

Genistein, Phycocyanobilin, and Melatonin May Prevent Hepatic Fibrosis by Suppressing Proliferation and Activation of Hepatic Stellate Cells

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Abstract

Hepatic fibrosis reflects hepatotoxin-mediated activation of hepatic stellate cells, resulting in their proliferation and transformation to myofibroblasts that secrete collagen. This activation is suppressed by estrogen, an effect which explains the decreased risk for hepatic fibrosis enjoyed by premenopausal women and by postmenopausal women receiving hormone replacement therapy. Since stellate cells have been found to express the beta but not the alpha isoform of the estrogen receptor, it can be predicted that nutritional intakes of the soy isoflavone genistein – a selective agonist for ERbeta in the low nanomolar plasma concentrations achievable with these intakes – have potential for suppressing hepatic fibrosis, in both men and women. The antiproliferative impact of estrogen on stellate cells is mediated at least in part by suppression of NADPH oxidase activity; oxidant production by this enzyme complex plays a crucial role in stellate cell activation. Alternatively, it may be feasible to inhibit NADPH oxidase with phycocyanobilin (PCB), a biliverdin homolog found in spirulina that has recently been shown to inhibit the NADPH oxidase activity of human cell cultures in low micromolar concentrations. The downstream effects of oxidants in stellate cells may be blunted by melatonin, which boosts expression of antioxidant enzymes in many tissues, and has demonstrated anti-fibrotic effects in rat models of hepatotoxicity. A regimen consisting of soy isoflavones, PCB, and nocturnal melatonin in appropriate doses might have considerable potential for prevention of hepatic fibrosis in at-risk subjects.

Estrogen Retards Hepatic Fibrosis

There is growing epidemiological evidence that premenopausal women afflicted with fibrogenic liver diseases such as hepatitis C are at lower risk for fibrosis and cirrhosis than are postmenopausal women or men with this disorder; furthermore, in postmenopausal women with hepatitis C, concurrent hormone replacement therapy is associated with slower progression of fibrosis.¹⁻³ This conclusion accords well with studies demonstrating that administration of estrogen or of estrogenic drugs attenuates hepatic fibrosis in rodents treated with hepatotoxic agents – whereas ovariectomy has the opposite effect.³⁻⁷ A likely site of estrogen's action in this regard is the hepatic stellate cell, which, in response to numerous hepatotoxic agents, converts to a myofibroblast phenotype and proliferates, depositing the excessive collagen and other ground substance proteins observed in hepatic fibrosis.⁸ In vitro, estrogen suppresses the proliferation and activation of stellate cells induced by various agents.^{3,9;10} In aggregate, these findings have prompted suggestions that postmenopausal women with hepatitis C or other fibrogenic liver disorders should be treated with hormone replacement therapy (HRT).^{1;2}

Genistein Targets Stellate Cells via ERbeta

Studies with cultured rat hepatic stellate cells have concluded that these cells do in fact express estrogen receptors – but these receptors are of the beta isoform; these cells do not express ERalpha.¹¹ Thus, the antifibrogenic hepatic effects of estrogen are likely to be mediated by ERbeta receptors. Genistein, a prominent isoflavone found in soy, is a potent and selective agonist for ERbeta – and the affinity of genistein for these receptors is in the low nanomolar range, a physiological concentration of free unconjugated genistein in people eating ample amounts of soy products or taking soy phytoestrogen supplements.¹²⁻¹⁴ Thus, it is not surprising that Chinese researchers were able to demonstrate that concentrations of genistein as low as 10 nM reduce the basal and PDGF-induced proliferation of stellate cells in culture, while also inhibiting their transformation to a myofibroblast phenotype - i.e. blocking α -smooth muscle actin expression.¹⁵ (While these investigators interpreted this effect as reflecting genistein's tyrosine kinase inhibitory activity, such activity in fact require micromolar concentrations of genistein that are of no physiological relevance.¹⁴) To date, there do not appear to be any published studies evaluating the impact of soy phytoestrogens or soy-rich diets on hepatic fibrosis in rodents. Nor have epidemiological studies examined the impact of soy-rich diets on susceptibility to hepatic fibrosis in humans.

These considerations suggest that ample daily intakes of soy phytoestrogens, achieved with a soy-rich diet or phytoestrogen supplements, could be useful for slowing the progression of fibrosis in patients with fibrogenic liver disorders. As compared with recommendations for HRT, this strategy would have several advantages. First, it could be implemented in males, who are at the highest risk for hepatic fibrosis. Secondly, it would not be expected to entail the increased risk for thrombogenic complications or breast cancer associated with HRT. And, finally, it might prove to be more effective. There is recent evidence that hepatic stellate cells are responsive to progesterone, and that this hormone promotes the proliferation and activation of stellate cells, in opposition to the down-regulatory impact of estrogen in this regard.¹⁰ Thus, the necessity of including a progestin in HRT (save in women who have been hysterotomized) might blunt the efficacy of the co-administered estrogen. In contrast, there is no need to administer a progestin in conjunction with genistein, since the latter is not uterotrophic in nutritional intakes; uterotrophic activity is mediated by ERalpha.^{14;16}

Obligate Role of NADPH Oxidase in Stellate Cell Activation

An additional nutraceutical strategy for suppressing proliferation and activation of hepatic stellate cells is suggested by the fact that NADPH oxidase activity is crucial to this activation. Agents which promote proliferation and activation of stellate cells have been found to stimulate NADPH oxidase activity in these cells, and inhibitors of this enzyme complex, as well as potent oxidant scavengers, have been shown to suppress the proliferation and activation of these cells.¹⁷⁻²² Furthermore, p47^{phox} double knockout mice are substantially protected from hepatic fibrosis following bile duct ligation, and the NADPH oxidase inhibitor DPI, as well as a cell-permeable SOD/catalase mimetic,

suppressed hepatic fibrosis induced by dimethylnitrosamine.^{17;18} Activation of p38 MAP kinase appears to mediate, at least in part, the impact of oxidant stress on stellate cell proliferation and phenotypic transformation.¹⁸ Recently, the ability of estrogen to suppress stellate cell activation has been traced, at least in part, to a reduction in NADPH oxidase activity.¹⁰

A Phytonutrient Inhibitor of NADPH Oxidase - Phycocyanobilin

Phycocyanobilin (PCB), a homolog of biliverdin that can constitute up to 1% of dry weight in spirulina, has recently been shown to inhibit the NADPH oxidase activity of human cell cultures in low micromolar concentrations that are likely clinically achievable (Toyoshi Inoguchi, personal communication). This inhibition probably reflects the fact that PCB can be converted by biliverdin reductase to phycocyanorubin, a homolog of bilirubin.²³ It is now becoming clear that unconjugated bilirubin functions physiologically as a very potent inhibitor of NADPH oxidase – active in the nanomolar concentrations that prevail inside cells.²⁴⁻²⁷ This offers a satisfying explanation for the remarkable antioxidant impact of the enzyme heme oxygenase-1 (HO-1), which generates biliverdin (rapidly reduced to bilirubin), carbon monoxide and free ferrous iron by degrading heme.²⁸ There are recent reports that induction of HO-1 in hepatic stellate cells or myofibroblasts suppresses their proliferative capacity;²⁹⁻³¹ moreover, portal injection of recombinant adenoviruses carrying the HO-1 gene has been reported to suppress the progression of micronodular cirrhosis in rats – an effect associated with a reduction in activated stellate cells.³² Bilirubin seems likely to mediate all or part of these protective effects of HO-1, as this agent mimics the impact of HO-1 induction on cultured hepatic myofibroblasts.³²

If PCB proves to be clinically useful, it will be used in moderate amounts that achieve only a partial inhibition of systemic NADPH oxidase activity; a more intense inhibition would probably entail an undue inhibition of antibacterial immune mechanisms and other adverse effects. But concurrent administration of genistein and PCB could be expected to achieve a very substantial inhibition of NADPH oxidase specifically in hepatic stellate cells, likely associated with a marked reduction in risk for hepatic fibrosis in those at risk for this disorder. When PCB becomes commercially available, studies should assess the joint impact of PCB and genistein on hepatic stellate cells, on rodent models of hepatic fibrosis, and – if these pre-clinical studies prove fruitful – on patients with fibrogenic liver disorders.

Until PCB per se becomes available as a nutraceutical, ingestion of whole spirulina will represent a practical strategy for obtaining the antioxidant benefits of this phytonutrient. In line with the dictum that “there is nothing new under the sun”, it is pertinent to cite a report from Ukrainian researchers who used oral spirulina to treat 60 patients with chronic liver disorders, as well as rats subjected to hepatotoxic measures; their results suggested that the spirulina was of benefit: “The hepatoprotective properties of spirulina are referable to its antiinflammatory, antioxidant, membrane-stabilizing, and immunocorrecting actions. In this way the employment of spirulina is believed to be

pathogenetically validated in chronic diffuse liver conditions, permitting stabilizing the process and preventing the transformation of chronic hepatitis into hepatocirrhosis.”³³

Melatonin – Boosting Antioxidant Enzyme Expression

Boosting the expression of antioxidant enzymes in stellate cells would seem likely to complement NADPH oxidase inhibition as a strategy for suppressing hepatic fibrosis. In many tissues, melatonin functions to increase such expression.^{34,35} Although the impact of melatonin on antioxidant enzyme activities of stellate cells has not been studied directly, melatonin has been shown to block the induction of an activation state in stellate cells exposed to exogenous hydrogen peroxide; in particular, melatonin suppressed the induction of TGF-beta and the production of type I collagen.³⁶ Other studies have shown that melatonin administration inhibits the development of hepatic fibrosis in rats treated with a range of hepatotoxins.³⁷⁻³⁹ These findings suggest that melatonin administered at bedtime – particularly in middle-aged or elderly subjects whose endogenous melatonin production is on the wane – may represent a practical clinical strategy for preventing hepatic fibrosis.

Ancillary Measures

It should also be feasible to slow hepatic fibrosis by suppressing up-stream signals generated by hepatocytes, Kupffer cells, or infiltrating leukocytes that contribute to stellate cell activation. Various nutraceuticals may have potential in this regard, including silymarin, lipoic acid, glycine, S-adenosylmethionine, selenium, and soy phospholipid.⁴⁰⁻⁴⁶ Whether any of these agents might influence stellate cell function more directly is not clear. The utility of these ancillary agents would likely vary as a function of the nature of the underlying hepatotoxicity. One can envision complex nutraceutical regimens that could do an effective job of suppressing hepatic fibrosis while also helping to preserve proper hepatocyte function.

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