL (42). This reaction most likely involves LPL-catalyzed retinyl ester hydrolysis but possibly also the well-characterized actions of LPL in facilitating whole particle uptake by cells (42,43). The importance of chylomicron-derived retinoid toward meeting target tissue needs is evidenced by RBP-deficient mice, which, aside from a mild vision phenotype, are phenotypically normal (44–46). As long as RBP-deficient mice are provided sufficient quantities of vitamin A in the diet, they are able to maintain normal retinoid-dependent functions and even to acquire retinyl ester stores in target tissues. When these mutant mice are deprived of dietary retinoid, they are at increased risk of developing symptoms of VAD because they are not able to mobilize hepatic retinyl ester stores through the actions of RBP (44–46). Thus, the delivery of retinoids by chylomicrons and their remnants can be an important route for retinoid delivery to target tissues.

Role of RBP in the Delivery of Retinol

As mentioned earlier, unesterified retinol is transported in the blood as retinol bound to RBP (47). RBP is primarily synthesized and secreted from the liver, where the majority of the body's retinoid is stored (48,49). However, RBP also is synthesized and secreted in lesser amounts by extrahepatic tissues, including the eye, adipose tissue, kidney, lungs, and heart (50). RBP forms a one-to-one protein–protein complex with another blood protein transthyretin (TTR), which prevents renal clearance of the retinal–RBP by the glomerulus (51–53). Hepatic secretion of holo-RBP is needed for the efficient export of retinol from liver stores (44–46). There is some controversy regarding how retinol is taken up by cells from the retinal–RBP–TTR complex (54). Retinol rapidly dissociates from RBP and rapidly flip–flops across the plasma membrane (55). Moreover, the rate of dissociation of retinol from RBP is not rate limiting for retinol uptake by cells, which argues that it is not necessary to invoke the actions of a cell surface receptor for RBP to account for retinol uptake into cells (54,56,57). Yet, other studies suggest that retinol uptake into cells involves a cell surface receptor for RBP (54,58–62). One possible receptor is coded by a retinoic acid-responsive gene, *STRA6* (*st*imulated by *r*etinoic *a*cid-6) (63). The STRA6 protein is a multispanning membrane protein that confers RBP binding to transfected cells and mediates the uptake of retinol (62). STRA6 is expressed in several blood–organ barriers, such as Sertoli cells, the yolk sac, chorioallantoic placenta, choroid plexi (64), and RPE (62,64). Mutations in *STRA6* cause severe malformations, such as anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, and mental retardation (65). However, patients affected by deleterious mutations in the RBP gene suffer from night-blindness early in life but are otherwise unaffected (66). A similar phenotype is seen in $Rbp^{-/-}$ mice, manifested as visual impairment early in life (44). These observations argue for additional roles for the STRA6 protein during development that are independent of its role as a receptor for RBP (67).

The retinoid needs for all tissues aside from the eye can be fully met through chylomicron delivery of retinoid or retinoid bound to serum albumin, as is evidenced by the visual phenotype of the $Rbp^{-/-}$ mice (44). Thus, the eye may rely on the STRA6 receptor to ensure sufficient retinoid uptake from RBP to support the visual cycle. However, because the complete absence of RBP does not impair retinoid-dependent functions in other tissues, a cell surface receptor for RBP may not be needed to ensure the normal uptake of retinol by such tissues in times of sufficient dietary vitamin A intake.

RA Delivery by Serum Albumin

RA is far less hydrophobic than retinol or retinyl ester, and it is not absorbed in chylomicrons but rather transported via the portal system bound to albumin because binding to RBP hinders its interaction with TTR (68). Pharmacokinetic studies of many of the synthetic analogues of RA indicate that they are absorbed primarily via the portal system like the natural acidic retinoid forms (69). However, depending on the lipophilic nature of a synthetic retinoid, some of its absorption may involve uptake via nascent chylomicrons, or it may be taken up solely via the portal route. This absorption will depend on the chemical properties of the particular retinoid.

In well-nourished humans and animal models, the circulating levels of all-*trans*-RA and 13-*cis*-RA bound to albumin are approximately 0.1 to 0.4% of those of retinal–RBP during fasting (70). Yet, this circulating RA can contribute significantly to tissue pools of RA in well-nourished animals (71). Tracer kinetic turnover studies in rats have shown that, for most tissues, 5–30% of the RA present within the tissue is derived from the circulating RA pool, whereas the brain and liver derive greater than 80% of their RA from the circulating RA pool (71). Thus, despite the low levels of all-*trans*- and 13-*cis*-RA in circulation, these acids are rapidly taken up by cells and tissues. Albumin-bound RA can be an important delivery pathway through which cells/tissues normally acquire retinoid. Like retinol, RA undergoes dissociation from albumin prior to traversing the membrane and entering into the cell, and RA is known to flip–flop readily across membrane bilayers. The delivery of synthetic RA analogues via albumin is pharmacologically important, and presumably, this process mimics RA uptake. However, there is little information available in the literature regarding how synthetic retinoids enter cells/tissues from the circulation.

Other Routes of Retinoid Delivery

In humans as well as in animal models, retinyl esters are normally secreted at low levels (1–2% of those of retinal–RBP) from the liver bound to nascent very low-density lipoprotein (VLDL) (72). The physiologic significance of VLDL retinyl ester has not been systematically investigated, but, invoking similarities between VLDL and chylomicron metabolism, it seems likely that the retinyl ester contained within VLDL is destined for uptake by extrahepatic tissues. The circulation also contains low levels of retinol-*β*-glucuronides and retinoyl-*β*-glucuronides (levels similar to those of all-*trans*- and 13-*cis*-RA) that are fully water soluble (54,73,74), hence more easily excreted. Alternatively, these retinoid-*β*-glucuronides may be used by the body to satisfy tissue retinoid needs because they can be readily hydrolyzed to retinol and RA (54,73,74).

ROLE OF LRAT IN THE ABSORPTION AND STORAGE OF RETINOL

As is true for the liver and RPE, LRAT is quantitatively the most important enzyme for catalyzing retinyl ester formation in most tissues (20,32,75). Consequently, LRAT is essential for the accumulation of retinoids within the liver and eye. In the liver, retinyl esters are found predominantly in hepatic stellate cells, which represent the main cellular retinoid storage site in the body. RPE cells also represent an important storage of retinyl esters reserved mostly for use in the production of the visual chromophore. The importance of LRAT in allowing tissues

to accumulate and store retinol is evidenced by the complete absence of retinyl esters in nearly all of the tissues that have been examined in $Lrat^{-/-}$ mice. The one exception to this general statement is adipose tissue, where retinyl ester levels are 2- to 3-fold elevated in $Lrat^{-/-}$ mice compared to those in age- and gender-matched wild-type mice (20,32). Although adipocytes have long been known to be important cellular sites of retinyl ester accumulation in the body accounting for the second largest retinoid store after the liver, little is known regarding how retinol is processed and becomes esterified within this cell type (76–80). LRAT is not needed to catalyze retinyl ester formation in adipose tissue but it is not presently clear which enzyme is responsible for retinyl ester formation within this tissue. The diacylglycerol acyltransferase 1 (DGAT1) enzyme, which catalyzes the formation of triglyceride from diacylglycerol and an acyl-CoA, catalyzes acyl-CoA-dependent retinol esterification *in vitro*, hence exhibiting ARAT activity (32,81,82). However, DGAT1, although highly expressed in adipose tissue, does not seem to be the only enzyme involved in catalyzing retinyl ester formation, because adipose tissue retinyl ester levels are the same for $Lrat^{-/-}$ mice and $Lrat^{-/-}/Dgat1^{-/-}$ double knockout mice (Blaner, W. et al., unpublished observations). Thus, it remains unclear which other enzymes can catalyze retinyl ester formation and facilitate the accumulation of retinoid stores within adipose tissue.

In addition to allowing for retinoid accumulation within tissues, the sequestration of retinol as retinyl ester also serves to limit the generation of RA during development (83) and acts as a protective mechanism following the consumption of high doses of dietary vitamin A (84). Although the levels of serum retinol and RA are within the normal range, $Lrat^{-/-}$ mice are not capable of sequestering retinol as retinyl esters, thus exhibiting signs of RA toxicity when maintained on a vitamin A-sufficient chow diet. This observation is also supported by the upregulation in $Lrat^{-/-}$ mice of Cyp26 genes that are known to have central roles in the detoxification (catabolism) of RA (75). In addition, when we employed microarray analysis to identify other differentially expressed genes in the livers of $Lrat^{-/-}$ mice and their wild-type counterparts, we found that among the genes upregulated in the liver of $Lrat^{-/-}$ mice maintained on a standard chow diet were several isoforms of the UDP-glucuronosyltransferase 1 (UGT1) family of enzymes (85) (Table 1). The UGT1 locus is known to be subject to regulation by RA (86). As mentioned above, *β*-glucuronidation products of all-*trans*-RA, 13-*cis*-RA, 4-oxo-RA, and 5,6-epoxy-RA are normally present at low levels in the blood and bile and at greater concentrations following supplementation with large doses of retinol or RA (87–89). Elevated expression levels of UGT1 in the livers of $Lrat^{-/-}$ mice could be taken to suggest that the liver is sensing excessive levels of RA, leading to the induction of detoxification mechanisms.

Esterification by LRAT leads to active recruitment of the retinoid from circulation as well as the establishment of a long-lived storage pool. This property of LRAT can be manipulated for therapeutic advantage for the delivery of retinol and other retinoids with an alcohol functional group. Another potential retinoid-based therapeutic retinylamine has a reactive amine group instead of alcohol, yet it can also be recruited and stored via amidation by LRAT (90) (Figure 2). The conversion of retinylamine to pharmacologically inactive retinylamides occurs *in vitro* and *in vivo* in the liver and RPE and leads to the safe storage of this potent inhibitor, which can be released after hydrolysis back to free retinylamine. This transformation leads to a prolonged therapeutic effect and lower toxicity of this compound in comparison with those of other proposed visual cycle inhibitors such as 13-*cis*-RA (90). In summary, the presence of LRAT in the target tissue can ensure the efficient and active uptake of retinol and other retinoids with a functional hydroxyl group as well as retinoids that can be amidated by LRAT.

ROLE OF CRBPS IN THE UPTAKE OF RETINOID BY SPECIFIC TISSUES

Three cellular-retinol-binding proteins, CRBP-I, II, and III, are known to facilitate the esterification of retinoids by LRAT. The physiologic roles of LRAT and the CRBPs in the

absorption and storage of retinol have been best studied in knockout animal models. CRBP-I is expressed in the liver, kidney, and eye, and $Crbp\text{-}I^{-/-}$ mice display lower levels of hepatic retinyl esters and reduced numbers of lipid droplets in hepatic stellate cells than wild-type mice. $Crbp\text{-}I^{-/-}$ mice succumb more easily to symptoms of VAD than wild-type mice if they are maintained on a vitamin A-restricted diet (91). CRBP-II is expressed primarily in the intestine, and its lack in $Crbp\text{-}II^{-/-}$ mice results in decreased levels of stored retinoids and increased neonatal mortality, especially during dietary vitamin A restriction (92). CRBP-III is expressed in the heart, muscle, adipose, and mammary tissues (93). $Crbp\text{-}III^{-/-}$ mice have difficulty incorporating retinol into milk. In some cases, the loss of one form of CRBP can be partly compensated for by the upregulation of other CRBPs, as is the case of the upregulation of CRBP-III in the absence of CRBP-I and vice versa (94). Although the studies of $Crbp^{-/-}$ mice demonstrate that the absence of these proteins results in a much less severe metabolic phenotype than that observed with $Lrat^{-/-}$ mice, these studies do show that like LRAT, the CRBPs have an important role in facilitating retinol uptake, esterification, and storage by cells (20,32,91–93).

The critical role of LRAT in catalyzing the esterification and thus the uptake and storage of retinol suggests that this enzyme may be a useful target for assuring the specific uptake of a retinoid therapeutic agent by a target tissue. Coupled with the hydrolysis of the esters, this potentially provides a way to localize release of the drug within a specific cell type or tissue. The release of stored retinoid drugs from their ester pools depends on the activity of retinyl ester hydrolases in the respective target tissues. Manipulating this reaction may provide a means for a controlled delivery of therapeutic retinoids, which, in principle, would allow for the usage of lower administered doses and hence lower toxicity. Moreover, the expression of LRAT is subject to regulation by RA (95,96). STRA6 was described as an RA-responsive gene (63), and modulation of the levels of STRA6 in WiDr colon adenocarcinoma cells by RA leads to the increased uptake of retinol in these cells (62). As a result, induction of the expression of LRAT or STRA6 using RA could increase the delivery of retinol to target tissues. Indeed, co-administration of RA effectively increases the delivery of retinol to lungs (97,98). In addition, the expression of CRBP-I is also upregulated via RAR action (99), whereas CRBP-II expression is controlled via RXR (100). In fact, agonists of RXR have been shown to affect the esterification of retinol in certain cell types (101). Possibly, ectopic expression of STRA6, LRAT, and/or CRBP via gene therapy or transcriptional regulation could help ensure the delivery of retinoids to specific target tissues or cell types. Both LRAT and CRBP are downregulated in many tumor cell types (102–117); therefore, less retinol is available for oxidation to produce RA in cancer cells. Upregulation of the retinoid pool in tumor cells might lead to higher endogenous levels of RA at the tumor site enabling the anticarcinogenic activities of this agent. Future studies could examine the feasibility and therapeutic effectiveness of increasing the intracellular retinoid pool through the upregulation of the enzymes and accessory proteins involved in the generation of retinyl esters.

RETINOL TOXICITY AND THE SIDE EFFECTS ASSOCIATED WITH RETINOID TREATMENT

Excess levels of vitamin A lead to a wide range of deleterious effects on health. Retinoids present a serious teratogenic risk and can lead to craniofacial, cardiac, thymic, and central nervous system malformations (118), which are the most severe manifestations of toxicity associated with retinoid therapy. Teratogenic effects are seen for both natural and synthetic retinoids used in clinical trials (119,120), but these are less pronounced for preformed retinol supplements (121). The administration of retinyl palmitate to rats, mice, and hamsters led to exencephaly, with frequent occurrences of cleft palate, spina bifida, eye defects, hydrocephaly, and shortening of the mandible and maxilla (122–125). The teratogenic effects are believed to stem mainly from the transcriptional activities of retinoids, mediated via the activation of RAR

or RXR. The teratogenic consequences of retinoid supplementation during pregnancy depend on the stage of development, the nature of the retinoid metabolite, and the genetic and environmental factors (120). Caution must be exercised in prescribing retinoids to ensure that pregnancy is avoided during treatment (126). Additionally, the administration of retinoids to young animals and children results in delayed growth as a result of premature epiphyseal plate closure (127,128).

Less severe consequences of retinoid supplementation result in acute and chronic forms of retinoid toxicity. Acute intoxication with vitamin A occurs following the ingestion of large amounts of vitamin A over a short period of time, but cases of acute toxicity are relatively rare. The clinical effective dose for vitamin A administration in the case of degenerative eye diseases such as retinitis pigmentosa (15,-000 IU/day) is below the maximal recommended dose (25,-000 IU/day) and was shown to be well tolerated after 12 years of treatment (129,130). In the few documented cases, recovery from acute retinoid toxicity is rapid once intake is reduced (131,132). Chronic effects are manifested as drying, ulceration and desquamation of the skin, anorexia, anemia, and bone loss. Liver damage leading to fibrosis and hepatic stellate cell activation are both seen in patients affected by hypervitaminosis A (133). Upon activation, hepatic stellate cells lose their capacity to store retinyl esters and secrete collagen, leading to cirrhosis (132,134). Oxidation of retinol generates RA, which stimulates osteoclast formation and activity leading to bone resorption and hypercalcemia (135,136). Bone loss could represent a health issue in the elderly, leading to osteopenia, osteoporosis, and increased risk of hip fracture (137,138).

CONCLUSIONS

Vitamin A metabolites, including RAs and their synthetic derivatives, collectively known as retinoids, modulate the rates of transcription of the genes involved in the regulation of cell proliferation and differentiation and thus exhibit chemotherapeutic and chemopreventive activities in a number of human cancers. Another vitamin A metabolite, 11-*cis*-retinal, serves as the visual chromophore, and several proposed retinoid-based therapies show promise in the treatment of eye diseases such as ADRP, LCA, and macular degeneration. Retinoid-based therapy is, however, often confounded by the toxicity of these compounds at pharmacological doses. Recent observations suggest that factors that regulate the uptake and storage of retinoids in cells may serve as targets for novel retinoid-based therapeutic strategies. Such factors include LRAT, the enzyme that catalyzes retinoid esterification and storage, and CRBPs, proteins that cooperate with LRAT in regulating retinoid uptake and metabolism. Manipulating the levels or activities of these proteins may allow for a better control of the levels of active retinoids in specific tissues, thereby reducing the amounts needed for efficacious therapy and targeting the agents to specific tissues.

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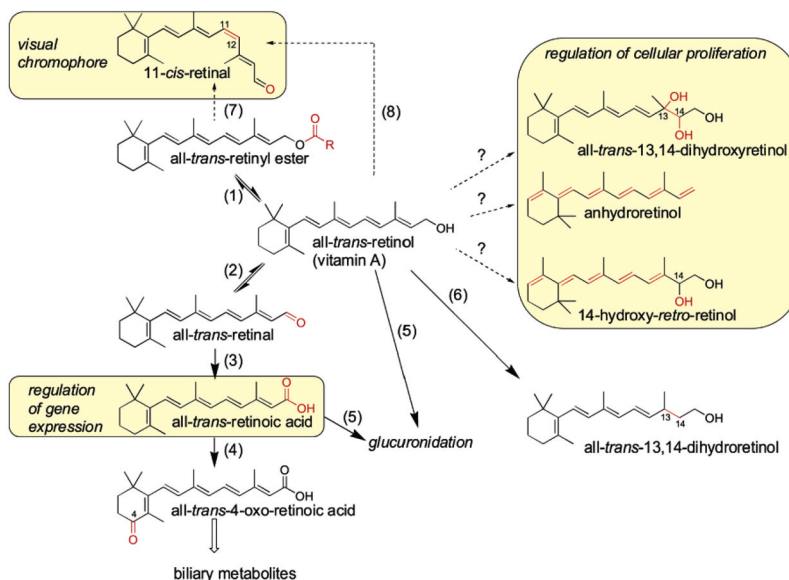
Figure 1.
Retinoid metabolism and bioactive retinoids in higher vertebrates. Known endogenous
bioactive retinoids are highlighted by yellow boxes and include all-*trans*-retinoic acid (RA),
which regulates gene expression via RAR, 11-*cis*-retinal, which is the chromophore of the
photoreceptor molecule rhodopsin, all-*trans*-13,14-dihydroxyretinol, and the *retro*-retinoids,
AR and 14-hydroxy-*retro*-retinol, which control cellular proliferation. All-*trans*-retinol can be
esterified by the LRAT or by the ARAT enzyme to produce retinyl esters that can be hydrolyzed
back to all-*trans*-retinol by retinyl ester hydrolase (REH) (reaction 1). The LRAT enzyme uses
phospholipid (PL) as acyl donors and prefers retinol complexed to CRBP as the substrate. The
oxidation of all-*trans*-retinol by short chain dehydrogenase/reductase (SDR) or ADH enzymes
leads to the formation of all-*trans*-retinal (reaction 2), which in turn is oxidized by
retinaldehyde dehydrogenases (RALDH) to all-*trans*-RA (reaction 3). All-*trans*-RA can be
converted to more polar metabolites through oxidation by cytochrome P450 CYP26 enzymes
(reaction 4) or through glucuronidation by UDP-glucuronosyltransferase (UGT) enzymes
(reaction 5). All-*trans*-retinol can be saturated by RetSat and leads to the formation of all-
trans-13,14-dihydroretinol (reaction 6), which can be oxidized by the same enzymes as those
of all-*trans*-retinol to produce all-*trans*-13,14-dihydroretinoic acid. The production of 11-*cis*-
retinal occurs in RPE cells and through esterification, isomerization, and oxidation reactions,
with isomerization involving the RPE65 enzyme (reaction 7). An alternate pathway for the
production of the visual chromophore for cones operates independently of RPE65 (reaction 8).
The enzymes involved in the production of all-*trans*-13,14-dihydroxyretinol, AR, and 14-
hydroxy-*retro*-retinol in vertebrates are not currently known, though a retinol dehydratase from
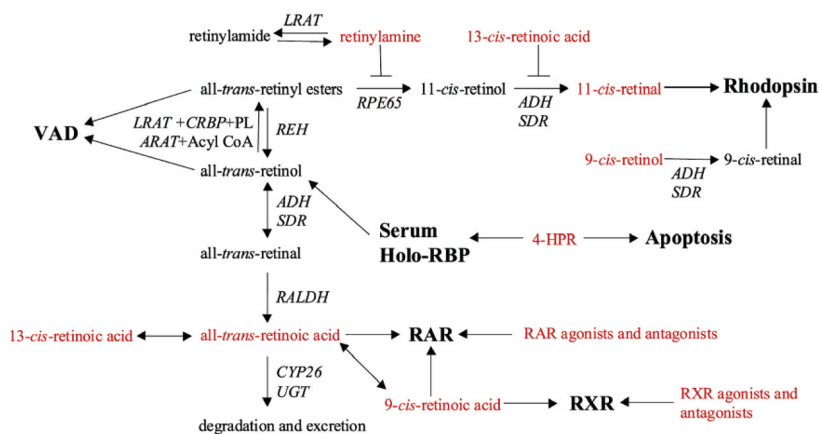insects was shown to catalyze the formation of AR.

Figure 2.
Targets of retinoid treatments. Both all-*trans*-retinol and all-*trans*-retinyl esters (palmitate) are effective in the treatment and prevention of vitamin A deficiency (VAD). The reconstitution of rhodopsin with 9-*cis*-retinal forms isorhodopsin and is an effective means of restoring visual function in several models of Leber congenital amaurosis (LCA). Inhibitors of the visual cycle, retinylamine and 13-*cis*-RA, can slow the rate of 11-*cis*-retinal formation in cases where excessive activation of rhodopsin can lead to phototoxicity and accumulation of all-*trans*-retinal and toxic metabolites as seen in Stargardt disease and age-related macular degeneration. A similar result can also be obtained with *N*-(4-hydroxyphenyl)retinamide (4-HPR or fenretinide), which binds RBP and leads to its excretion through glomerular filtration. Retinylamine can be amidated by the LRAT enzyme, allowing for the storage of the drug in an inactive form with lower toxicity. Independently of RAR, 4-HPR can lead to the apoptosis of tumor cells. RAR can be modulated by all-*trans*-RA, 9-*cis*-RA, and many synthetic agonists and antagonists. The retinoid X receptor can be modulated by 9-*cis*-RA and several synthetic agonists and antagonists. Both 13-*cis*- and 9-*cis*-RA can isomerize to all-*trans*-RA *in vivo*. The drug targets are shown in bold font, and the retinoids used in various therapies are shown in red font.

Table 1

Differentially Expressed Genes in the Liver of $Lrat^{-/-}$ Mice vs WT[a]

gene symbol	gene description	accession no.	fold change $Lrat^{-/-}$ WT
	upregulated in $Lrat^{-/-}$ mice vs WT		
Ugt1a9	UDP-glucuronosyltransferase 1 family A9	S57479	7.9
Apoa4	apolipoprotein A-IV	M64249	6.0
Mt1	metallothionein 1	BC027262	4.4
Alas1	aminolevulinate synthase 1	M63245	4.4
Gdpd3	Glycerophosphodiester phosphodiesterase domain containing 3	NM_024228	3.6
Cxcl14	chemokine (C-X-C motif) ligand 14	AF352785	3.3
Hspa8	heat shock protein 8	X05837	3.1
Serpina4-ps1	serpin A4, pseudogene 1	BC024071	3.0
Hsp110	heat shock protein 110	AK075731	3.0
Dhtkd1	dehydrogenase E1 and transketolase domain containing 1	AK050057	2.7
Hspa1b	heat shock protein 1B	M12573	2.5
Lcn13	Lipocalin 13	NM_153558	2.5
Cap1	adenylate cyclase-associated protein 1	NM_007598	2.4
Tppp	tubulin polymerization-promoting protein	NM_182839	2.3
Tsc22d3	TSC22-related inducible leucine zipper 3	AF024519	2.3
	downregulated in $Lrat^{-/-}$ mice vs WT		
Clec2h	C-type lectin domain family 2, member h	NM_053165	0.49
MGC60813	Unknown	BC058613	0.45
Tubb2a	tubulin, beta 2 a	NM_009450	0.44
Cml5	camello-like 5	AK007530	0.43
Cg10671	Cg10671 like	BC048823	0.43
Mm.2121	Unknown	NM_026790	0.38
Tiam2	T-cell lymphoma invasion and metastasis 2	NM_011878	0.38
Cebpe	CCAAT/enhancer binding protein epsilon	NM_207131	0.37
Hsd3b5	Hydroxy-delta-5-steroid dehydrogenase	NM_008295	0.32
Camk2b	calcium/calmodulin-dependent protein kinase II, beta	AK083344	0.19
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AK002237	0.15

[a]Total RNA from the three livers of 3 month old $Lrat^{-/-}$ (or WT) mice maintained on a standard diet were pooled to generate one sample. We used two different pools of hepatic RNA from $Lrat^{-/-}$ mice to compare them with two different pools of WT mice. Each sample of pooled RNA was detection-labeled and hybridized in duplicate on a mouse genomic microarray using a service provided by NimbleGen System, Inc. (Madison, WI) as described previously (139). The microarray contained 37,364 genes and covered the entire mouse transcriptome as represented by the University of California, Santa Cruz database (build HG 17), using a minimum of 11 probes per gene. The expression of genes was normalized according to the probe signal, and the average signal for each gene was normalized for each sample replicate. Array data for samples across the whole study were normalized by NimbleGen Systems, Inc. (Madison, WI), using the robust multichip analysis feature of the data analysis package found at http://www.bioconductor.org. Project-wide spreadsheets of robust multichip analysis results were exported to Microsoft Excel. Expression level ratios for all of the possible pairwise comparisons, comprising a four-way comparison of two $Lrat^{-/-}$ and two WT samples, were calculated. These pairwise ratios were imported to Microsoft Access and mined for credible fold changes. Changes in gene expression greater than or equal to 2-fold in at least three of the four comparisons were considered significant.