Less Smad2 Is Good for You! A Scientific Update on Coffee’s Liver Benefits

Olav A. Gressner

Scientists at the National Institutes of Health have reported that increased coffee consumption is associated with a slower progression of fibrogenesis in patients with chronic and particularly alcoholic liver disease and a reduced incidence of hepatocellular carcinoma. However, a causal mechanistic explanation was pending. New results indicate that the methylxanthine caffeine—a major component of coffee and the most widely consumed pharmacologically active substance in the world—might be responsible for this phenomenon, because it inhibits the synthesis of connective tissue growth factor (CTGF/CCN2) in liver parenchymal and nonparenchymal cells, primarily by inducing degradation of Smad2 (and to a much lesser extent Smad3) and thus impairment of transforming growth factor β (TGF-β) signaling. CTGF and TGF-β play crucial roles in the fibrotic remodeling of various organs, and, ultimately, carcinogenesis. This article summarizes the clinical-epidemiological observations as well as the pathophysiological background and provides suggestions for the therapeutic use of (methyl)xanthine derivatives in the management of fibro-/carcinogenic (liver) diseases. (HEPATOLOGY 2009;50:970-978.)

Coca-Cola, regular coffee, and cappuccino are only a few of the uncountable types of caffeinated beverages without which modern everyday life would be unthinkable. However, apart from the enjoyment frequently associated with a tasty cup of coffee on a Sunday morning, recent scientific reports point to the ability of this beverage to minimize the progression of chronic fibrogenic liver disease and the susceptibility to develop hepatocellular carcinoma (HCC).

Liver fibrosis, most commonly caused by chronic alcohol abuse and hepatitis C, and characterized by replacement of functional liver tissue by fibrotic scar tissue as well as regenerative nodules which may, ultimately, result in cirrhosis and the development of HCC, is a major health burden worldwide.1 Disease progression and fibrogenic activity show significant interindividual variability, allowing discrimination between slow, intermediate, and rapid fibrosers. Both environmental and host genetic factors are suspected to modify disease susceptibility and progression rate.2

Data on 5,994 adult patients with chronic liver disease collected by scientists during the third National Health and Nutrition Examination Survey (NHANES III)3,4 of the Centers of Disease Control and Prevention proposed a hepatoprotective effect of increased coffee consumption. Similar results were obtained previously by NHANES I as well as during a recent study by the National Institute of Diabetes and Digestive and Kidney Disease (Bethesda, MD).5 These studies provided evidence that patients with higher coffee consumption displayed a milder course of fibrosis,4,5 especially in alcoholic liver disease3,4,6 and lower serum activities of alanine aminotransferase and gamma glutamyl transpeptidase.3,6 According to NHANES III, two cups of coffee per day were sufficient to markedly reduce the risk of fibrosis progression.4

Japanese scientists evaluating 9-year data of a total of 60,107 subjects for the association of coffee intake and the
risk of developing HCC found that the risk of malignancy was significantly reduced compared with subjects who did not consume coffee. This was confirmed by the Japan Collaborative Cohort Study for Evaluation of Cancer Risk, which calculated a hazard ratio of only 0.50 for death due to HCC for drinkers of one or more cups of coffee per day. A Swedish meta-analysis evaluating the data of 2,260 cases and 239,146 noncases confirmed this inverse relation between coffee consumption and risk of HCC and calculated a 43% reduced risk of developing HCC in patients that consumed at least two cups of coffee per day.

Despite these striking epidemiological data, the cellular and molecular mechanisms underlying the antifibrotic and tumor-suppressive effects of coffee consumption in patients with chronic liver disease remains obscure.

**Caffeine: Chemical Structure, Metabolism, and Mechanism of Action**

At the behest of Johann Wolfgang von Goethe, Friedlieb Ferdinand Runge, a pharmacochemist from Wrocław, Poland, was the first to look for the psychoactive substance in coffee. In 1820, he finally extracted chemically pure caffeine, from which the German scientists Christoph-Heinrich Pfaff and Justus von Liebig successfully deduced the structural formula $C_8H_{10}N_4O_2$ by way of burning analysis. In his 1875 professorial dissertation, the Würzburg chemist and pharmacist Ludwig Medicus transferred this structural formula into the chemical structure 1,3,7-trimethylxanthine. However, after major disputes with Hermann Emil Fischer, who was based in Berlin and was the Nobel Prize winner for chemistry in 1902, Ludwig Medicus’ chemical structure only received public acceptance after the first chemical synthesis of caffeine by Fischer in 1895.

Today, global consumption of caffeine has been estimated at 120,000 tons per year, making it the most widely consumed pharmacologically active substance in the world. It is completely absorbed by the stomach and small intestine within 45 minutes of ingestion, and is eliminated by first-order kinetics.

Caffeine is metabolized in the liver, particularly in liver parenchymal cells (hepatocytes), by the cytochrome P450 oxidase enzyme system (CYP1A2) into the three metabolic dimethylxanthenes paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine). In three-dimensional shapes, atoms are color coded: C, grey; N, dark blue; O, red; H, white.

Fig. 1. Hepatocellular metabolism of caffeine by the cytochrome P450 oxidase enzyme system (subtype 1A2) into the three metabolic dimethylxanthenes paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine). In three-dimensional shapes, atoms are color coded: C, grey; N, dark blue; O, red; H, white.
CTGF/CCN2 Expression in the Liver: Regulation and Functional Relevance

CTGF is a 36- to 38-kDa cysteine-rich, heparin-binding, and secreted protein synthesized by various cell types. It is now classified as the second of six members of the CCN gene family containing CTGF itself, cyp61, NOV, and others, which share approximately 40% to 60% sequence similarity and are characterized as mosaic proteins that comprise four conserved structural modules. These modules are important for the pleiotropic functions of CTGF including—among others—matrix production, cell migration, cell adhesion, and cellular differentiation. As may be deduced from these effects, CTGF has reached considerable pathophysiological relevance because of its involvement in the pathogenesis of fibrotic diseases, carcinogenesis, atherosclerosis, or skin scarring, and other conditions with excess production of extracellular matrix components.

The competence of hepatocytes for the synthesis of CTGF was recently shown, and that it is sensitively up-regulated by exogenous and endogenous TGF-β. From this, hepatocytes are now recognized as a quantitatively important source of CTGF.

CTGF gene activation by TGF-β is mediated by a functional Smad-binding element, which resides within the CTGF promoter. In hepatocytes, TGF-β-driven CTGF gene expression is regulated primarily by Smad2 and, to a much lesser extent, Smad3.

The fibro-/carcinogenic mechanisms in the liver are dependent on an interplay of many profibrotic/antifibrotic, oncogenic/tumor suppressive and proinflammatory/anti-inflammatory cytokines. The hierarchy of profibrogenic growth factors most importantly includes TGF-β, designated as a fibrogenic master cytokine with multiple effects on extracellular matrix turnover, hepatic cellular apoptosis, proliferation and liver regeneration, inflammation and immunosuppression, and, ultimately, carcinogenesis. The natural antagonist of many actions of TGF-β is bone-morphogenetic protein 7 (BMP-7), a member of the TGF-β superfamily.

Even though the molecular mechanism of action of CTGF is still not known in detail yet, a modulator role in the epithelial-to-mesenchymal transition (EMT) of adhering hepatocytes into cells with reduced intercellular adhesion, increased motility, and mesenchymal, fibroblast-like properties, is discussed. This process is gaining more and more importance in the pathogenetic understanding of hepatic fibrogenesis, but accumulating evidence also points to a critical role of EMT-like events during tumor progression and malignant transformation.
endowing the incipient cancer cell with invasive and metastatic properties.52

The prototype of the currently most powerful inducer of EMT is TGF-β.53 In contrast, BMP-7 not only inhibits EMT, but can even induce a mesenchymal-epithelial transition.50 Recent reports gave evidence that up-regulation of CTGF inhibits BMP-7 signal transduction in the diabetic kidney.54 Furthermore, Abreu et al.49 presented data describing CTGF as extracellular trapping protein for BMP and TGF-β, preventing its binding to the specific BMP receptors. Of note, the opposite effect, enhancement of receptor binding, was observed for TGF-β.51 From this, CTGF would act profibrogenic and possibly procarcinogenic, which is supported by reports of increased CTGF expression in various tumor tissues55-60 as well as in the fibrotic liver (Fig. 2),28,61,62 and, even more important, by recent studies, in which in vivo knockdown of CTGF by small interfering RNA leads to substantial attenuation of experimental liver fibrosis.63,64 Thus, modulators of CTGF expression will have a great pathogenetic relevance for fibrosis.

**Inhibition of Hepatocellular CTGF Synthesis by Caffeine Through Enhanced Proteasomal Degradation of the TGF-β Effector Smad 2**

Recent observations have yielded evidence that caffeine is able to enforce proteasomal Smad2 degradation by enhancing the activity of Smurf2, a member of the family of E3 ubiquitin ligases (Fig. 3),65-67 with the consequence that Smad2 is increasingly bound to ubiquitin and proteasomally degraded.66,67 This finding seems to be of particular relevance for clinical situations of TGF-β activation such as viral hepatitis and tumor growth.66,67,69 as degradation of Smad2 in response to TGF-β requires receptor-mediated phosphorylation of its C-terminal serine.66,67,70 This indicates a stimulation of proteasome-mediated degradation which is largely specific for Smad2, as previously observed by Lo and Massague.66 The induction of Smad3 degradation by caffeine was much less pronounced, but its phosphorylation by the TβRII Alk5 receptor was clearly impaired (Fig. 3), which may be explained by the fact that Smurf2 is also able to bind the activated TGF-β receptor complex, leading to TβRII degradation, resulting in an inhibition of phosphorylation of Smad3 and even in a partial degradation of total Smad3 protein allosterically bound to the type 1 receptor in the Smad3/Sara (Smad anchor for receptor activation)/TβRII kinase complex.57-71 Nevertheless, receptor-phosphorylated Smads do not seem to be interchangeable, and each one seems to follow specific metabolic routes.

It is not known how caffeine triggers proteasomal degradation; however, earlier works have claimed that the cAMP/PKA dependent pathway can directly regulate the activity of the ubiquitin-proteasome system72,73 and that phosphorylation by PKA can alter the proteasomal degradation rate of the phosphorylated protein.74,75 Therefore, a link between hepatocellular cAMP accumulation and ubiquitin ligase activity may be presumed.
Prevention of N-Acetyl-D-Galactosamine-6-Sulfate Induced Hepatic Expression of CTGF by Intraperitoneal Application of Caffeine

All the findings discussed above were based exclusively on data obtained by in vitro experiments. Even more exciting were the results of very recent investigations aimed at more closely mimicking the situation of therapeutic caffeine application, which impressively demonstrated the capability of caffeine to suppress hepatic CTGF expression also in the experimental rat model of toxic hepatitis induced by N-acetyl-D-galactosamine-6-sulfate in vivo (Fig. 4). It was shown that caffeine applied every 4 hours over a period of 24 hours markedly reduced D-galactosamine–induced CTGF expression in the damaged liver and reduced the spillover of hepatic-derived CTGF into the circulation. Caffeine-treated animals were alive and in good condition.

Inhibiting Effect of the Primary Caffeine-Derived Metabolites on Hepatocellular CTGF Synthesis

The 50% inhibitory dose (ID$_{50}$) of caffeine for hepatocellular CTGF synthesis was calculated as being 4.42 mM (Fig. 5). Based on these rather high, therapeutically impracticable concentrations, further studies were initiated to investigate the respective repressive capacities of...
the demethylated primary caffeine metabolites paraxanthine, theobromine, and theophylline. Paraxanthine was identified as the most potent caffeine-derived pharmacological repressor of CTGF expression in hepatocytes, with an ID$_{50}$ of 1.15 mM—3.84-fold lower than what is observed for caffeine (Fig. 5). Paraxanthine simultaneously displayed the least cytotoxicity of all tested metabolites.$^{77}$ At the toxicological threshold concentration of 1 mM for paraxanthine,$^{78,79}$ an inhibition of hepatocellular CTGF synthesis by 44% was observed. Even in the presence of 0.13-1 $\mu$g/L exogenously added TGF-$\beta$, paraxanthine (1.25 mM and 2.5 mM) still reduced hepatocellular CTGF synthesis by an average of 27% and 45%.$^{77}$

Transdifferentiation-Dependent Inhibition of CTGF and Collagen $\alpha 1$ Type 1 Synthesis in Rat Hepatic Stellate Cells by Paraxanthine

We learned in detail how caffeine and its primary metabolite paraxanthine suppress Smad2 (and Smad3) regulated CTGF expression in hepatocytes. However, their effect on hepatic stellate cells (HSCs), activated as a result of hepatic injury and then undergoing transition from quiescent vitamin A–rich cells into proliferative, fibrogenic, and contractile myofibroblasts was not yet addressed, even though the relevance of this mesenchymal cell type in the pathogenesis of hepatic fibrogenesis is still considered unequivocally substantial.$^{80}$

Recently, some light was shed on this burning question by emerging data dealing with the effect of paraxanthine on HSC activation, in addition to data on related changes in the synthesis of extracellular matrix components by this cell type (unpublished data by Gressner et al.). These data showed a transdifferentiation-dependent inhibitory effect of paraxanthine on CTGF protein expression and promoter activity also in HSCs, being particularly effective in the progressive stage of transdifferentiation. This reduction of CTGF expression was accompanied by an increasing, paraxanthine-dependent inhibition of expression of collagen $\alpha 1$ type 1 (Col1) but not of $\alpha$-smooth muscle actin ($\alpha$-SMA) during transdifferentiation (Fig. 6) (unpublished data by Gressner et al.).

Such a transdifferentiation-dependent increasing sensitivity of this mesenchymal cell type toward paraxanthine-induced inhibition of CTGF (and also Col1) expression may be the result of an evolution of Smad signaling and an enhancement in TGF-$\beta$–binding and responsiveness during their activation and transdifferentiation to myofibroblasts, facilitating an autocrine stimulation by TGF-$\beta$. This would also explain the lacking effect on $\alpha$-SMA expression, as the transcriptional regu-
loration of α-SMA in HSCs is largely TGF-β-independent, whereas its reorganization to stress fibers during transdifferentiation is not.81,82 Regardless, next to its effects in hepatocytes, these results were the first to show the suitability of paraxanthine to also antagonize transdifferentiation-dependent sensitization of HSCs toward TGF-β actions, such as induction of CTGF and Col1 expression.

Conclusions and Perspectives

Taken together, the studies introduced above point to an important role of (methyl)xanthine derivatives such as caffeine or paraxanthine in interrupting the TGF-β/Smad2 (and, to a lesser extent, TGF-β/Smad3) signaling pathway in hepatocytes as well as activated HSCs. These findings may partially explain earlier results showing that the activation of the TGF-β response element (TβRE), sharing partial homology with the consensus sequence of the cAMP response element, is inhibited in the presence of cAMP analogs or agents elevating intracellular cAMP concentrations. Duncan et al.83 presumed a direct interaction between cAMP and the TβRE; however, the presented data suggest that the phenomenon described by Duncan et al. might not rely on a direct interaction of this promoter sequence with cAMP, but much more on a modulation of (phosphorylated) Smad2 (and Smad3) concentrations by cAMP-elevating substances such as caffeine or paraxanthine.

Of course, the data discussed above still have limitations in terms of their immediate therapeutic relevance. For example, the specificity of caffeine and/or paraxanthine in repressing profibrogenic (such as CTGF) but not anti-fibrogenic (such as BMPs) target genes has not been assessed yet.84 Also, the activation of other, non–PKA-mediated hepatocellular signaling pathways by caffeine, such as AMP-activated protein kinases,85 cannot be entirely excluded at present and require further evaluation.

However, because the overall pivotal role of (TGF-β and) CTGF in the pathogenesis of liver fibrosis has been convincingly proven in experimental rat liver fibrosis with silenced CTGF,63,64 and because a strong overexpression of CTGF is found in both fibrotic and tumor tissues,28,55-62 the findings summarized above suggest a suppressive effect of caffeine, paraxanthine, or related (methyl)xanthine derivatives also on human liver fibrosis. This suggestion is further supported by recent data showing that blocking adenosine A2A receptors reduces peritoneal fibrosis in two independent experimental models in vivo.86 Therefore, (dimethyl) derivatives of xanthine may eventually be proposed as a family of drugs useful in the treatment of chronic fibrogenic (or even carcinogenic) disorders, not only of the liver. The presented findings will hopefully initiate further studies in this direction. And while they are on their way, enjoy your cup of coffee!

References

24. Mayr B, Montminy M. Transcriptional regulation by the phosphoryla-
1220.
26. Heusinger-Ribeiro J, Eberlein M, Wahab NA, Goppelt-Stuhrme M. Ex-
1861.
27. Kothapalli D, Hayashi N, Grotendorst GR. Inhibition of TGF-beta-stimu-
29. Leask A, Abraham DJ. All in the CCN family: essential matricellular sig-
naling modulators emerge from the bunker. J Cell Sci 2006;119:4805-
4810.
32. Gressner OA, Lahme B, Siluschek M, Rehbein K, Weiskirchen R, Gressner AM. Connective tissue growth factor is a Smad2 regulated amplifier of transform-
33. Gressner OA, Lahme B, Demirci I, Gressner AM, Weiskirchen R. Differ-
40. Liu LX, Han YC, Wu SH, Lv ZH. Expression of connective tissue growth factor in tumor tissues is an independent predictor of poor prognosis in patients with gastric cancer. World J Gastroen-
lated connective tissue growth factor expression in hepatocytes via PPAR-
46. Lo RS, Massague J. Ubiquitin-dependent degradation of TGF-beta-acti-
47. Zhang Y, Chang C, Gehling DJ, Hemmati-Brivanlou A, Derynck R. Regu-
49. Qiu BY, Lam SS, Correia JJ, Lin K. Smad3 allostery links TGF-beta recep-