Administration of Bilins and High-Dose Biotin May Replicate the Beneficial Impact of Heme Oxygenase-1 Induction on Pathogenic Fibrosis

Mark F. McCarty, Jorge Barroso-Aranda, and Francisco Contreras,
Oasis of Hope Hospital, Tijuana, Mexico

Abstract

Clinical fibrotic syndromes are usually driven by excessive transforming growth factor-beta (TGF-beta) activity. Heme oxygenase-1 (HO-1), which is induced by TGF-beta in many tissues, has antifibrotic effects which are at least partially attributable to down-regulation of TGF-beta signal transduction. This down-regulation is mediated by bilirubin – a physiological inhibitor of NADPH oxidase that suppresses the oxidant stress required for optimal TGF-beta activity – and by carbon monoxide, which acts via guanylate cyclase, cGMP, and protein kinase G to inhibit the nuclear translocation of phosphorylated Smads while also impeding TGF-beta activation by blocking thrombospondin-1 transcription. It may be feasible to mimic these protective effects of HO-1 in clinical fibrotic syndromes by administering bilins (biliverdin or phycocyanobilin) and high-dose biotin – the former can function as clinical NADPH oxidase inhibitors, whereas the latter stimulates guanylate cyclase activity. This strategy may prove useful in the prevention and management of clinical fibrotic syndromes in which TGF-beta activity plays a central mediating role – likely including hepatic cirrhosis, glomerulosclerosis, interstitial fibrosis, ventricular hypertrophy, atrial fibrosis with fibrillation, pulmonary hypertension, cystic fibrosis, idiopathic pulmonary fibrosis, asthma, pancreatic fibrosis, scleroderma, and fibrostenosis in Crohn’s disease.

Heme Oxygenase-1 is Protective in Fibrotic Syndromes

Boosting the activity of heme oxygenase-1 (HO-1), either by administration of inducing agents, or transfection with the HO-1 gene, has shown marked antifibrotic effects in rodent models of hepatic, renal, pulmonary, and cardiac fibrosis. Conversely, human HO-1 deficiency is associated with severe renal interstitial fibrosis. Transforming growth factor-beta (TGF-beta) activity is a central mediator of fibrosis in most fibrotic syndromes; this reflects its ability to promote synthesis of extracellular matrix proteins, while suppressing secretion of proteases and boosting that of protease inhibitors. TGF-beta also promotes HO-1 expression at the transcriptional level, and there is reason to suspect that this represents a protective feedback mechanism, helping to prevent an overexuberant fibrogenic response, as we shall see, HO-1 acts in several complementary ways to oppose TGF-beta signal transduction.

Modulators of TGF-beta Signal Transduction

The main intracellular effects of TGF-beta are believed to reflect serine phosphorylations of the transcription factors Smad2 and Smad3, catalyzed by activated TGF-beta receptors. These phosphorylated Smads, after binding to Smad4, can be translocated to the nucleus, where they act as
transcriptional activators for TGF-beta target genes. However, TGF-beta also exerts other effects – including activation of MAP kinases – via routes that are still somewhat obscure.\textsuperscript{36-38} In particular, TGF-beta induces oxidant stress in many tissues by increasing NADPH oxidase activity.\textsuperscript{39-48} This often reflects a dramatic up-regulation of Nox4 at the transcriptional level – likely reflecting Smad2/3 activation.\textsuperscript{45-47} However, TGF-beta also promotes rapid activation of pre-existing NADPH oxidase in some tissues, an effect which may contingent on concurrent activation of Src family kinases.\textsuperscript{42-44} In many tissues, the pro-fibrotic effects of TGF-beta are largely contingent on the increased oxidative stress evoked by NADPH oxidase activation, as demonstrated by the fact that inhibitors of this enzyme complex, as well as certain antioxidants (e.g. N-acetylcysteine, catalase), suppress the ability of TGF-beta to induce increased expression of downstream targets such as collagen type 1 and smooth muscle alpha-actin.\textsuperscript{39-42, 45, 47, 49}

Antioxidants also abrogate the pro-proliferative impact of TGF-beta on smooth muscle cells.\textsuperscript{46} In some cell systems, the oxidative stress induced by TGF-beta up-regulates the ability of this hormone to catalyze activating phosphorylations of Smads 2 and 3.\textsuperscript{39-41, 47} Oxidative stress may also influence Smad transcriptional activity by promoting activation of p38 and other MAP kinases.\textsuperscript{39, 50} A number of studies indicate that p38 and/or Erk activity plays a co-factor role in Smad-dependent transcriptional activation, for reasons that remain unclear.\textsuperscript{51-61}

Oxidant stress also promotes TGF-beta activity by boosting transcription of the TGF-beta1 gene; in this way, TGF-beta can promote its own synthesis.\textsuperscript{62-67} This inductive effect appears to be mediated by AP-1 and NF-kappaB transcription factors, which are often activated during oxidant stress.\textsuperscript{68-70} Oxidant stress also promotes activation of the latent TGF-beta protein; TGF-beta is synthesized in a latent precursor form that requires activation before TGF-beta can express its hormonal activity. Oxidant stress can achieve TGF-beta activation by a direct effect,\textsuperscript{71, 72} and possibly also by stimulating production of thrombospondin-1, a protein which binds to latent TGF-beta in a way that achieves its activation.\textsuperscript{73-75} Angiotensin II promotes transcription of the thrombospondin-1 gene via activation of the p38 and JNK MAP kinases,\textsuperscript{76, 77} and it is reasonable to expect that oxidants may have a comparable effect. The ability of oxidant stress to promote the synthesis and activation of TGF-beta presumably explains why certain hormones which activate NADPH oxidase in their target tissues – most notably angiotensin II, endothelin, and perhaps marinobufagenin – tend to promote fibrosis in these tissues.

Another way in which oxidant stress can promote TGF-beta activity is by interfering with nitric oxide activity; as is well known, superoxide directly quenches nitric oxide, and the resulting peroxynitrite can inhibit NO synthase activity by oxidizing its cofactor tetrahydrobiopterin.\textsuperscript{78, 79} A key role of nitric oxide is to activate guanylate cyclase, leading to increased activity of cGMP-dependent protein kinase G (PKG). PKG activity has been shown to antagonize TGF-beta signaling in several types of cells, apparently because PKG inhibits the nuclear translocation of phosphorylated Smads.\textsuperscript{80, 81} This effect, in turn, may reflect increased proteasomal degradation of activated Smads.\textsuperscript{82} Not surprisingly, various measures which activate PKG have shown antifibrotic effects in rodent studies.\textsuperscript{82-88}

PKG activity can also oppose activation of TGF-beta. Recent studies with rat mesangial cells show that PKG inhibits the transcription of thrombospondin-1 by decreasing the availability of the USF2 transcription factor; this in turn is associated with reduced TGF-beta bioactivity.\textsuperscript{89-91}
HO-1 Opposes TGF-beta Activity via Bilirubin and Guanylate Cyclase

What does any of this have to do with HO-1 activity? HO-1 generates both biliverdin and carbon monoxide (CO) by degrading heme.92, 93 This biliverdin is rapidly reduced to bilirubin by the ubiquitously expressed enzyme biliverdin reductase.94, 95 Bilirubin, in the nanomolar concentrations that can be achieved intracellularly, is now known to function physiologically as a highly potent inhibitor of NADPH oxidase96-98 – a phenomenon that at last rationalizes the potent antioxidant activity of this compound in cell cultures.99 Oxidative stress – including that induced by TGF-beta – promotes HO-1 induction via interaction of Nrf2 with antioxidant response elements;100-102 the resulting production of bilirubin often achieves feedback inhibition of this oxidant stress by suppressing NADPH oxidase activity. Thus, HO-1 activity would be expected to interfere with TGF-beta signal transduction by inhibiting TGF-beta’s ability to evoke oxidant stress.

One of the key functions of the CO produced by HO-1 activity is to stimulate guanylate cyclase activity, an effect analogous to that of nitric oxide (albeit the maximal response to nitric oxide is considerably greater).103-106 In the context of oxidant stress, the production and stability of nitric oxide is often compromised – whereas superoxide fails to interact with CO. Thus, when HO-1 is induced by oxidant stress, the CO that is generated may be viewed as “pinch-hitting” for nitric oxide, at least with respect to guanylate cyclase activation. As noted above, guanylate cyclase activity, via PKG, impedes TGF-beta’s Smad-mediated signaling activity, while also suppressing TGF-beta activation. Thus, both the bilirubin and CO generated by HO-1 activity have the potential to dampen TGF-beta’s bioactivity.

CO also promotes activation of p38 MAP kinase via MKK3 in some cell lines; this effect is independent of guanylate cyclase activation.106, 107 This activation of p38 MAP kinase may have a countervailing impact on TGF-beta bioactivity, as inhibitors of this kinase negatively influence TGF-beta bioactivity and activation. Nonetheless, the net effect of HO-1 activity appears to be anti-fibrogenic in most models studied. Thus, TGF-beta-mediated HO-1 induction may be viewed as a protective feedback mechanism that prevents an overexuberant fibrotic response.

Orally Administrable Bilins and High-Dose Biotin May Mimic HO-1’s Antifibrotic Activity

Some authors have suggested that inducers of HO-1 might be used clinically to treat or prevent fibrotic syndromes. However, an alternative approach may be feasible. Oral administration of biliverdin or of structurally homologous phycobilins found in cyanobacteria (e.g., phycocyanobilin in spirulina) has the potential to achieve systemic suppression of NADPH oxidase activity99,108 – mimicking the impact of locally induced HO-1 in this regard. Moreover, in slightly supraphysiological concentrations, the vitamin biotin achieves a moderate (several-fold) activation of guanylate cyclase comparable to that produced by CO.109, 110 Biotin is well tolerated in high doses (e.g. 100 mg daily or more),111 and intriguing effects of high-dose biotin in rodents or humans have been interpreted as reflecting guanylate cyclase activation.112-116 Potentially, biotin’s efficacy in this regard could be complemented in certain tissues by phosphodiesterase 5 inhibitors such as sildenafil.117, 118

These considerations suggest that, once biliverdin and or phycobilins are commercially available for oral administration, and once the pharmacokinetics of these agents and of high-dose biotin have been clarified in humans, it may be feasible to prevent and treat a range of fibrotic clinical syndromes by supplementing with bilins and high-dose biotin. Furthermore, rodent studies suggest that orally administered
Phycocyanobilin may be bioavailable when administered in whole spirulina or in phycocyanin (the spirulina protein which carries phycocyanobilin as a covalently-linked chromophore); if this is also true in humans, ample dietary intakes of spirulina might be expected to have clinically useful anti-fibrotic activity.

The range of disorders in which these measures could conceivably be employed for prevention or therapy might include such disparate clinical syndromes as hepatic cirrhosis, glomerulosclerosis, tubulointerstitial fibrosis, cystic fibrosis, idiopathic pulmonary fibrosis, asthma, ventricular hypertrophy, atrial fibrosis with fibrillation, pulmonary hypertension, post-radiation fibrosis, pancreatic fibrosis, scleroderma, and fibrostenosis in Crohn’s disease – in all of which TGF-beta is suspected to play a central pathogenic role.

References


(5) Pachori AS, Melo LG, Zhang L, Solomon SD, Dzau VJ. Chronic recurrent myocardial ischemic injury is significantly attenuated by pre-emptive adeno-associated virus heme oxygenase-1 gene delivery. J Am Coll Cardiol 2006 February 7;47(3):635-43.


Iglesias-de la Cruz MC, Ruiz-Torres P, Alcamí J, ez-Marques L, Ortega-Velazquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D. Hydrogen peroxide increases


(84) Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, Gonzalez-Cadavid NF. L-arginine and phosphodiesterase (PDE) inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. *Nitric Oxide* 2003 December;9(4):229-44.


(99) McCarty MF. "Iatrogenic Gilbert syndrome" - a strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. *Medical Hypotheses* 2007; accepted for publication.


transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. *J Immunol* 2004 September 1;173(5):3467-81.


