rence rate, which may include “incidence of appearance of new lesions”, should also be evaluated, because the overall survival can be easily affected by second or subsequent treatments for recurrence.

Dr. Fujita also states, “it appears that RFA has become an equally effective alternative to surgical resection for early HCC”. But, what is his opinion based on? We beg to disagree with this statement, because it is not based on any concrete data. At least our data [1] do not lend support to the notion of equality in clinical effects between surgery and RFA.

Finally, Dr. Fujita has proposed that we conduct a subgroup analysis according to the size and number of tumors. We agree with him, because a subgroup analysis would theoretically be useful in a large volume study like ours. However, we abandoned this idea, because the critical limitation of the short follow-up period in our study can still not be overcome. We shall consider a subgroup analysis in a future study, using the latest data being collected now.

References


Intraperitoneal application of caffeine prevents D-galactosamine-induced hepatic expression of connective tissue growth factor (CTGF/CCN2) in the rat

To the Editor:

A recent report from our group published in this Journal gave evidence that caffeine suppresses TGF-β-dependent and -independent Connective Tissue Growth Factor (CTGF/CCN2) expression in hepatocytes via a cyclic adenosine monophosphate (cAMP-)-dependent mechanism that involves upregulation of the nuclear receptor PPARγ and thus sensitization towards the natural PPARγ ligand 15-deoxy-D12,14-Prostaglandin J2, as well as enhanced degradation and inhibition of phosphorylation of the TGF-β effector Smads 2 and 3 [1]. This study finds clinical parallels in studies performed by the NIH/NIDDK which have recently suggested that coffee consumption may protect against cirrhosis, especially against alcoholic cirrhosis [2], and is of particular interest as it was shown that silencing of the CTGF gene in vivo almost entirely inhibits fibrotic remodeling of livers in mice previously subjected to hepatotoxic agents [3].

Based on these findings, we aimed at transferring the results to the in vivo situation by investigating the effect of intraperitoneal caffeine injection on CTGF expression in the N-acetyl-D-galactosamin-6-sulfate (D-GalN) injured rat livers in vivo and on serum CTGF concentrations. Therefore, 4 adult Sprague–Dawley rats (body weight 140–145 g) were included in this study. 2 rats were given a total of 6 intraperitoneal (i.p.) injections of each 50 mg/kg body weight caffeine dissolved in 0.9% NaCl solution every 4 h. One caffeine treated rat as well as one previously untreated rat received an i.p. injection of D-GalN (500 mg/kg body weight). D-GalN was applied 1 h after the first caffeine injection. The control rat received i.p. injections of 0.9% NaCl solution every 4 h. One caffeine treated rat as well as one previously untreated rat received an i.p. injection of D-GalN (500 mg/kg body weight). D-GalN was applied 1 h after the first caffeine injection. The control rat received i.p. injections of 0.9% NaCl instead of caffeine or D-GalN. Rats were killed by abdominal exsanguination and livers were perfused with 0.9% NaCl solution for 4 h. One caffeine treated rat as well as one previously untreated rat received an i.p. injection of D-GalN (500 mg/kg body weight). D-GalN was applied 1 h after the first caffeine injection. The control rat received i.p. injections of 0.9% NaCl instead of caffeine or D-GalN. Rats were killed by abdominal exsanguination and livers were perfused with 0.9% NaCl, dehydrated in 70–100% ethanol and eventually fixed in 4% (w/v) paraformaldehyde buffered in PBS, pH 7.4, and embedded in paraffin. Immunohistochemis-
try for CTGF, a cAMP ELISA, and an in-house CTGF ELISA were performed as described previously [1,4,5]. Furthermore, serum levels of ALT, AST (all units/L) and creatinine (to exclude renal insufficiency; mg/dL) were determined on a Roche Modular Analytics P i auto-analyzer™.

As shown in Fig. 1, in vivo toxicity by i.p. administration of D-GalN induced hepatic CTGF expression already after 24 h, which resembled the increase of serum transaminases in this group (AST – control: 54 116 U/L; D-GalN: 257 U/L; caffeine: 113 U/L; D-GalN + caffeine: 132 U/L; ALT – control: 64 U/L; D-GalN: 61 U/L; D-GalN + caffeine: 85 U/L), whereas the liver of the control animal and of the one treated with caffeine alone remained CTGF negative. No difference in creatinine was found between the animals. Concomitant application of caffeine with D-GalN markedly reduced CTGF expression in the damaged liver. Previously, we gave evidence that caffeine mediated its inhibitory effect on hepatocellular CTGF expression, at least in part, through an elevation of intracellular cAMP concentrations. We, therefore, investigated concentrations of cAMP in whole liver lysates of the animals described above. Application of caffeine raised intrahepatic cAMP levels approximately 2.2-fold (76 vs. 181 pmol/lg DNA) while D-GalN alone even slightly reduced hepatic cAMP levels (65 pmol/lg DNA). The animal treated i.p. with caffeine and D-GalN showed a significant increase of cAMP levels compared to the rat treated with D-GalN alone (98 pmol/lg DNA) and also slightly elevated cAMP levels compared to the control rat. As expected from previous observations [4], the rat with isolated D-GalN injection displayed higher CTGF serum-levels than the one treated with caffeine alone (218% vs. 94% of control) or the untreated animal (set as 100%). Of note, concomitant application of caffeine to the D-GalN treated rat significantly lowered serum concentrations of CTGF when compared to the rat treated with D-GalN alone (139% of control). However, remaining CTGF concentrations were still significantly elevated compared to the untreated control rat.

This is the first relevant demonstration of the capability of caffeine to suppress hepatocellular CTGF expression not only in vitro, but also in the experimental model of toxic hepatitis induced by D-GalN in vivo, and to markedly reduce the spill-over of hepatic derived CTGF into the circulation. As shown in the previous in vitro experiments, this inhibition is accompanied by an elevation of intracellular cAMP concentrations. Even though because of the small size of the study group, the presented results can only provide a hint on a possible transferability of the previously obtained in vitro data to the in vivo situation, and further large-scale studies in this direction are certainly required, a suppressive effect of caffeine on experimental and human liver fibrosis may be suggested, which could eventually propose methylxanthines as a family of drugs useful in the treatment of chronic fibrogenic liver diseases or other fibrotic disorders. The presented findings hopefully initiate further studies in this direction.

References

To the Editor:

Type 1 hepatorenal syndrome (HRS) is characterized by a marked splanchnic arterial and systemic vasodilatation ultimately resulting in an acute functional renal failure. Two recent, large, randomized controlled trials confirmed that terlipressin improved renal function, only in 35% of these patients [1,2]. We report an improvement of renal function using bosentan, a nonselective endothelin-receptor antagonist, and N-acetylcysteine (NAC) association in a terlipressin/albumin unresponsive type 1 HRS case.

A 49-year-old man with post-hepatitis B cirrhosis (Child-Pugh score 8, large ascites) presented with a 3-day history of decompensation with jaundice (serum bilirubin, 61 \( \mu \)mol/L), coagulopathy (international normalized ratio, 2.70), grade II encephalopathy, and oligo-anuria (urine output less than 100 mL/24 h, serum creatinine, 3.85 mg/dL) after resolution of spontaneous bacterial peritonitis under adequate anti-biotherapy. Medical past history was remarkable for chronic kidney disease related to biopsy proven post-hepatitis B membranoproliferative glomerulonephritis (baseline serum creatinine, 1.70 mg/dL, proteinuria 3 g/day and hematuria 200 red blood cells/high power field). On admission, there was no evidence of shock (mean arterial pressure, 80 mm Hg), gastrointestinal bleeding, or treatment with nephrotoxic drugs. Proteinuria and hematuria were stable compared to previous renal status, and the kidneys appeared normal on ultrasonography. Urinary sodium/potassium ratio was less than 1. Although the patient presented with medical history of glomerulonephritis, this acute renal failure episode had all the features of type 1 HRS [3], and the decision was made to start a treatment with terlipressin and albumin. Despite 72 h intravenous terlipressin 8 mg/24 h associated with adequate intravenous fluid and albumin infusion (1 g/kg at day 1 followed by 40 g/day), the patient worsened: his serum creatinine: 4.90 mg/dL urine output dropped to 10 mL/24 h, and he developed pulmonary oedema necessitating hemodialysis. Treatment including oral endothelin antagonist (bosentan, 62.5 mg twice daily) and N-acetylcysteine (150 mg/kg over 2 h, followed by a dose of 100 mg/kg daily for 5 consecutive days) was instituted leading to early clinical improvement: urine output increased to 2000 mL daily, serum creatinine decreased to baseline values (1.97 mg/dL) and dialysis could be withheld. Table 1 illustrates the end-stage liver disease score and renal parameters before and 2 weeks after the above mentioned treatment.

Table 1
Effect of bosentan + N-acetylcysteine in type 1 HRS: renal and liver outcomes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Terlipressin + albumin treatment</th>
<th>Bosentan + NAC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>90/50</td>
<td>95/50</td>
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<tr>
<td>Urine output (L/24 h)</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.85</td>
<td>4.90</td>
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<tr>
<td>Transaminases (ALT/AST)</td>
<td>76/64</td>
<td>116/86</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>61</td>
<td>68</td>
</tr>
<tr>
<td>MELD score</td>
<td>35</td>
<td>38</td>
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</tbody>
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HRS, hepatorenal syndrome, MELD, model for end-stage liver disease.