



Hepatic Complexities of Retinoid Therapy

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Retinoids stimulate hepatocyte proliferation, a subject thoughtfully reviewed by Bushue and Wan in a recent issue of the *Journal of Experimental and Clinical Medicine*.¹ The stimulation is paradoxical, because the active metabolite of vitamin A, tretinoin (all-trans retinoic acid), usually stimulates tissue differentiation, cell cycle arrest, and apoptosis.² These actions are mediated by nuclear receptor transcription factors, the retinoic acid receptors (RAR). Based on these inhibitory effects, three RAR agonists—tretinoin, isotretinoin, and fenretinide—have contemporary applications in cancer therapy.³ Moreover, retinoids are used for the treatment of acne, other skin diseases, and even inflammatory conditions. Although there are no specific indications for liver disease, liver exposure is a consequence of all retinoid therapy, even topical application.

The liver expresses three RARs (α , β and γ). All three have been inactivated in mutant mice;⁴ the mutations in these mice cause growth retardation, bone malformations, and epithelial changes, but not obvious liver defects. Aside from proliferative effects, no studies have demonstrated that retinoids control critical regulations of hepatocytes. The importance of RARs in the liver therefore appears relatively minor, although hepatocellular carcinoma is among the tumors inhibited by retinoids in experimental systems.

The liver also expresses three retinoid X receptors (RXR α , β and γ), for which alitretinoin (9-cis retinoic acid) is the strong natural activator. Two RXR-specific drugs, i.e., *rexinoids*, alitretinoin and bexarotene, are currently used for therapy of cutaneous T-cell lymphoma and sometimes other cancers. Tretinoin and other RAR ligands also activate RXR and are therefore less specific than rexinoids.

In contrast to the limited scope of RAR responses in liver, RXRs regulate a wide variety of metabolic pathways

as the dimeric partners of many nuclear receptor transcription factors. RARs are among these RXR partners, but others are far more important in the liver. Four families of RXR partners—liver X receptors (LXR α , β), the farnesoid X receptor (FXR), peroxisome proliferator activated receptors (PPAR α , β , γ), and thyroid hormone receptors (TR α , β)—regulate hepatic metabolism. LXRs are receptors of oxysterols that control genes of lipid and carbohydrate metabolism.⁵ FXR, a bile acid receptor, regulates genes of bile and cholesterol metabolism, and lipoproteins.⁶ PPARs regulate genes of fat uptake and lipid metabolism.⁷ TRs regulate genes of lipid metabolism and oxidative phosphorylation.⁸ Two other RXR partners, CAR and PXR, are xenobiotic receptors that regulate drug inactivation. Although the RXR knockout mutation causes embryonic death, mice with a liver-targeted mutation are viable. The profound changes in these mutant mice verify the central importance of RXR. Deficiency causes severe fatty liver, extreme hyperlipidemia, and global changes of liver gene expression.⁹

In experimental animals, both tretinoin and alitretinoin induce moderate levels of hepatocyte proliferation, but alitretinoin has a stronger effect. Only tretinoin is an RAR ligand, but both are RXR ligands. This has led Bushue and Wan to propose a novel way to link the responses, invoking the *active partner effect*. When a specific ligand activates one of the liver-enriched nuclear receptors, RXR is a passive dimeric partner that does not require its own ligand. Some combinations (RXR/CAR, RXR/PXR, RXR/LXR, RXR/PPAR, and RXR/TR) are *permissive*;^{10,11} i.e., addition of RXR ligand increases their stimulation of target genes. As a result, retinoid causes off-target stimulation of genes regulated by all of these other receptors. Among these, CAR,¹² TR,¹³ and PPAR¹⁴ are known activators of hepatocyte proliferation in rodents. Costimulation of any one of these receptors

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could therefore account for the mitogenic effects of retinoid.

The active partner effect leads to three important clinical issues. One is whether the proliferative response is clinically useful, to replace hepatocytes in liver damage or induce growth after transplantation. Clinical utility is questionable, however, because tretinoin and alitretinoin are only weak mitogens compared to direct activators of CAR, PPAR, or TR. Phenobarbital, for example, which stimulates proliferation by activating CAR, has a much stronger effect. None of these proliferative responses in rodents is confirmed in human liver, and one, PPAR-induced proliferation, has been ruled out. In an elegant study, the mouse PPAR α gene was mutated, and its function replaced by a transgene encoding human PPAR α . Human and mouse PPAR α both gave identical activation of metabolic gene targets, but only the mouse gene activated proliferation.¹⁵ Since both were compared within mouse liver, subtle differences between human and mouse PPAR α must account for the differing effects on proliferation. Demonstration that human CAR or TR can stimulate proliferation will require similar experiments. A study in rat, however, showed that thyroid hormone can significantly augment the poor proliferative responses observed after 95% hepatectomy, or in old age.¹³ Thus, identification of direct mitogenic pathways in human liver could lead to valuable clinical studies.

Phenobarbital is a well-known cancer-promoting agent, which raises a second issue, the possibility that retinoid-induced hepatocyte proliferation will cause cancer. Since retinoids promote experimental carcinogenesis in mouse liver, it is likely that they will also promote human cancers that occur as a consequence of liver damage. Nevertheless, the promotion will be weak compared to drugs that directly activate the CAR response pathway. Such drug effects are already part of the complexity of managing liver disease, and it is very difficult to compare the magnitude of theoretical cancer risk with the benefits of drug therapy.

The third and probably most important issue is drug interaction. The active partner effect can simultaneously stimulate both CAR- and PXR-mediated drug response pathways. Retinoids can therefore induce an unusually broad spectrum of drug inactivations, and at the same time potentiate the metabolic effects of PPAR, LXR, and TR activation. Moreover, costimulation of the CAR response pathway might enhance the toxicity of

acetaminophen. Retinoid therapy may therefore add unexpected complexity to drug treatment in many therapeutic situations.

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