EDITORIALS

β1 Integrin is a Long-sought Sensor for Tauroursodeoxycholic Acid

More than a thousand years ago at the time of the gleaming Tang dynasty in China (618-907 A.C.), a golden age of Chinese poetry, dried black bear's bile was recommended for treatment of jaundice (but also of intractable diarrhea in the summer and heart complaints) as documented in the *Tang Materia Medica,* the first state pharmacopoeia. The major organic constituent of black bear's bile is ursodeoxycholic acid (UDC), which may form up to 60% of black bear's total bile acids.[1] UDC has potent anticholestatic and anti-apoptotic properties[1] and is today regarded as the standard treatment of primary biliary cirrhosis (PBC)[2,3] and intrahepatic cholestasis of pregnancy (ICP).[3] It exerts anticholestatic effects in numerous other conditions of hepatocellular (as in ICP) or cholangiocellular cholestasis (as in early PBC).[1,3] Of note, UDC is enriched from 1%-2% to 40% of total bile acids in bile of patients with PBC and healthy volunteers treated with therapeutic doses of UDC (15 mg/kg daily).[4]

How does UDC exert its choleretic and anticholestatic properties at the level of the hepatocyte? In contrast to hydrophobic bile acids such as chenodeoxycholic (CDC) or lithocholic acid (LC), UDC does not markedly affect transport protein expression *in vivo* at the transcriptional level to modulate transport capacity.[5] The impact of limited posttranscriptional modification of carrier expression[5] for the choleretic and anticholestatic effects of UDC, possibly by way of modulation of expression of specific microRNAs,[6] is yet unclear.

The first hints that UDC may act as a potent intracellular signaling molecule come from the early 1990s when UDC was unraveled as a Ca^{++} agonist[7–10] and an activator of protein kinase C (cPKCα),[11–13] mitogen-activated protein kinases (MAPK: Erk1/2, $p38^{MAPK}$),[14,15] and $\alpha_5\beta_1$ integrins[16] in hepatocytes (later confirmed in cholangiocytes[17]). The concept that UDC conjugates, by activating intracellular signaling cascades, might enhance the secretory capacity of hepatocytes (and cholangiocytes) by stimulating vesicular exocytosis and, thereby, insertion of key transport proteins into their target membrane was independently developed by Häussinger et al. (then in the context of bile acid-induced cell swelling)[18] and Beuers et al. (as a Ca^{++}-/cPKCα-dependent vesicle fusion process).[10,12] These groups and others were subsequently able to show in experimental models that UDC conjugates exert choleretic effects by apical carrier insertion by way of a dual MAPK- and integrin-dependent mechanism in healthy liver[14–16] and anticholestatic effects by stimulation of impaired apical carrier insertion by way of Ca^{++}-/type II inositol-1,4,5-triphosphate (InsP3R) receptor/cPKCα/PKA-dependent mechanisms in cholestatic liver.[19–23] The intracellular effects of UDC appear to be dependent on taurine (T) or glycine (G) conjugation of the bile acid.[1,24] Thus, TUDC and GUDC appear to be the secretory saviors under (patho-) physiological conditions in liver.

UDC conjugates exert their intracellular signaling in liver cells after cell entry,[25,26] but not by extracellular membrane/receptor interactions. This explains why hepatocytes equipped with the bile acid membrane carrier Na^+/taurocholate cotransporting peptide (NTCP) are more than any other cell type sensitive to the effects of UDC conjugates at low micromolar concentrations. Still, an intracellular receptor/sensor specifically for UDC conjugates which might initiate one or the other signaling cascade had so far not been identified.

In the present issue of HEPATOLOGY, Gohlke et al.[26] present in an elegant series of experiments the landmark finding that low micromolar TUDC, but not taurocholic acid (TC), TCDC, GCDC, and TLC-sulfate within a minute after cell entry transfers the β_1 unit of mainly cytosolic $\alpha_5\beta_1$ integrins into its active conformation in perfused rat liver and human Ntcp-transfected HepG2 hepatoma cells. This leads to rapid phosphorylation and

activation of Erk1/2, epidermal growth factor receptor (EGFR), and other downstream events.[26] The effect of TUDC on kinase activation was inhibited by the absence of Ntcp, by high levels of TC, after knockdown of β1 integrin, or after application of an integrin-antagonistic hexapeptide.[26] Thus, β1 integrin appears to be a long-sought intracellular sensor of UDC conjugates.

Of note, TUDC-induced β1 integrin activation was observed mainly in cytoplasm rather than at the plasma membrane. In contrast, hypo-osmotic cell swelling was associated with β1 integrin activation at the plasma membrane.[26] Thus, TUDC and hypo-osmotic cell swelling activate similar, but not identical integrin-dependent pathways in human hepatocytes.

Gohlke et al.[26] performed molecular dynamics simulations of the $\alpha_5\beta_1$ integrin complex. This computational method is used to investigate the structure and time dependent dynamics of biological molecules, e.g. conformational changes of proteins in the absence and presence of potential ligands. The authors performed simulations in the absence and presence of TUDC, TC, and a β1 integrin inhibitory hexapeptide. These intriguing simulations suggested that TUDC, but not TC or the hexapeptide when bound to the head region of β1 integrin, induces an allosteric conformational change known to be associated with β1 integrin activation.

How is β1 integrin activation by TUDC related to the anticholestatic effect of TUDC? We cannot yet assess the relevance of the actual findings for the cholestatic patient. In the experimental model of TLC-induced cholestasis, TUDC exerted anticholestatic effects by Ca^{++}-, type II InsP3R receptor, cPKCα- and PKA-dependent,[19–23] but not by MAPK (Erk 1/2 and p38^{MAPK}) -dependent mechanisms.[27] Thus, it remains unclear whether TUDC-induced β1 integrin-dependent induction of choleresis is related to the anticholestatic and hepatoprotective effects of TUDC in cholestasis. It appears possible that a second β1 integrin-independent sensor for TUDC may initiate an alternative signal cascade leading to Ca^{++}/ type II InsP3R receptor/cPKCα- and PKA-dependent apical carrier insertion under cholestatic conditions.

The authors are to be congratulated for their elegant and sophisticated work. Their landmark finding of an intracellular sensor of TUDC represents a key for understanding the choleretic effect of TUDC in intact liver cells at the molecular level.

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