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Bile Acids are Important Contributors of AKI Associated with Liver Disease: **COMMENTARY**

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Advanced cirrhosis is frequently complicated by acute kidney injury (AKI).[1] The first diagnosis that comes to mind in such cases is type 1 hepatorenal syndrome (HRS-AKI) . This form of AKI occurs when cirrhosis-induced systemic vasodilation triggers profound renal vasoconstriction, precipitating a fall in glomerular filtration. Accordingly, it is considered a functional form of AKI, that ought to improve if the abnormal hemodynamics are corrected. However, this is frequently not the case; even our most effective therapies only work in a minority of patients, implying that the renal dysfunction has progressed beyond that of a purely functional form of AKI. Several factors have been identified that predict poor responsiveness to vasoconstrictor therapy.[2] One such factor is hyperbilirubinemia (and the concomitant rise in bile acids),[2] raising the possibility that these cholephiles play a causative role in cirrhosis-associated AKI. Two additional observations support this notion. First, despite not being harmful at physiologic levels, these cholephiles are cytotoxic at high concentrations (thus fulfilling the Paracelsian dictum, "the dose makes the poison"). For instance, the hyperbilirubinemia seen in neonatal jaundice is cytotoxic to the brain and liver.[3] Likewise, high doses of bile acids increase oxidative stress and induce cell death pathways, resulting in tissue dysfunction.[4,5] And second, increased delivery and filtration of these cholephiles during cirrhosis result in high enough intrarenal levels to be cytotoxic. However, despite this strong biologic plausibility, the role of cholephiles in cirrhosis-associated AKI is unclear. The debate in this issue of Kidney360 tackles the question of whether bilirubin and/or bile acids play a causative role in the development and/or progression of AKI or are merely prognosticators of non-responsive disease. This question has important therapeutic ramifications and raises the broader question of whether therapeutic targets need to expand beyond hemodynamic and general support.

On the PRO side of the debate, Drs. Fickert and Rosenkranz present persuasive evidence of bile acid-mediated renal toxicity in rodents with cholestasis due to common bile duct ligation (CBDL). This model is characterized by increased oxidative stress, mitochondrial injury, apoptosis, and cell membrane damage, all of which result in renal dysfunction. The primary evidence implicating bile acids in renal injury is its dependency on the bile acid receptor, the farnesoid X receptor (FXR). Indeed, knocking out FXR, or reducing its activation by administering hydrophilic bile acids, ameliorates renal injury.[4-6] However, since FXR is expressed in several organs (liver, heart, kidney, vasculature and intestine), bile acid-induced renal injury may be due to either direct or indirect effects on the kidney (e.g. via exacerbation of hemodynamics and inflammation). The abundance of FXR in the kidney, together with the low response rates to vasoconstrictors in humans, make it tempting to conclude that the bile pigments exert their toxicity via direct effects on the kidneys. The debaters also provided human data supporting this view; histologic evidence of cholemic nephropathy was present in 54% of patients with advanced cirrhosis.[7] Not surprisingly, the mean bilirubin level was higher in patients with bile casts (26 vs 15 mg/dl). Interestingly, although 30% of these patients were diagnosed as HRS-AKI (implying normal kidney morphology), 85% had tubular bile cast.[7] Perhaps, this explains why terlipressin was effective in only 13% of HRS-AKI patients with serum bilirubin >10 mg/dl, compared to 67% of patients with serum bilirubin <10 mg/dL.[2] Altogether, these findings suggest that the cholephile toxicity should be considered as a pathogenic mechanism of AKI in patients with cholestasis and cirrhosis.

On the CON side, Drs. Allegretti and Belcher counter several of the arguments supporting a causative role for cholephiles in cirrhosis-associated AKI. They highlight the lack of a dose-response

relationship between bilirubin and kidney damage, and the limitations in the clinicopathologic HRS-AKI studies that reported a high prevalence of tubular bile casts. In fact, they present evidence suggesting that bilirubin may be renoprotective. Next, they highlight the paucity of definitive animal models implicating bilirubin as the cause of kidney dysfunction; a) the CBDL models do not exhibit consistent morphologic evidence of cholemic nephropathy, and b) the Gunn rat (which has isolated hyperbilirubinemia) is protected against hypertension and renal injury.[8,9] Finally, they contend that the overlapping pathogenetic mechanisms present during cholestasis/cirrhosis, complicates the interpretation of morphologic findings, and suggest that alternative explanations may be more likely.

This debate highlights the complexities of establishing causation in multifaceted diseases. We address this by focusing on 2 questions. First, *are cholephiles nephrotoxic?* There is good evidence that hydrophobic bile acids (the most common subtypes) are cytotoxic to a variety of cell types in vitro.[4,10] Moreover, cholestasis-induced hemodynamic abnormalities and renal dysfunction, are abated by knocking out, or decreasing the activity of the FXR, again suggesting. While these latter studies did not discern the mechanism of renal injury, we agree with Allegretti and Belcher that is likely due to a combination of systemic hemodynamic and intrarenal alterations. It should be noted, that even in the absence of obvious renal dysfunction, the systemic changes may render the kidney more susceptible to a second hit. Indeed, CBDL rodents developed worse ischemia/reperfusion-induced AKI than controls.[11] Thus, while we agree that the evidence is incomplete, the available data suggests that bile acids are cytotoxic. On the other hand, the evidence for bilirubin is conflicting. Evidence supporting cytotoxicity comes from the neuro- and hepatotoxic effects of hyperbilirubinemia in patients with Rh disease or genetic diseases of bilirubin metabolism (*e.g.* Type 1 Crigler-Najjar syndrome).[12] Moreover, administering unconjugated bilirubin to renal cortical slices is cytotoxic to proximal tubule cells.[10,13] Clinically, this manifests as a proximal tubulopathy, which resolves if bilirubin levels decrease.[14] Conversely, there is also substantial evidence against bilirubin playing a causative role in cirrhosis-associated AKI. First, neither hypotension nor renal dysfunction are prominent features of the genetic forms of hyperbilirubinemia. Second, bilirubin, has antioxidant properties making it cytoprotective. Indeed, increasing its levels via induction of the heme oxygenase system, or as in experimental models of isolated hyperbilirubinemia (*e.g.* Gunn rats or bilirubin infusions) protects against several forms of renal injury.[8,9,15,16] Third, indirect clinical evidence also supports a protective role for bilirubin; mild hyperbilirubinemia (2 to 4 mg/dl) in cirrhotic patients is associated with less tubulointerstitial damage and lack of acute tubular necrosis.[17] Our interpretation of the evidence is that mild to moderate elevations of bilirubin are likely protective. However, it may become toxic at very high levels or in the presence of specific factors or conditions.

Accepting the potential cytotoxicity of bile acids, the second question is *do cholephiles contribute to the pathogenesis of cirrhosis-associated AKI?'* The evidence for a toxic effect of cholephiles in humans is associative, not causal. For instance, despite bile casts being common in autopsies of patients with cirrhosis-associated AKI, this does not prove that the cholephiles contributed to the AKI (or were even present at the onset of the AKI). Conversely, their absence does not exclude bile acid toxicity either. Indeed, the toxic effects of bile acids in cell culture experiments are independent of bile acid precipitates, [18] and cholestasis-induced exacerbation of AKI occurs in the absence of bile casts.[11] Thus, the available data, though provocative, is purely associative. The root problem with establishing causation is the lack of a biomarker that specifically identifies cholephile-induced renal injury. Since

the likelihood of this biomarker existing is virtually zero, the only way of demonstrating causation would be to show that removing the cholephile, or inactivating its target (receptor), prevents renal injury. Two approaches are via the use of a) hydrophilic bile acids (ursodeoxycholic and norursodeoxycholic acid),[19] as used in patients with primary sclerosing cholangitis, and b) extracorporeal albumin dialysis (e.g. molecular adsorbent recirculating system and single-pass albumin dialysis) to decrease circulating cholephiles.[20] However, large-scale studies testing the efficacy of these approaches are lacking and proving a benefit of any approach will likely be difficult because of the multifaceted aspects of cirrhosis and our lack of knowledge regarding the therapies (*e.g.* what, when, and how much to target).

All things considered, while we agree that conclusive data is lacking, we are of the opinion that bile acids are likely active contributors to the pathogenesis of cirrhosis-associated AKI. It is just too hard to ignore the experimental data supporting this possibility, in the absence of opposing evidence. That is, most of our doubts regarding their role arises from the absence of definitive evidence, rather than evidence of absence. Having said that, we are not suggesting that we immediately start targeting bile acids. Rather, we would like to see their role examined in a comprehensive manner, answering fundamental questions. For instance, we need to more accurately establish the circulatory levels of bile acids (such measurements are not routinely available) and determine the threshold for bile acid toxicity, as well as identify confounding factors which can alter this threshold (*e.g.* cirrhosis-induced hemodynamic impairments, anabolic steroid use, etc.). We would emphasize that cirrhosis-associated AKI does not likely abide by the pathophysiologic equivalent to Occam's razor. Rather, it follows Hickam's Dictum; 'the patient can have as many diseases (or causes of HRS-AKI) as they damn well please'. Therefore, targeting a single factor will almost certainly result in only a modest benefit at best, as seen with terlipressin. This should not deter us from incorporating them into what will likely be a multitargeted approach to cirrhosis-associated AKI. Identifying diverse contributing factors, and learning on how to best utilize them (*e.g.* when and how), while minimizing adverse effects is the true challenge to treating these patients.

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Author Contributions

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