Endothelin receptor antagonist TAK-044 arrests and reverses the development of carbon tetrachloride induced cirrhosis in rats

C Thirunavukkarasu, Y Yang, V M Subbotin, S A K Harvey, J Fung, C R Gandhi

Background and aims: Hepatic concentrations of the powerful vasoconstrictor and fibrogen endothelin 1 (ET-1) and its receptors increase in human and experimental cirrhosis, suggesting a major role for ET-1 in the pathology of chronic liver disease. We investigated whether ET-1 receptor antagonism, after the development of fibrosis and cirrhosis, arrests/reverses the progression of chronic liver disease.

Methods: Chronic liver injury was induced in rats by carbon tetrachloride (CCl₄) treatment (0.15 ml/kg intraperitoneally twice a week) in conjunction with phenobarbital in drinking water (0.4 g/l) for four groups (1: fibrosis and eight (group 2: cirrhosis) weeks. Rat were then treated concurrently with the ET-1 receptor antagonist TAK-044 (10 mg/kg/day) and CCl₄/phenobarbital for a further four weeks.

Results: Histopathological examination revealed significant arrest of progression to cirrhosis in group 1 and reversal of cirrhosis in group 2 rats. TAK-044 treatment caused significant amelioration of portal hypertension, systemic hypotension, and liver injury (reduced activities of serum aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase), and improved hepatic synthetic capacity (increased serum albumin concentration) in both groups of rats relative to vehicle treated rats. TAK-044 reduced formation collagen synthesis, as evidenced by decreased hepatic hydroxyproline content, mRNA expression of collagen-α type I, and tissue inhibitors of matrix metalloproteinases 1 and 2, and mRNA and protein expression of a potent fibrogenic cytokine, transforming growth factor β1.

Conclusions: The results emphasize the role of ET-1 in the development of cirrhosis and strongly suggest that blockade of its actions can be a rational therapy for chronic liver disease and its complications.

Abbreviations: ET, endothelin; MAP, mean arterial pressure; MMP, matrix metalloproteinase; NO, nitric oxide; PAF, platelet activating factor; SMA, smooth muscle actin; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteinases; CCl₄, carbon tetrachloride; TGF-β, transforming growth factor β; γGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; RT-PCR, reverse transcriptase-polymerase chain reaction; QRT-PCR, quantitative real time PCR; ECM, extracellular matrix.
Reversal of cirrhosis by TAK-044

![Graph A](Image)

![Graph B](Image)

**Figure 1** Effect of carbon tetrachloride (CCl4) and TAK-044 treatment on hepatic endothelin 1 (ET-1) and preproET-1 mRNA. Rats were treated with CCl4 for 4, 8, and 12 weeks. During CCl4 treatment between four and eight weeks and eight and 12 weeks, 50% of the rats each received TAK-044 or saline. Hepatic ET-1 content and mRNA expression of preproET-1 were determined in tissue samples from three rats in each group, each assay performed in triplicate. Details are described in the methods section. (A) Hepatic ET-1 concentration. (B) Hepatic preproET-1 mRNA expression normalised with respect to that of β-actin with equal amounts of cDNA used in the polymerase chain reaction. *p<0.05, **p<0.01 versus control; ††p<0.01 versus CCl4.

**MATERIALS AND METHODS**

**Induction of liver cirrhosis**

The experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee according to the guidelines of the National Institutes of Health. Chronic liver injury was induced in male Sprague-Dawley rats (230–250 g), as described previously.692 5 by CCl4 administration (0.15 ml/kg intraperitoneally twice a week) in conjunction with phenobarbital (0.4 g/l) in drinking water. Control rats received injection of the carrier (peanut oil) and phenobarbital water. At the end of four weeks of treatment, femoral arterial and portal venous pressures were measured in three control and three CCl4 treated rats, as described previously.8 25 26 Briefly, under pentobarbital anaesthesia, a PE-50 catheter was inserted into the femoral artery to monitor arterial blood pressure. The hepatic trigone was exposed via laparotomy and the portal vein was skeletonised. The intestines and abdominal cavity were covered with warm saline soaked sponges during the entire experimental period. A 23 gauge needle in a PE-50 catheter was inserted into the portal vein to measure portal pressure. After stable recordings of arterial and portal venous pressure, blood was drawn from the femoral artery. The liver was excised, washed in ice cold phosphate buffered saline, and snap frozen in liquid nitrogen, after storing a slice of left lateral lobe in 10% buffered formalin.

**Determination of ET-1 and its receptors**

ET-1 was extracted and its concentration measured by ELISA (Peninsula Laboratories, Sunnyvale, California, USA). Hepatic ET-1 receptors were determined by saturation and competition binding assays, essentially as described previously.9 25

**Biochemical parameters**

Serum total protein, albumin, bilirubin, γ-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were estimated using a kit from Stanbio Laboratory (Boerne, Texas, USA).

**Morphology and scoring of liver cirrhosis**

Sections of 4 μm thickness were prepared from formalin fixed liver slices embedded in paraffin and stained with haematoxylin-eosin and Masson’s trichrome stain.27 Liver pathology was graded by VMS in a blinded manner using an established scoring system, as described previously27; a score of “5” was considered as complete cirrhosis and “0” as normal. Sections were also treated with anti-α-smooth muscle actin (SMA) antibody (Dako, Carpinteria, California, USA;
smooth muscle actin-1A4) to determine activated stellate cells/myofibroblasts, as described previously.26,27

**Determination of RNA expression**

Relative mRNA expressions of preproET-1, ET-1 receptors, collagen α type I, and TGF-β1 were determined by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR), essentially as described previously.26 The same amount of cDNA was used to determine expression of β-actin mRNA for normalisation. The number of PCR cycles employed was in the linear range of the reaction for each product.

Quantitative real time PCR (QRT-PCR) was performed for mRNA expression of tissue inhibitors of metalloproteinases (TIMPs) using a Prism 7700 Sequence Detector from Applied Biosystems Inc. (ABI, Foster City, California, USA) which measures 1.7a. Assay of cDNA levels was performed using Taqman Universal Master Mix (ABI) and the primer probe sets from Invitrogen (Rockville, Maryland, USA). Primers were designed using Primer Express software (ABI). Threshold cycle numbers were determined for TIMP-1 and TIMP-2 and corrected using the equivalent data for β-actin.

**Determination of hydroxyproline and collagenase**

Hepatic hydroxyproline content was measured as described previously using the chloramine T/p-dimethylaminobenzaldehyde procedure. Enzyme activity was determined for gelatinases (MMP-2), but not gelatinases (MMP)-1, MMP-8, and matrix degrading metalloproteinases (MMP)-13. The liver was homogenised in 0.3 M Tris HCl buffer (pH 7.4) and centrifuged at 200 rpm for five minutes. Enzyme activity was determined for collagenase, the liver was homogenised in 2.5 N acetic acid containing 10 M urea, 100 μg/ml PMSF, and 10 μg/ml pepstatin, and incubated for 20 minutes at room temperature in polypropylene tubes. After neutralisation with 2.7 N NaOH in 1 M HEPES, the homogenate was centrifuged at 20 190 g for 20 minutes. TGF-β1 content was determined in the supernatant by ELISA (Promega, Madison, Wisconsin, USA).

**Statistical analysis**

Results are expressed as mean (SEM). Physiological, histopathological, and biochemical findings represent averages of seven rats (CCl4 or CCl4 + TAK-044 treatment) and three rats (control) for each time point. Results of molecular assays represent averages of samples from at least three rats in each group, each analysed in duplicate or triplicate. Statistical significance was derived by the non-parametric Mann-Whitney two tailed variance test using the SPSS program to determine significance between multiple groups. A p value of <0.05 was considered statistically significant.

**RESULTS**

**Endothelin and its receptors**

Hepatic concentrations of ET-1 increased by threefold, fourfold, and sevenfold, respectively, after 4, 8, and 12 weeks of CCl4 treatment (fig 3A); similar increases were also observed in the preproET-1 mRNA transcript (figs 1B, 2A). ET-1 concentration was 40% and 20% less in TAK-044 treated rats than in saline treated rats at eight and 12 weeks, respectively. ETα receptor density increased by 40%, 60%, and 80% after 4, 8, and 12 weeks of CCl4 treatment, respectively (fig 3A). ETβ receptor density increased by 1.5-fold at four weeks, and by 3.5-fold at eight and 12 weeks of CCl4 treatment (fig 3C). TAK-044 treatment between four and eight weeks or eight...
and 12 weeks did not alter ETA or ETB receptor density. ETA receptor mRNA increased by 50% at four weeks, and by 70–90% at eight and 12 weeks of CCl4 treatment (figs 2B, 3B); ETB mRNA expression increased by 25%, 85%, and 110% at 4, 8, and 12 weeks of CCl4 treatment, respectively (figs 2B, 3D). ETA as well as ETB mRNA expression was approximately 20% less in TAK-044 treated rats than in saline treated rats both at eight and 12 weeks (figs 2B, 3B, 3D).

### Table 1 General characteristics of the study animals

<table>
<thead>
<tr>
<th></th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CCl4</td>
<td>Control</td>
<td>CCl4</td>
<td>Control</td>
<td>CCl4</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>360 (4)</td>
<td>270 (22)*</td>
<td>390 (12)</td>
<td>313 (16)*</td>
<td>332 (19)*</td>
<td>416 (16)</td>
</tr>
<tr>
<td>Spleen wt (g)</td>
<td>0.83 (0.03)</td>
<td>0.77 (0.1)</td>
<td>0.86 (0.05)</td>
<td>1.5 (0.21)*</td>
<td>1.17 (0.03)*</td>
<td>0.83 (0.06)</td>
</tr>
<tr>
<td>Ascertes (g)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11 (3)</td>
<td>–</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>7.13 (0.1)</td>
<td>6.43 (0.1)**</td>
<td>7.1 (0.1)</td>
<td>6.5 (0.12)*</td>
<td>6.7 (0.3)</td>
<td>7.1 (0.1)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.73 (0.03)</td>
<td>4.34 (0.06)*</td>
<td>4.7 (0.09)</td>
<td>3.99 (0.17)*</td>
<td>4.38 (0.11)</td>
<td>4.61 (0.10)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.85 (0.1)</td>
<td>3.5 (0.16)*</td>
<td>2.83 (0.08)</td>
<td>3.62 (0.11)*</td>
<td>3.02 (0.14)</td>
<td>2.89 (0.13)</td>
</tr>
<tr>
<td>LDH (U/dl)</td>
<td>284 (17)</td>
<td>424 (24)*</td>
<td>268 (19)</td>
<td>397 (17)*</td>
<td>296 (13)**</td>
<td>285 (13)</td>
</tr>
<tr>
<td>ALT (U/dl)</td>
<td>19 (1)</td>
<td>54 (3)***</td>
<td>18 (1)</td>
<td>306 (22)***</td>
<td>172 (12)***</td>
<td>23 (2)</td>
</tr>
<tr>
<td>AST (U/dl)</td>
<td>47 (1)</td>
<td>113 (10)***</td>
<td>71 (4)</td>
<td>251 (15)***</td>
<td>184 (19)***</td>
<td>44 (6)</td>
</tr>
<tr>
<td>ALP (U/dl)</td>
<td>54 (2)</td>
<td>60 (3)</td>
<td>53 (2)</td>
<td>181 (8)***</td>
<td>146 (10)***</td>
<td>45 (6)</td>
</tr>
<tr>
<td>γ-GT (U/dl)</td>
<td>1.7 (0.3)</td>
<td>14.0 (1.3)**</td>
<td>1.3 (0.1)</td>
<td>17.2 (1.2)***</td>
<td>7.1 (0.6)**</td>
<td>2.1 (0.3)</td>
</tr>
</tbody>
</table>

Rats were treated with vehicle or carbon tetrachloride (CCl4) for 4, 8, and 12 weeks. In the last two groups, 50% of rats each were treated with TAK-044 or saline between four and eight weeks or eight and 12 weeks. Various determinations were made, as described in the methods section.

Results are means (SEM) of three control, seven CCl4 treated, and seven TAK-044+CCl4 treated rats.

For biochemical parameters, each assay was performed in duplicate for each rat. LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γ-GT, γ-glutamyl transferase.

* p < 0.05, ** p < 0.01, *** p < 0.001 versus control; †p < 0.05, ‡p < 0.01 versus CCl4.
General characteristics (table 1)
The body weight of CCl₄ treated rats was 25%, 20%, and 30% lower than that of paired controls at 4, 8, and 12 weeks, respectively. TAK-044 treatment of rats receiving CCl₄ improved body weight by 5% and 23%, respectively, at eight and 12 weeks. The weight of the spleen increased significantly after eight and 12 weeks of CCl₄ treatment; TAK-044 prevented the increase in spleen weight at eight weeks to a small extent but not at 12 weeks. All of the CCl₄ treated rats developed ascites at eight and 12 weeks; the volume of ascites was nearly eight times greater at 12 weeks than at eight weeks of CCl₄ treatment. TAK-044 treatment caused 50% and 75% reduction in ascites at eight and 12 weeks, respectively. The synthetic capacity of the liver was reduced in CCl₄ treated rats, as indicated by decreased serum albumin, which was normalised by TAK-044 treatment. Liver injury, as estimated by serum LDH, AST, ALT, and γ-GT, also improved significantly in TAK-044 treated rats. No mortality occurred in TAK-044+CCl₄ treated rats, and mortality was less than 5% in saline+CCl₄ treated rats at eight and 12 weeks of treatment.

Histological assessment
Figure 4A shows normal hepatic architecture in control rats. Liver fibrosis was evident, with the beginning of septum formation after four weeks of CCl₄ treatment (fig 4B). In control livers, α-SMA positive cells were present in portal veins and hepatic arteries (fig 5A) but staining of cells in

Figure 5  Expression of α smooth muscle actin (α-SMA) during the course of cirrhosis development with or without concurrent TAK-044 treatment. Rats were treated with carbon tetrachloride (CCl₄) for 4, 8, and 12 weeks. During CCl₄ treatment between four and eight weeks or eight and 12 weeks, 50% of rats each received TAK-044 or saline. Liver sections were immunostained for α-SMA. (A) Control livers treated with phenobarbital only (eight weeks). α-SMA positive cells are restricted to the portal vein and hepatic artery walls only. (B) Four weeks of CCl₄ treatment. In addition to portal and arterial walls, α-SMA positive cells appear within thin fibrous septae and liver parenchyma. (C) Eight weeks of CCl₄ treatment with saline treatment between four and eight weeks. Abundant α-SMA staining in the fibrous areas indicate the presence of activated hepatic stellate cells/myofibroblasts. (D) Eight weeks of CCl₄ treatment with concurrent TAK-044 treatment between four and eight weeks. The number of α-SMA positive cells in the fibrous areas is reduced significantly. (E) Twelve weeks of CCl₄ treatment with saline treatment between eight and 12 weeks. Cells in wide fibrous septum facing liver parenchyma as well as intralobular cells express α-SMA. (F) Twelve weeks of CCl₄ treatment with concurrent TAK-044 treatment between eight and 12 weeks. Number of α-SMA positive cells is significantly decreased. Original magnification x200.

Figure 6  Histopathology score in TAK-044 treated cirrhotic rats. Histopathological assessment of cirrhotic livers after four weeks of carbon tetrachloride (CCl₄) treatment, eight weeks of CCl₄ treatment with four weeks of concurrent saline or TAK-044 treatment, and 12 weeks of CCl₄ treatment with four weeks of concurrent saline or TAK-044 treatment. Scores were rated between “5” (cirrhotic) and “0” (normal morphology). Results are means (SEM) of three CCl₄ treated rats at four weeks, and seven rats each for CCl₄+saline or CCl₄+TAK-044 treatment at eight and 12 weeks. *p<0.02 versus control.
Reversal of cirrhosis by TAK-044

Effect of carbon tetrachloride (CCl₄) and TAK-044 treatment on collagen α type I mRNA. Rats were treated with CCl₄ for 4, 8, and 12 weeks. During CCl₄ treatment between four and eight weeks or eight and 12 weeks, 50% of rats each received TAK-044 or saline. (A) Representative gel showing mRNA expression of collagen α type I and β-actin using the same amount of cDNA in reverse transcriptase-polymerase chain reaction. (B) Bar graph showing mRNA expression of collagen α type I normalised with respect to that of β-actin, representing averages from duplicate determinations from three rats in each group.

Collagen mRNA, hydroxyproline, collagenase, and TIMP mRNA

mRNA expression of collagen α type I increased respectively by 4, 9, and 13-fold at 4, 8, and 12 weeks of CCl₄ treatment (fig 7). mRNA expression of collagen I in rats treated concurrently with TAK-044 and CCl₄ was 50% less than in saline+CCl₄ treated rats at both eight and 12 weeks (fig 7).

CCl₄ treatment increased hepatic hydroxyproline by two-fold at four weeks and by 10-fold at eight and 12 weeks (fig 8). A significant (35%) reduction in hydroxyproline occurred in rats receiving TAK-044 relative to those receiving saline between four and eight weeks as well as eight and 12 weeks of CCl₄ treatment.

Hepatic collagenase activity increased by 64% at four weeks and by twofold at eight and 12 weeks of CCl₄ treatment (fig 9). Concurrent TAK-044 and CCl₄ treatment caused 30% and 20% decreases in collagenase activity at eight and 12 weeks, respectively (fig 9).

Matrix metalloproteinases (MMPs) are important in liver fibrogenesis and its resolution but we were unable to obtain any conclusive evidence for changes in expression of MMP-1 and MMP-13 in CCl₄ and TAK-044+CCl₄ treated rat livers (not shown). TIMP-1 and TIMP-2 modulate fibrosis by inhibiting MMPs. QRT-PCR demonstrated 33%, 300%, and 150% increases in TIMP-1 mRNA, and 65%, 260%, and 300% increases in TIMP-2 mRNA at eight, 12, and 24 weeks of CCl₄ treatment, respectively.
Mean arterial and portal venous pressure

Mean arterial pressure (MAP) was not significantly different between control and CCl4 treated rats at four weeks (fig 12A). At both eight and 12 weeks of CCl4 treatment, MAP decreased by nearly 30% compared with paired controls. Between four and eight weeks of concurrent TAK-044 treatment, MAP tended to rise but the change did not reach statistical significance. On the other hand, significant improvement (almost to normal levels) in MAP occurred on treatment with TAK-044 between eight and 12 weeks (fig 12A).

Portal venous pressure increased significantly in rats treated with CCl4 for eight and 12 weeks (100–150% increase), indicative of portal hypertension (fig 12B). In both groups, TAK-044 reduced portal hypertension remarkably, with portal pressure being only 30–50% higher than in control rats.

**DISCUSSION**

The profound haemodynamic and metabolic effects of ET-1 in the normal liver,14 15 progressive increase in the ET-1 system from initiation of CCl4 induced liver injury to development of cirrhosis,16 and upregulation of the ET-1 system in experimental biliary cirrhosis17 and human cirrhosis of various aetiologies18 19 indicate that ET-1 is a major factor in the pathogenesis and complications of the chronic cirrhosis.20

**TGF-β1**

Hepatic TGF-β1 content increased respectively by 0.8, 8, and 11-fold at 4, 8, and 12 weeks of CCl4 treatment (fig 11A). TAK-044 caused nearly 60% reduction in TGF-β1 content in CCl4 treated rats (which was similarly increased at 4, 8, and 12 weeks) also decreased on treatment with TAK-044 (fig 11B, 11C).

**Figure 10** Real time polymerase chain reaction (PCR) for tissue inhibitor of metalloproteinase (TIMP)-1 ([A] and TIMP-2 ([B]) mRNA expression. TIMP-1, TIMP-2, and β-actin were measured using quantitative real time PCR, as described in the methods section. TIMP-1 and TIMP-2 values were corrected using β-actin, and normalised to four week controls to facilitate comparison. Values are means (SEM) for duplicate or triplicate determinations in three separate experiments. *p<0.05, **p<0.01 versus control; †p<0.05, ††p<0.01 versus CCl4.

310% increases in TIMP-2 mRNA after 4, 8, and 12 weeks of CCl4 treatment, respectively (fig 10). TAK-044 reduced mRNA expression of TIMP-1 by 36% and 63%, and of TIMP-2 by 34% and 22% at eight and 12 weeks, respectively.

**Figure 11** Effect of carbon tetrachloride (CCl4) and TAK-044 treatment on transforming growth factor β1 (TGF-β1). Rats were treated with CCl4 for 4, 8, and 12 weeks. During CCl4 treatment between four and eight weeks or eight and 12 weeks, 50% of rats each received TAK-044 or saline. Hepatic TGF-β1 content and its mRNA expression were determined. (A) Hepatic TGF-β1 content. Values are means (SEM) of three control and three CCl4 treated rat livers (four weeks) and three control, seven CCl4 treated rats (eight weeks) or eight and 12 weeks, each sample assayed in triplicate. (B) Representative gel showing mRNA expression of TGF-β1 and β-actin using the same amount of cDNA in reverse transcriptase-polymerase chain reaction. (C) mRNA expression of TGF-β1 normalised with respect to that of β-actin. Values shown are means (SEM) of three control and three CCl4 treated rat livers (four weeks) and three control, three CCl4 treated rat livers (eight weeks) or eight and 12 weeks, each sample assayed in duplicate. *p<0.0, **p<0.01, ***p<0.001 versus control; †††p<0.001 versus CCl4.
Rats were treated with CCl4 for 4, 8, and 12 weeks. During CCl4 treatment, mean arterial pressure (MAP) and portal pressure of cirrhotic rats were determined. Values are means (SEM) of three control and TAK-044 treated rats each received TAK-044 or saline. (A) MAP and (B) portal venous pressure were measured. Values are means (SEM) of three control and three CCl4 treated rat livers (four weeks) and three control, seven CCl4-treated rats, and seven CCl4+TAK-044 treated rat livers at eight and 12 weeks. *p<0.05, **p<0.01 versus control; †p<0.05 versus CCl4.

Figure 12: Effect of carbon tetrachloride (CCl4) and TAK-044 treatment on mean arterial pressure (MAP) and portal pressure of cirrhotic rats. Rats were treated with CCl4 for 4, 8, and 12 weeks. During CCl4 treatment between four and eight weeks or eight and 12 weeks, 50% of rats each received TAK-044 or saline. (A) MAP and (B) portal venous pressure were measured. Values are means (SEM) of three control and three CCl4 treated rat livers (four weeks) and three control, seven CCl4-treated rats, and seven CCl4+TAK-044 treated rat livers at eight and 12 weeks. *p<0.05, **p<0.01 versus control; †p<0.05 versus CCl4.

Increased deposition of collagen in the fibrotic liver is a result of its enhanced synthesis and reduced degradation. Paradoxically, hepatic collagenase activity measured in vitro is significantly increased in CCl4 treated rats (present study, and also Okazaki and Maruyama and Montfort and Perez-Tamayo) and decreased by TAK-044 treatment. Either collagen synthesis far exceeds its degradation or collagenase activity measured in vitro is strongly suppressed in vivo by TIMPs which are increased in the fibrotic liver. The rapid decrease in TIMP expression seen during spontaneous recovery from CCl4 induced advanced fibrosis likely increases collagenase activity in vivo, reversing ECM deposition. This is consistent with our finding of decreased TIMP-1 and TIMP-2 expression in rats receiving TAK-044.

The present 58% and 60% decreases in portal hypertension in TAK-044 treated rats at eight and 12 weeks, respectively, are combinations of the resolution of fibrosis and inhibition of the contractile component. Acute infusion of TAK-044 in cirrhotic rats affects only the contractile component, giving a more modest (20%) decrease in portal hypertension. Both ET-1 receptor subtypes appear to be critical in the haemodynamic component of portal hypertension, with sinusoidal and presinusoidal constriction caused by ETA and ETB receptors, respectively. Mixed ETA+ETB antagonists reduce portal hypertension in cirrhotic rats but do not affect portal pressure in normal rats. Thus the haemodynamic component of portal hypertension in cirrhosis includes major participation of the upregulated ETa and ETb receptors, and increased endogenous ET-1.

Decreased MAP in cirrhosis is attributed to increased production of several vasodilatory mediators, including NO and PAF. In the present study, MAP was found to increase in rats treated with TAK-044. Therefore, inability of the acute treatment with ET-1 receptor antagonist to improve systemic hypotension suggests requirement of long term antagonism of ET-1 receptors in the liver, and possibly in the systemic vasculature. As ETa antagonist is unable to alter arterial pressure in cirrhotic rats, our results also suggest that ETb plays a primary role in systemic hypotension in cirrhosis. Indeed, ETb has been shown to stimulate the synthesis of NO and PAF, respectively, in endothelial cells and Kupffer cells; the latter effect is augmented in cirrhosis. ETa on the other hand, seems to limit systemic hypotension as intra-arterial infusion of the ETa antagonist BQ-123 caused a greater increase in forearm blood flow in cirrhotic patients compared with normal volunteers.

Cirrhosis causes upregulation of the hepatic ET-1 system in both rats and humans. Both ETA and ETB receptors appear to play important roles in the development and complications of cirrhosis, and review of the literature suggests that simultaneous antagonism of both receptor subtypes is necessary to achieve beneficial effects. Rockey and Chung found that essentially concurrent administration of the mixed antagonist bosentan and CCl4 (bosentan from day 0, CCl4 from day 2) significantly decreased hepaticactivation, proliferation, and fibrogenesis in stellate cells, and mRNA and protein expression of the powerful fibrogenic cytokine TGF-β1 was reduced in TAK-044 treated rats. Type I collagen is a major component of the ECM deposited in liver fibrosis, and both TAK-044 and CCl4 treatment between four and eight weeks, and reduced its expression remarkably at 12 weeks. TAK-044 also caused marked reduction in hepatic hydroxyproline content. Together, these results suggest that ET-1 plays an important role in fibrogenesis by modulating TGF-β1 and collagen synthesis. However, such effects of ET-1 may be indirect as ET-1 stimulates synthesis of collagen and TGF-β1 in normal but not in activated stellate cells.

Increased deposition of collagen in the fibrotic liver is a result of its enhanced synthesis and reduced degradation. Paradoxically, hepatic collagenase activity measured in vitro is significantly increased in CCl4 treated rats (present study, and also Okazaki and Maruyama and Montfort and Perez-Tamayo) and decreased by TAK-044 treatment. Either collagen synthesis far exceeds its degradation or collagenase activity measured in vitro is strongly suppressed in vivo by TIMPs which are increased in the fibrotic liver. The rapid decrease in TIMP expression seen during spontaneous recovery from CCl4 induced advanced fibrosis likely increases collagenase activity in vivo, reversing ECM deposition. This is consistent with our finding of decreased TIMP-1 and TIMP-2 expression in rats receiving TAK-044.

The present 58% and 60% decreases in portal hypertension in TAK-044 treated rats at eight and 12 weeks, respectively, are combinations of the resolution of fibrosis and inhibition of the contractile component. Acute infusion of TAK-044 in cirrhotic rats affects only the contractile component, giving a more modest (20%) decrease in portal hypertension. Both ET-1 receptor subtypes appear to be critical in the haemodynamic component of portal hypertension, with sinusoidal and presinusoidal constriction caused by ETA and ETB receptors, respectively. Mixed ETA+ETB antagonists reduce portal hypertension in cirrhotic rats but do not affect portal pressure in normal rats. Thus the haemodynamic component of portal hypertension in cirrhosis includes major participation of the upregulated ETa and ETb receptors, and increased endogenous ET-1.

Decreased MAP in cirrhosis is attributed to increased production of several vasodilatory mediators, including NO and PAF. In the present study, MAP was found to increase in rats treated with TAK-044. Therefore, inability of the acute treatment with ET-1 receptor antagonist to improve systemic hypotension suggests requirement of long term antagonism of ET-1 receptors in the liver, and possibly in the systemic vasculature. As ETa antagonist is unable to alter arterial pressure in cirrhotic rats, our results also suggest that ETb plays a primary role in systemic hypotension in cirrhosis. Indeed, ETb has been shown to stimulate the synthesis of NO and PAF, respectively, in endothelial cells and Kupffer cells; the latter effect is augmented in cirrhosis. ETa on the other hand, seems to limit systemic hypotension as intra-arterial infusion of the ETa antagonist BQ-123 caused a greater increase in forearm blood flow in cirrhotic patients compared with normal volunteers.

Cirrhosis causes upregulation of the hepatic ET-1 system in both rats and humans. Both ETA and ETB receptors appear to play important roles in the development and complications of cirrhosis, and review of the literature suggests that simultaneous antagonism of both receptor subtypes is necessary to achieve beneficial effects. Rockey and Chung found that essentially concurrent administration of the mixed antagonist bosentan and CCl4 (bosentan from day 0, CCl4 from day 2) significantly decreased hepatic
collagen mRNA (stellate cells) and protein (liver section) at day 22. This short term (~3 week) study also showed that bosentan inhibited activation (measured as α-SMA expression) of stellate cells. In contrast, a long term experiment53 interest.

C R Gandhi, C Thirunavukkarasu, Y Yang, J Fung, Department of Surgery, Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Department of Pathology, Veterans Administration Medical Center, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

REFERENCES


35 Tran Thi TA, Kawada N, Demaille K. Regulation of endothelin-1 action on the perfused rat liver. FEBS Lett 1993;318:553–7.


EDITOR’S QUIZ: GI SNAPSHOT

Answer

From question on page 1000

During menses, colonoscopy showed a large multilobulate polyp (3.5 cm in diameter) of the sigmoid colon with a hyperaemic and friable mucosa (fig 1). Histological examination showed a normal mucosa with the presence of some branched crypts and marked inflammation in the lamina propria.

During the intermenstrual period (on the eighth day of the cycle), polyp size had decreased; its surface appeared only slightly lobulate without friability and with hyperaemia, which was reduced at two red spots (fig 2). Histopathological evaluation showed the presence of endometriotic foci in the context of a mucosa containing branched crypts.

The patient was submitted to a gynaecological examination which was found to be normal. Pelvic and vaginal ultrasounds did not show evidence of endometriotic lesions. Magnetic resonance imaging detected a 1.8 cm area with a T1 hyperintense and T2 hypointense signal in the sigmoid wall.

Medical treatment with leuprolide acetate depot, a gonadotropin releasing hormone agonist (GnRH-a) (3.75 mg intramuscularly every four weeks for three months) was started. Abdominal pain and bloody diarrhoea promptly disappeared. At the four month follow up she was still free of symptoms and anaemia had reversed (haemoglobin 12.3 g/dl). At the one year follow up, colonoscopy showed a flat lesion without hyperaemia or friability at the site of the endometriotic polyp. Histological examination of multiple biopsies was normal and a magnetic resonance imaging showed complete disappearance of the previously detected lesion. After 18 months of follow up, the patient remains completely asymptomatic.

Endometriosis is defined as the presence of endometrial tissue in sites other than the uterus. The rectosigmoid is the most frequently involved site. Heterotopic endometrium generally adheres to serosal surfaces and may invade the bowel wall. However, appearance as a polyp is rarely reported. Infiltration of the mucosa is uncommon and therefore the endoscopic biopsy may not be diagnostic. The variable appearance on endoscopy is related to the cyclic endometrial tissue changes.

Surgical resection is indicated for massive rectal bleeding or obstruction but the role of surgery is less clear when the clinical course is not life threatening. Medical treatment with a GnRH-a is commonly used in pelvic endometriosis but studies on its efficacy in intestinal endometriosis are still lacking.

To our knowledge this is the first case reported in the literature showing that an endometriotic colonic polyp may be successfully treated by medical therapy with a GnRH-a. This case further illustrates that endometriosis may appear as a colonic polyp and that polypectomy is not curative.
Endothelin receptor antagonist TAK-044 arrests and reverses the development of carbon tetrachloride induced cirrhosis in rats

C Thirunavukkarasu, Y Yang, V M Subbotin, S A K Harvey, J Fung and C R Gandhi

Gut 2004 53: 1010-1019
doi: 10.1136/gut.2003.026534

Updated information and services can be found at:
http://gut.bmj.com/content/53/7/1010

These include:

References
This article cites 56 articles, 7 of which you can access for free at:
http://gut.bmj.com/content/53/7/1010#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/