Retinoids May Increase Fibrotic Potential of TGF-β: Crosstalk Between Two Multi-functional Effectors

Key events in liver fibrosis include the accumulation of extracellular matrix, the activation of hepatic stellate cells (HSC) to proliferating myofibroblast-like cells, and a reduction in stellate-cell lipid droplets containing retinyl esters (vitamin A). These findings in vivo are recapitulated in culture, in which rat stellate cells grown on uncoated plastic lose retinyl esters and transform into proliferating myofibroblast-like cells with a 10-fold increased secretion of collagen (mainly type I). The cytokine, transforming growth factor β (TGF-β), has a central pro-fibrogenic role; it may act in a predominantly autocrine manner such that its release specifically by stellate cells is central to fibrogenesis.

The role of retinoids (normal analogs, synthetic analogs, and metabolites of retinol) in liver fibrosis has become intriguing. In vitro, the activation of quiescent stellate cells to dividing myofibroblast-like cells may be inhibited by the addition of retinol and retinoic acid to the medium; in that process, proliferation and collagen production is effectively free retinol because in excess this compound can partition specifically by stellate cells is central to fibrogenesis.

In vitro and metabolites of retinol) in liver fibrosis has become intriguing. In vitro, the activation of quiescent stellate cells to dividing myofibroblast-like cells may be inhibited by the addition of retinol and retinoic acid to the medium; in that process, proliferation and collagen production is effectively free retinol because in excess this compound can partition specifically by stellate cells is central to fibrogenesis.

Parallel data in vivo have been reported by Senoo and Wake; in their study, they injected 15 mg retinol/kg of body weight into rats subcutaneously twice a week for 2 weeks before repeated injection of carbon tetrachloride (CCL₄). The treatment resulted in increased storage of retinyl esters in HSC, and suppressed experimental hepatic fibrosis induced by CCL₄. Beta-carotene treatment, which also increases retinyl ester storage in stellate cells, similarly attenuates CCL₄-induced hepatic fibrosis. Consistent with this, vitamin A deficiency, with low levels of retinyl esters in stellate cells, promotes CCL₄-induced liver fibrosis.

In this issue of Hepatology, Okuno et al. report in a well-designed, technically sound study that retinoids exacerbate liver fibrosis, an observation that appears in conflict with the observations discussed above. First, day-7 cultures of stellate cells, in which the majority of cells have a myofibroblast-like phenotype, increased their secretion of total TGF-β upon treatment with 9-cis or all-trans retinoic acid. The effect of retinoic acid could be observed in as little as 3 hours after its addition to the cells. Second, treated cells released active TGF-β into the culture medium. These data demonstrate that retinoic acid both induces the secretion of latent TGF-β and stimulates its activation. Finally, the authors found that surface-associated plasminogen activator was increased in cultures exposed to 9-cis retinoic acid, suggesting that plasmin may mediate the activation of latent TGF-β in this system.

A review of retinoid metabolism and signaling is necessary to address this apparent paradox between previous data and the data by Okuno et al. Dietary vitamin A is presented to the liver as retinyl esters within chylomicron remnants. Parenchymal uptake and hydrolysis of retinyl esters yields retinol, which combines with retinol-binding protein in the endoplasmic reticulum and is secreted from the hepatocytes. Most of the secreted RBP-bound retinol is then taken up by the stellate cells and is re-esterified to retinyl ester. Intracellular binding proteins help maintain low concentrations of free retinol because in excess this compound can partition into membranes and disrupt normal structure and function. Under normal circumstances, about 50% to 80% of vitamin A is stored in the HSC (HSC) in mammals as retinyl esters. HSC are instrumental in providing tissues and cells with optimal amounts of vitamin A despite huge fluctuations in daily vitamin A intake.

Of the four cytoplasmic binding proteins that are specific for retinol and retinoic acid, cellular retinol binding protein type I (CRBP-I) is most prominent in HSC. Each stellate cell in normal rats has been calculated to contain 10⁶ to 10⁸ molecules of CRBP-I. CRBP-I directs retinol to the enzyme lecithin:retinol acyltransferase (LRAT) for esterification. Retinol acyltransferase also is present in a relatively high concentration in HSC. CRBP-I seems to be rate limiting for retinol uptake and storage by stellate cells; vitamin A-deficient rats exhibit a marked reduction in stellate-cell CRBP-I and concomitantly reduced retinol uptake, esterification, and storage. CRBP-I may also facilitate the irreversible oxidation of retinol to the more biologically active retinoic acid, but the mechanism for oxidation of retinol has not been proven for HSC. It is important to note that once retinoic acid is formed, it cannot be reduced to retinol and stored as retinyl esters.

The potent biological effects of vitamin A are, in most cases, mediated by six different nuclear retinoic acid receptors, five of which have been identified in stellate cells. These receptors regulate gene expression by binding to short DNA sequences (retinoic acid response elements) in the vicinity of target genes. The most important ligand for the retinoid X receptor (RXR) subfamily (RXR-α, RXR-β, and RXR-γ) is the 9-cis isomer of retinoic acid, whereas both all-trans and 9-cis retinoic acids are the most important ligands for the retinoic acid receptor subfamily (RAR-α, RAR-β, and RAR-γ). The receptors can also be activated by other...
natural and synthetic retinoids. An active research have elucidated a complex pathway of activation of retinoids, which involve homodimerization of RXRs, heterodimerization between RARs and RXRs, as well as between RXRs and other members of the nuclear receptor family, interaction of the nuclear receptors with coactivators and coactivators, activation of the receptors by different retinol and retinoic acid metabolites, and activities of different types of responsive elements. This high degree of complexity is essential to tightly regulate the activity of retinoids.

Although retinoids seem generally to inhibit liver fibrosis, the data of Okuno et al. clearly suggest that there are conditions in which they stimulate fibrosis. It should be noted, however, that the present study found no effect of retinoic acids at concentrations below 100 nmol/L. Half of the maximal effect on the secretion of total TGF-β was seen at 400 nmol/L. The physiological concentration of retinoic acids in most cells and body fluids may be as low as 10 nmol/L, although the data on this are limited. Pharmacological intake of concentrated forms of retinol or retinoic acid can increase the retinoic acid concentration significantly.

The anti-proliferative effect of retinoic acid is more pronounced in HSC than in myofibroblasts. In addition, RAR-β and RXR-α messenger RNA and retinoic acid response elements binding activity of nuclear extracts are reduced in myofibroblasts compared with stellate cells, which is an indication of impaired retinoic acid receptor signaling in activated myofibroblasts. Changes in nuclear receptor receptors could, therefore, play a key role in the observation by Okuno et al., retinoids stimulate RARE-regulated genes such as CRBP(I), RXR-α and RAR-β in stellate cells. The latter, complexed with retinoic acid, block the activity of AP-1 (the fos/jun family), a transcription factor important for TGF-β. As HSC lose retinoids, if their expression of RAR-β, RXR-α, or CRBP changes, AP-1 activity may increase, leading to increased transcription of AP-1 responsive genes, such as TGF-β and, thence, to fibrogenesis. As the stimulation of TGF-β activation by retinoic acid occurs under circumstances in which the tonic inhibitory effect of retinoids (which presumably is mediated via nuclear receptors) on fibrogenesis is diminished, mechanisms apart from nuclear retinoic acid receptors, such as retinoylation or direct effects on membrane proteins, might be involved.

At present, from the data available it may be postulated that the retinoyl ester storage is a key determinant in preserving the quiescent phenotype in stellate cells; however, this remains to be proven. The newly discovered active retinol metabolites (14-hydroxy-4, 14-retro-retinol, and 4-oxoretinol) are candidates for sensors of retinyl esters storage that potentially could mediate such an effect. If this is the case, retinoic acid, which cannot be converted to the active retinol metabolites, does not have this possibility of protective action.

Although much remains to be learned, in linking retinoic acid exposure to TGF-β activation, the study by Okuno et al. adds a potentially important new dimension to the role of vitamin A metabolites in liver injury.

REFERENCES


