Several epidemiological studies suggest that coffee drinking is inversely correlated with the risk of development of liver fibrosis. However, a causal, mechanistic explanation has long been pending. New results indicate that the methylxanthine caffeine, major component of coffee and the most widely consumed pharmacologically active substance in the world, might be responsible for this phenomenon as it, and even more potently its derived primary metabolite paraxanthine, inhibits transforming growth factor (TGF)-β-dependent and -independent synthesis of connective tissue growth factor (CTGF/CCN2) in liver parenchymal cells in vitro and in vivo. CTGF plays a crucial role in the fibrotic remodeling of various organs which has therefore frequently been proposed as therapeutic target in the management of fibrotic disorders.

This article summarizes the clinical–epidemiological observations as well as the pathophysiological background of the antifibrotic effects of coffee consumption and provides suggestions for the therapeutic use of caffeine and its derived metabolic methylxanthines as potentially powerful drugs in patients with chronic fibrogenic liver disease by their inhibitory effect on (hepatocellular) CTGF synthesis.
ify disease susceptibility and progression rate (Gressner et al., 2008a).

Data on 5994 adult patients with chronic liver disease, collected by US-American scientists during the third National Health And Nutrition Examination Survey (NHANES III) (Ruhl and Everhart, 2005a,b) of the National Centers of Health Statistics, Disease Control and Prevention (CDC; Atlanta/Georgia, USA) 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/National Institute of Health (NIDDK/NIH; Bethesda/MD, USA) (Modi et al., 2007).

In summary, these studies gave evidence that patients with higher coffee consumption displayed a milder course of fibrosis (Ruhl and Everhart, 2005b; Modi et al., 2007), especially in alcoholic liver disease (Ruhl and Everhart, 2005a; Tanaka et al., 1998) and lower serum activities of alanin-aminotransferase (ALT) and γ-glutamyltransferase (GGT) (Ruhl and Everhart, 2005a,b; Tanaka et al., 1998). According to Ruhl et al., two cups of coffee daily were sufficient, to markedly reduce the risk of fibrosis progression (Ruhl and Everhart, 2005b).

Scientists from Tohoku University Hospital in Sendai/Japan who evaluated 9-year data of coffee consumption of 60,107 subjects for the association of coffee intake and the risk of developing primary liver cancer (hepatocellular carcinoma; HCC) found that regular coffee consumption (Shimazu et al., 2005). The Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study) investigated 110,688 cohort members aged 40–79 years in respect of their average coffee intake and calculated a hazard ratio of 0.50 for death due to HCC for drinkers of one and more cups of coffee per day. In contrast, the ratio for drinkers of less than one cup per day was 0.83, which therefore confirmed an inverse association between coffee consumption and HCC mortality (Kurozawa et al., 2005).

These findings were supplemented with a Swedish meta-analysis of the Karolinska Institute, Stockholm which evaluated the data of 9 cohort and case–control studies involving a total of 2260 cases and 239,146 non-cases. All epidemiological studies that were considered reported an inverse relation between coffee consumption and risk of liver cancer, and in 6 studies the association was statistically significant. Overall, this meta-analysis revealed an association of an increase in consumption of 2 cups of coffee per day and a 43% reduced risk of developing HCC (Larsson and Wolk, 2007).

However, despite of these striking epidemiological data, the cellular and molecular mechanisms underlying the antifibrotic and tumor-suppressive effects of coffee consumption remained obscure.

2. Caffeine: what is it all about?

Most of the world’s coffee today comes from either South America or Indonesia, but coffee originated in the highlands of Ethiopia and did not reach Europe for thousands of years (Weinberg and Bealer, 2000). It was not until the 16th century that the introduction of coffee to Europe took place. Coffee arrived from the Middle East where it had achieved a near cult like following, with the first coffeehouses being established in Istanbul (Weinberg and Bealer, 2000). Once accepted by Islamic law (it was very nearly banned, like alcohol) the beverage followed the spread of Islam across Africa and Eastern Europe. Venice, which relied heavily upon trade with the Muslim east, was first introduced to the invigorating liquid in the 1570s (Weinberg and Bealer, 2000). However coffee would remain a luxury item at this time and not drunk for refreshment, but as a medicinal drink. However once coffee was transplanted to European colonies in Asia and South America the bean thrived and became accessible to the public (Weinberg and Bealer, 2000). The 17th century saw the opening of the first European coffeehouse in Venice, which later spawned over two hundred others along its canals. Coffee spread quickly at this point and other coffeehouses were founded in the major cities of Italy. Some of these dignified and elegant establishments are still in existence in Venice, Turin and Rome; virtual palaces to the national stimulant (Weinberg and Bealer, 2000).

At the behest of the German writer Johann Wolfgang von Goethe, Friedlieb Ferdinand Runge, a chemist and pharmacist from Wroclaw, was the first to investigate coffee beans with the objective of finding the psychoactive substance in coffee (Weinberg and Bealer, 2000). In 1820, he finally extracted chemically pure caffeine, from which the German scientists Christoph Heinrich Paff and Justus von Liebig successfully deduced the structural formula C8H10N4O2 by burning analysis (Weinberg and Bealer, 2000). In his 1875 professorial dissertation, the Würzburg chemist and pharmacist Ludwig Medicus transferred this structural formula into the chemical structure 1,3,7-trimethylxanthine (Weinberg and Bealer, 2000). However, after major disputes with Hermann Emil Fischer, based in Berlin and Nobel Prize winner in 1902, Ludwig Medicus’ chemical structure only received public acceptance after the first chemical synthesis of caffeine by Fischer in 1895 (Weinberg and Bealer, 2000).

Today, global consumption of caffeine has been estimated at 120,000 tonnes per annum, making it the most widely consumed pharmacologically active substance in the world (Weinberg and Bealer, 2000). In North America, 90% of adults consume caffeine daily (Weinberg and Bealer, 2000). It is completely absorbed by the stomach and small intestine within 45 min of ingestion, and is eliminated by first-order kinetics (Newton et al., 1981).

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; 84%), theobromine (3,7-dimethylxanthine; 12%), and theophylline (1,3-dimethylxanthine; 4%) (Barone and Roberts, 1996; Gates and Miners, 1999; Ullrich et al., 1992; Roberts et al., 1994) (Fig. 1). Further demethylation and oxidation form urates and uracil derivatives. About a dozen metabolites can be recovered in the urine of regular coffee consumers (Barone and Roberts, 1996; Ullrich et al., 1992).

Caffeine and its metabolites act through multiple mechanisms involving both action on receptors and channels on the cell membrane, as well as intracellular action on calcium and cAMP pathways (Nguyen et al., 2007). Even though the major caffeine derivatives, i.e. paraxanthine, theobromine, and theophylline, have common mechanisms of action, the fraction, by which any of the pathways is affected, differs between them.

By virtue of its purine structure, caffeine can act on some of the same targets as adenosine related nucleosides and nucleotides, i.e. activation of intracellular Ryanodine receptors [which are the physiological target of cADPR (cyclic ADP ribose)] in vitro as well as competitive inhibition of adenosine receptors (particularly to subtypes 1 and A2) and of the cyclic adenosine monophosphate phosphodiesterase (cAMP-PDE) in vitro and in vivo (Belibi et al., 2002; Jafari and Rabbani, 2004; Fisone et al., 2004; Daly, 1993; Etteng et al., 1997). Inhibition of the latter, in particular, results in an accumulation of cAMP within the cell.

cAMP was one of the first identified second messengers transmitting signals via G-protein coupled receptors and protein kinase A (PKA) from the cell surface to the nucleus (Montminy, 1997).
In the unactivated state, PKA resides in the cytoplasm. Induction by cAMP liberates the catalytic subunits of PKA, which then are capable of diffusing into the nucleus where they phosphorylate transcription factors, i.e. CAMP response element binding protein (CREB) (Sassone-Corsi, 1998). PKA phosphorylates CREB at serine 133, which then transactivates cAMP-responsive genes by binding as a dimer to a conserved, 8 bp, palindromic cAMP response element (CRE), TGACGTCA. Over 100 genes with functional CREs have been identified so far and a modulation of various cell signaling proteins by cAMP has been reported (Mayr and Montminy, 2001).

3. Connective tissue growth factor (CTGF/CCN2): structure and protein family

We just concluded that caffeine and its primary metabolites act as competitive intracellular inhibitors of cAMP-PDE, which converts cAMP to its non-cyclic form (Jeon et al., 2005), thus allowing cAMP to build up in cells. This aspect is of particular relevance, as cAMP was shown to inhibit transforming growth factor (TGF-β) expression (Heusinger-Ribeiro et al., 2001; Kothapalli et al., 1998).

CTGF, a 36–38 kDa cysteine-rich, heparin-binding, and secreted protein synthesized by various cell types, is now classified as the second of six members of the CCN gene family containing CTGF itself, cyr61, NOV, and others (Rachfal and Brigstock, 2003), which share approximately 40–60% sequence similarity and are characterized as mosaic proteins that comprise four conserved structural modules (Leask and Abraham, 2006). These modules are important for the pleiotropic functions of CTGF including among others matrix production, cell migration, cell adhesion, and cellular differentiation (Moussad and Brigstock, 2000; Rachfal and Brigstock, 2005).

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, skin scarring, and other conditions with excess production of connective tissue (Fig. 2) (Rachfal and Brigstock, 2005).

4. Modulation of CTGF expression by the TGF-β superfamily of cytokines

Recently, the ability of hepatocytes for the synthesis of CTGF was shown by detailed cell culture studies, which clearly demonstrate CTGF expression in parenchymal liver cells, and that it is sensitively up-regulated by exogenous Transforming Growth Factor (TGF-β) (Fig. 2) (Gressner et al., 2007; Weng et al., 2007). In addition, hepatocellular CTGF expression also occurs spontaneously in TGF-β-free culture conditions due to intracellular activation of latent TGF-β (Gressner et al., 2008b). Thus, hepatocytes are now recognized as a quantitatively important source of CTGF, which responds to TGF-β.

TGF-β belongs to a superfamily of cytokines, which comprises further ligands, such as bone morphogenetic proteins (BMPs), and Activin A. All TGF-β superfamily ligands bind to a type II receptor dimer, which recruits a type I receptor dimer forming a hetero-tetrameric complex with the ligand (Gressner et al., 2002), resulting in the phosphorylation of the type I receptor. The activated type I receptor then phosphorylates receptor-regulated SMADs (R-SMADs), which can now bind the coSMAD SMAD4, R-SMAD/coSMAD complexes accumulate in the nucleus where they act as transcription factors and participate in the regulation of target gene expression (Gressner et al., 2002).

CTGF gene activation by TGF-β is mediated by a functional Smad-binding element, which resides within the CCN2 promoter (Rachfal and Brigstock, 2005). In hepatocytes TGF-β-driven CTGF gene expression is primarily controlled by Smad2 and its transcriptional cofactors and, to a much lesser extent, by Smad3 (Gressner et al., 2009), which confirms data obtained in other cellular systems (Gressner et al., 2008b,c). A large number of Smad2-associated transcriptional co-activators, including CREB binding protein (CBP) and p300, have been identified to possess intrinsic acetyltransferase activities that are important for their abilities to enhance transcrip-
tion by this downstream mediator of TGF-β actions (Bannister and Miska, 2000; Brown et al., 2000; Chen et al., 2001; Kouzarides, 2000; Marmorstein, 2001; Marmorstein and Roth, 2001; Ogrzyzko, 2001; Roth et al., 2001). In particular DNA binding activity and association with target promoters of Smad2 are tightly regulated by CBP/p300-mediated acetylation of this Smad in response to TGF-β signaling (Simonsson et al., 2006).

5. The role of CTGF in epithelial to mesenchymal transition

The fibrogenic mechanisms in the liver are dependent on an interplay of many pro- and anti-fibrotic/-inflammatory cytokines (Gressner, 1996; Pinzani and Rombouts, 2004). The hierarchy of pro-fibrogenic growth factors most importantly includes TGF-β, designated as “fibrogenic master cytokine” with multiple effects on extracellular matrix turnover (Gressner et al., 2002; Bissell et al., 1991; Gressner et al., 1997, 1996), proliferation and liver regeneration (Bissell et al., 2001; Inagaki and Okazaki, 2007; Huang and Huang, 2005), inflammation and immunosuppression (Cerwenka and Swain, 1999), and cancerogenesis (Elliott and Blobe, 2005). The natural antagonist of many actions of TGF-β is bone-morphogenetic protein 7 (BMP-7), a member of the TGF-β superfamily (Chen et al., 2004). Thus, the balance of both growth factors, i.e. TGF-β and BMP-7, will be crucial for development of fibrosis and outcome of (chronic) liver disease, i.e. risk for the development of HCC.

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 (Abreu et al., 2002). This process is gaining more and more importance in the pathogenetic understanding of hepatic fibrogenesis (Zeisberg et al., 2003, 2007), 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 (Larue and Bellacosa, 2005).

The protoype of the currently most powerful inducer of EMT is TGF-β (Zavadil et al., 2004), activating this pathway via induction of Smad2/3 phosphorylation and the Snail transcription factor (Zavadil et al., 2004). In contrast, BMP-7, the most important molecular counterpart of TGF-β, not only inhibits EMT, but can even induce a mesenchymal–epithelial transition (reverse EMT = MET) (Zeisberg et al., 2003). Recent reports gave evidence that up-regulation of CTGF inhibits BMP-7 signal transduction in the diabetic kidney (Nguyen et al., 2008). Abreu et al. furthermore presented data describing CTGF as extracellular trapping protein for BMP and TGF-β (Abreu et al., 2002). According to their functional studies on Xenopus laevis, CTGF directly binds BMP and TGF-β through their cysteine-rich (CR) domain, thus antagonizing BMP activity by preventing its binding to BMP receptors. Of note, the opposite effect, enhancement of receptor binding, was observed for TGF-β (Abreu et al., 2002). These results suggest that CTGF inhibits BMP and activates TGF-β signals by direct binding in the extracellular space. From this, CTGF would act pro-fibrogenic (Fig. 3).

A central role of CTGF in liver fibrogenesis and tumor growth, which may thus be expected, is documented by reports on increased CTGF expression in various tumor tissues (Gressner and Gressner, 2008; Liu et al., 2008; Mullis et al., 2008; Munemasa et al., 2007; Boag et al., 2007; Kidd et al., 2007) as well as in fibrotic liver tissue (Fig. 4) (Rachfal and Brigstock, 2003; Hayashi et al., 2002; Paradis et al., 1999), and, even more important, by recent studies, in which knock-down of CTGF by siRNA leads to substantial attenuation of experimental liver fibrosis (George and Tsutsumi, 2007; Li et al., 2006). Thus, modulators of CTGF-expression will have a great pathogenetic relevance for fibrosis.

6. Caffeine suppresses TGF-β-dependent and -independent synthesis of the hepatocyte primarily through an inhibition of the TGF-β effector Smad2

We previously talked about Smad2 (and, to a lesser extent, Smad3) as key mediator of TGF-β-induced CTGF expression in hepatocytes (Gressner, 2009). Very recent observations gave evidence, that caffeine is able to enforce proteasomal Smad2 degradation in hepatocytes (Fig. 6) by...
Fig. 3. The impact of connective tissue growth factor (CTGF/CCN2) on hepatocellular epithelial-to-mesenchymal transition during hepatic fibrogenesis. Hepatocytes are induced to undergo epithelial-to-mesenchymal transition (EMT) or apoptosis. The resulting fibroblast-like cells lose their ability to express albumin while they become positive for the fibroblast-specific protein-1 (FSP1). In all these processes, TGF-β acts as a strong inductor of EMT while BMPs have an opposing effect. The balance of both cytokines is modulated by CTGF that increases TGF-β and reduces BMP activities.

enhancing, the activity of SMURF2, a member of the family of E3 ubiquitin ligases (Lo and Massague, 1999; Zhang et al., 2001), with the consequence that Smad2 is increasingly bound to ubiquitin and proteasomally degraded (Gressner et al., 2008d; Lo and Massague, 1999; Zhang et al., 2001; Wicks et al., 2006). This finding seems to be of particular relevance for clinical situations of TGF-β activation such as viral hepatitis and tumor growth (Gressner et al., 2002; Elliott and Blobe, 2005; Massague et al., 2000; Murawaki et al., 1998), as degradation of Smad2 in response to TGF-β requires receptor-mediated phosphorylation of the C-terminal serines (Lo and Massague, 1999; Zhang et al., 2001), which would suggest a normal or even stimulated Alk5-dependent phosphorylation, i.e. intra- or extracellular presence of TGF-β (Gressner et al., 2008b). Enhanced degradation of Smad3 was not observed in the presence of caffeine, but an inhibition of its phosphorylation (Fig. 6), which indicates a stimulation of proteasome-mediated degradation, specifically of Smad2, by caffeine. Such a high degree of specificity of SMURF2 to preferentially degrade Smad1 and Smad2 but not Smad3, was previously described by Lo et al. (Lo and Massague, 1999; Zhang et al., 2001). The caffeine-induced inhibition of phosphorylation of Smad3, however, may still be secondary to enhancement of SMURF2 activity, as this ubiquitin ligase is also

Fig. 4. Immunohistochemical staining of CTGF in normal and bile-duct ligated fibrotic rat liver (Gressner et al., 2008). Localization of CTGF in cytokeratin 18 positive hepatocytes and in few desmin-positive (myo-)fibroblasts is shown.
Caffeine upregulates the expression of the nuclear peroxisome proliferator-activated receptor γ in hepatocytes

Peroxisome proliferator-activated receptors (PPARs) comprise a group of nuclear receptor isoforms intimately connected to cellular lipid metabolism and cell differentiation. Three types of PPARs have been identified: α, γ and δ (β). Without going into pathophysiological detail, earlier reports demonstrated that the prostaglandin analog and PPARγ ligand 15-deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2) has a potent inhibitory effect TGF-β1-induced CTGF expression in the liver (Sun et al., 2006; Zhao et al., 2006), suggesting that hepatic CTGF is a PPARγ-regulated gene. However, in human aortic smooth muscle cells it was shown that activation of PPARγ abrogates TGF-β-induced CTGF expression by directly interfering with the Smad3 signaling pathway (Fu et al., 2001) and the p300 system, which are necessary cofactors for target gene activation by the Smad2/3 transcriptional complex (Fu et al., 2001; Hazra et al., 2004; Yavrom et al., 2005).

The expression of type 2 PPARγ (PPARγ2) was previously described as being directly regulated by cAMP/CREB, in that phosphorylated CREB binds to the promoter of PPARγ2 together with activating transcription factor 1 (ATF1), initiating gene transcription (Fox et al., 2006; Inoue et al., 2005). Thus, it is not surprising that upregulation of PPARγ expression by caffeine, in particular in combination with TGF-β, was identified as a further mechanism of the inhibitory effects of caffeine on CTGF expression in hepatocytes (Gressner et al., 2008d). As previously observed for aortic smooth muscle cells (Fu et al., 2001), binding of 15-d-PGJ2 to PPARγ also leads to a dissociation of Smad2 (and -3) transcriptional complex, that involves the cofactors p300 and CREB binding protein (CBP) within the hepatocyte, thus preventing the transcriptional activation of TGF-β target genes such as CTGF (Fig. 6) (Simonsson et al., 2006; Gressner et al., 2008d). However, it has to be considered that controversial data on the role of CBP/p300 in CTGF promoter activation are communicated, depending on the cell type (Fu et al., 2001; Hazra et al., 2004; Yavrom et al., 2005; Leask et al., 2001).

Still, this finding is of interest insofar as patients with fibrogenic liver disease or with HCC originating from nonfibrotic livers display strikingly higher serum concentrations of 15-d-PGJ2 compared to healthy controls and non-liver disease sick (Fig. 7) (Gressner et al., in press-a). Thus, it may be suggested that these patients display particular sensitivity towards anti-fibrotic therapy approaches with PPARγ inducing drugs such as caffeine.

8. Intraperitoneal application of caffeine prevents d-galactosamine induced hepatic expression of CTGF

All the findings discussed above were based exclusively on results of in vitro experiments. However, results obtained from in vivo studies are often not directly applicable to the in vivo situation. Even more exciting were the results of very recent investigations, aiming at more closely mimicking the situation of therapeutic caffeine application, that impressively demonstrated the capability of caffeine to suppress hepatocellular CTGF expression not only in vitro, but also in the experimental rat model of toxic hepatitis induced by N-acetyl-d-galactosamin-6-sulfate (d-GalN) in vivo (Fig. 8) (Gressner et al., in press-c). These data show that concomitant application of caffeine in a total of 6 intraperitoneal injections of each 50 mg/kg body weight caffeine every 4 h over 24 h markedly reduced d-GalN (500 mg/kg body weight) induced CTGF expression in the damaged liver, and that it raised intrahepatic cAMP levels approximately 2.2-fold compared to controls (Gressner et al., in press-c). Furthermore, caffeine markedly reduced the spill-over of hepatic derived CTGF into the circulation when compared to rats treated with d-GalN alone, which displayed significant higher CTGF expression.
Fig. 7. (Left) 15-d-PGJ2 serum concentrations in healthy controls, patients with extrahepatic manifestation of disease (NLD) and in patients with liver fibrosis (HCV). (Right) 15-d-PGJ2 serum concentrations in healthy controls and with hepatocellular carcinoma (HCC). Serum levels of 15-d-PGJ2 in patients with liver fibrosis (n = 289) are significantly higher than those found in NLD patients (n = 307; p < 0.0001) and in the control group (n = 139; p < 0.0001), whereas no significant difference is found between NLD patients and controls. Also, 15-d-PGJ2 serum concentrations are significantly elevated in patients with HCC (n = 43) compared to healthy controls (n = 63, p < 0.0001). Box plots are displayed, where the dotted line indicates the median per group. The box represents 50% of the values and horizontal lines show minimum and maximum values of the calculated non-outlier values. Open circles indicate outlier values.

serum concentrations than the control animals (Gressner et al., in press-c). Caffeine treated animals were alive and in good condition (Gressner et al., in press-c).

9. Identification of paraxanthine as the most potent caffeine-derived inhibitor of connective tissue growth factor expression in liver parenchymal cells

Above, we discussed the capacity of caffeine to almost entirely inhibit spontaneous CTGF synthesis at a calculated 50% inhibitory dose (ID₅₀) of 4.42 mM (Fig. 5) (Gressner et al., in press-c). Based on this, further studies were initiated to investigate the repres- sive capacities of the primary demethylated caffeine metabolites paraxanthine, theobromine, and theophylline on hepatocellular CTGF expression. The data suggest paraxanthine as the most potent caffeine-derived pharmacological repressor of hepatocellular CTGF expression with an ID₅₀ of 1.15 mM, i.e. 3.84-fold lower than what is observed for caffeine (Fig. 9), by simultaneously displaying least cytotoxicity of all tested metabolites (Gressner et al., in press-c).

At the toxicological threshold concentration of 1 mM for paraxan-

Fig. 8. Hepatic CTGF expression following toxic liver injury by α-GalN is strongly reduced by intraperitoneal application of caffeine in vivo. (A) Immunohistochemical detection of CTGF in liver (40× magnification). Paraffined rat liver slices were incubated with two different polyclonal antibodies against CTGF (lower two rows, CTGF expression is displayed by a reddish coloration) and respective non-immune control immunoglobulin factions (upper rows). (B) Western blot analysis of CTGF in whole liver lysate obtained from untreated, only α-GalN treated as well as caffeine and α-GalN treated rats.
thine, defined by the US-American Hazardous Materials Information System® III (HMIS® III) [NPCA, 2008] and the National Fire Protection Association [NFPA, 2008], an inhibition of hepatocellular CTGF synthesis by still 44% was observed (Gressner et al., in press-c). As for caffeine, this effect was strongly reverted in the presence of a specific competitive cAMP inhibitor, indicating that also paraxanthine mediates its inhibitory effect on CTGF synthesis through an elevation of intracellular cAMP concentrations (Gressner et al., in press-c). Furthermore, paraxanthine (1.25 and 2.5 mM) also reduced TGF-β-induced hepatocellular CTGF synthesis by in average 27% and 45%, respectively, and thus, next to caffeine itself, proved to be the strongest inhibitor also of CTGF expression caused by exogenous TGF-β (Gressner et al., in press-b).

10. Summary and future perspectives

Taken together, the studies introduced above point to two major mechanisms how caffeine might act on the suppression of hepatocellular CTGF synthesis: (A) reduction of the steady state concentration of total Smad2 protein, Smad2 is the Alk5-receptor-dependently phosphorylated mediators of TGF-β signaling to the CTGF promoter (Gressner et al., 2007, 2008b,d, in press-c), and (B) upregulation of the PPARγ-receptor resulting in enhanced sensitivity of PC towards natural PPARγ ligands such as 15-PGJ2. They all have the common consequence of an interruption of the Smad2 signaling pathway. 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 (CRE) [Duncan et al., 1999], is inhibited in the presence of cAMP analogs or agents elevating intracellular cAMP levels [Duncan et al., 1999]. Previously, a direct interaction between cAMP and the TβRE was thus suggested [Duncan et al., 1999]. However, the presented data propose that this 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) Smad levels and on a reduction of activity of the Smad2/3 transcriptional complex (via PPARγ) by cAMP elevating substances such as caffeine or paraxanthine.

Without doubt, the data discussed above still have limitations in terms of their immediate therapeutic relevance. For example, investigations on the effect of these methylxanthines on classical profibrogenic target genes in hepatic stellate cells (e.g. α-smooth muscle actin, or type 1 collagen) is missing, as the contribution of hepatic stellate cells to the pathogenesis of hepatic fibrosis is unequivocally substantial (Friedman, 2008a,b). Furthermore, the specificity of caffeine and/or paraxanthine in repressing profibrogenic (i.e. CTGF) but not antifibrogenic target genes (i.e. BMPs) has not been assessed yet.

However, as the overall pivotal role of CTGF in the fibrogenic process of the liver has been convincingly proven in experimental rat liver fibrosis with silenced CTGF [George and Tsutsui, 2007; Li et al., 2006], as strong overexpression of CTGF is found in both, fibrotic and tumor tissues (Rachfal and Brigstock, 2003; Gressner and Gressner, 2008; Liu et al., 2008; Mullis et al., 2008; Munemasa et al., 2007; Boag et al., 2007; Kidd et al., 2007; Hayashi et al., 2002; Paradis et al., 1999), and as caffeine is able to prevent d-GalN induced hepatic expression of CTGF in the rat in vivo, the molecular–biological mechanisms summarized above suggest a suppressive effect of caffeine, paraxanthine or cAMP analogs on human liver fibrosis, which could eventually propose methylxanthines as a family of drugs useful in the treatment of such disorders. The presented findings hopefully initiate further studies in this direction.

Conflict of interest

There are no conflict of interests.

References


