BINGE DRINKING AND NITRIC OXIDE METABOLITES IN CHRONIC LIVER DISEASE

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Abstract — Aims: The effect of binge drinking in the production of nitric oxide metabolites has not been studied in patients with chronic viral liver disease. Methods: We therefore studied serum levels of nitrates and nitrates (NOx) in 13 patients with chronic viral hepatitis and nine patients with compensated viral cirrhosis, after administration of 80 g alcohol. 15 patients with compensated alcoholic cirrhosis and seven healthy individuals were used as controls. Serum NOx levels were measured by a modification of the Griess reaction before and at 2, 12 and 24 h after alcohol consumption. Results: An increase of serum NOx levels, that was statistically significant at 12 h, was found in healthy controls (P < 0.05). A similar pattern of NOx levels was observed in patients with chronic hepatitis. By contrast, in patients with cirrhosis, either viral or alcoholic, no significant increase was found after alcohol administration. However, basal levels in cirrhotics were significantly elevated (82.2 ± 13.8 vs. 43.1 ± 7.2 μM/l, P < 0.01) compared to healthy controls. Conclusions: Binge drinking causes a significant increase of serum NOx evident after 12 h with a return after 24 h at pre-drinking levels in healthy controls and patients with chronic viral hepatitis. In cirrhosis, such an increase is not observed serum levels being constantly elevated throughout the study period.

BACKGROUND

Binge alcohol consumption has been arbitrarily defined as five drinks in a row for men and four drinks in a row for women, consumed over a short period of time. It seems that this type of alcohol consumption is not uncommon, at least in the western world. In 1993, 39% of college students binged at least three times every month, while in 1995 an increase to 52% of students was noted (Wechsler et al., 1998). Binge drinking has been experimentally shown to exacerbate acetaminophen induced centrilobular injury (De Leve et al., 1997). Moreover, in rats alcohol binging caused activation of Kupffer cells with resultant increase of phagocytic activity, prolonged release of TNFα and persisting superoxides for 24 h, although blood alcohol levels had returned to normal 12 h after the last dose of alcohol (Abril et al., 1999).

Nitric oxide (NO) plays an important role in the regulation of portal vein pressure and influences splachnic vasodilatation in cirrhotic patients (Vallance et al., 1991). Moreover, NO overproduction precedes hyperdynamic splachnic circulation in rats with experimental portal hypertension (Wiest et al., 1999). It is known that in rats’ Kupffer cells excessive production of NO is diminished by chronic alcohol administration (Kimura et al., 1996). The effect of binge drinking on the production of NO metabolites in patients with chronic liver disease has not been studied before.

We therefore studied the effect of acute alcohol administration on NOx serum levels in patients with viral and alcoholic cirrhosis and compared them with healthy controls and patients with chronic viral hepatitis.

PATIENTS AND METHODS

Patients

Thirty-seven patients [26 male, age 30–72 (median 53) years, 20 smokers] and seven healthy controls [four male, age 24–53 (median 35) years, three smokers] participated in the study.

In all patients diagnosis had been confirmed by liver biopsy. Nine patients were diagnosed with viral cirrhosis (five male, seven HCV, two HBV, six Child’s A, three Child’s B, four smokers). Fifteen patients had alcoholic cirrhosis, (12 male, 10 Child’s A, five Child’s B, 10 smokers). Thirteen patients had chronic viral hepatitis (nine male, nine HCV, four HBV, six smokers).

No patient was receiving any diuretic or corticoid treatment. In the group of chronic viral hepatitis patients, eight of the 13 patients (six HCV, two HBV) had received interferon treatment that was concluded 6 months or more before the study began. Healthy controls were recruited from the personnel of the Gastroenterology Department. Patients and controls remained on ward during 24 h following binge drinking and consumed a standard hospital diet of 1800–2200 kcal. Permission from the Ethics Committee of the Hospital was obtained and all patients and controls gave an informed consent for participation in the study.

Study design

Ethanol at a total of 80 g as a 40% solution was ingested in the morning after an over-night fast. Ethanol administration was completed within 30 min. Whole blood was collected before drinking and at 2, 12 and 24 h after completion of alcohol consumption. Serum was separated by centrifugation and kept at −70°C until NOx measurements. Patients were kept in a normal hospital diet after ethanol administration.

Nitric oxide measurements

Total serum nitrite (NO3) and nitrate (NO2) concentration was measured by a modification of the Griess reaction as...
previously described (Matrella et al., 2001). Briefly, 100 µl serum sample was incubated for 30 min at 37°C in the presence of 0.2 U/ml Aspergillus nitrate reductase, 5 µmol/l FAD and 0.1 mmol/l NADPH in 50 mmol/l HEPES buffer (total volume 500 ml) for the conversion of nitrate to nitrite. Following the incubation, 5 µl lactate dehydrogenase (1500 U/ml) and 50 µl 100 mmol/l pyruvic acid were added to each tube to oxidize any unreacted NADPH (reduced pyridine nucleotides strongly inhibit the Griess reaction). Samples were then incubated for 10 min at 37°C. Finally, 1 ml pre-mixed Griess reagent was added to each tube. After a 10-min incubation time at room temperature, the absorbance of each sample was determined at 543 nm on a Hitachi U-2000 spectrophotometer (Moriyama et al., 1997).

**Statistical analysis**

Differences in NOx levels within each group at 2, 12 and 24 h were evaluated by the Student’s test for paired data, using levels before alcohol intake as reference values. Comparisons among groups were performed by the Student’s t-test for unpaired data and P < 0.05 was considered as statistically significant. All statistical comparisons were performed using the SPSS for Windows statistical package (SPSS, Chicago, IL).

**RESULTS**

In Table 1, all NOx levels before, 2, 6, 12, and 24 h after ingestion of alcohol are presented for all patient and control groups.

**Basal NOx levels**

Mean values for cirrhotic patients were significantly higher from both healthy controls and patients with chronic viral hepatitis (P < 0.01 and P < 0.05 respectively, Fig. 1). There was no significant difference between patients with alcoholic or viral cirrhosis (Fig. 2).

**Serum NOx response after binge drinking**

In the control group there was a gradual albeit not significant increase of serum NOx levels that was evident after 2 h. This increase reached a maximum at 12 h (P < 0.05 compared to basal levels) and then gradually returned to pre-drinking levels after 24 h (P < 0.05 compared to 12 h levels) as shown in Fig. 1.

A similar but steeper increase at 12 h was observed for patients with chronic hepatitis (0–12 h P < 0.01, 12–24 h P < 0.01). By contrast, in cirrhotic patients irrespective of aetiology this increase at 12 h was not observed (Fig. 2).

In general, serum levels of NOx in cirrhotics at any given time were similar to those obtained in healthy controls after binge drinking.

**DISCUSSION**

Nitric oxide has been proposed as a mediator of hyperdynamic circulation in cirrhosis (Wiest et al., 1999). Increased serum levels have been reported in cirrhotic patients with and without ascites (Genesca et al., 1999) as well as with hepatocellular carcinoma (Moriyama et al., 1997; Notas et al., 2001).

![Fig. 1. Serum NO levels after binge drinking in patients with viral and alcoholic cirrhosis and healthy controls.](image1)

![Fig. 2. Serum NO levels after binge drinking in patients with viral cirrhosis compared to patients with cirrhosis (irrespective of aetiology) and healthy controls.](image2)

**Table 1. Mean NOx levels for healthy controls and patients with chronic hepatitis, viral and alcoholic cirrhosis before, 2, 12 and 24 h after ingestion of 80 g ethanol**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Controls</th>
<th>Chronic hepatitis</th>
<th>Viral cirrhosis</th>
<th>Alcoholic cirrhosis</th>
<th>Total cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43.1 ± 7.2</td>
<td>67.9 ± 5.6**</td>
<td>81.7 ± 11.9**</td>
<td>82.5 ± 21.2**</td>
<td>82.2 ± 13.8**</td>
</tr>
<tr>
<td>2</td>
<td>59.1 ± 12.9</td>
<td>66.8 ± 7.5</td>
<td>73.6 ± 9.6</td>
<td>83.5 ± 15.3</td>
<td>73.9 ± 10.1</td>
</tr>
<tr>
<td>12</td>
<td>95.2 ± 24.5*</td>
<td>131.2 ± 22.4**</td>
<td>80.6 ± 8.2</td>
<td>96.0 ± 11.8</td>
<td>90.2 ± 8.1</td>
</tr>
<tr>
<td>24</td>
<td>48.2 ± 8.8</td>
<td>76.2 ± 7.5*</td>
<td>57.6 ± 4.5</td>
<td>79.2 ± 11.4*</td>
<td>71.1 ± 7.5*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.05, **P < 0.01.
In-vitro evidence also suggests that overproduction of NO during inflammation might affect vital hepatocyte functions like detoxification of xenobiotics (Veihelmann et al., 1997).

Ethanol has been experimentally shown to influence nitric oxide production in the rat (Kato et al., 1996). It has been proposed that NO has a possible protective effect by attenuating the detrimental effects of ethanol on liver microcirculation (Oshita et al., 1994; McCuskey et al., 1995; Nanji et al., 1995). However, chronic ethanol administration seems to lead to NO-induced liver injury (Chamuli trat et al., 1996). Moreover, chronic ethanol feeding in the rat has been found to reduce both nitric oxide production and anti-tumour activity of Kupffer cells (Kato et al., 1996).

In human alcoholic liver disease there is evidence that the high haemodynamic state of patients with advanced cirrhosis is possibly due to either increased endothelial synthesis of NO (Calver et al., 1994; Campillo et al., 1995) or smooth muscle derived NO (Ryan et al., 1996). Peripheral blood monocytes from patients with alcoholic hepatitis secrete more nitric oxide than monocytes from patients without alcoholic hepatitis, (Hunt et al., 1992) a finding not confirmed in another study (Criado-Jimenez et al., 1995). Peripheral blood monocytes from patients with alcohol induced cirrhosis produce higher levels of NO compared to monocytes from healthy controls (Criado-Jimenez et al., 1995; Sanchez-Rodriguez et al., 1998).

Binge drinking has been experimentally shown to influence metabolic pathways in liver cells (Arteel et al., 1996; Rivera et al., 1998). Gluconeogenesis was found to be delayed in isolated perfused rat liver (Deaciuc et al., 1992) and F-actin content was found to be upregulated in leucocytes of female rats (Zhang et al., 1997). Acute ethanol intoxication has been reported to stimulate superoxide anion production in the perfused rat liver (Bautista et al., 1992) and ethanol induced oxidative stress has been shown to cause massive mitochondrial DNA degradation (Mansouri et al., 1999). Acute alcohol intoxication may increase free radical release due to activation of Kupffer endothelial cells (Bautista et al., 1994, 1996, 1999; Yokoyama et al., 1999) and hepatocytes (Bailey et al., 1998). Acute ethanol intoxication also influences expression of interleukin-6 surface receptors in both Kupffer cells and hepatocytes (Deaciuc et al., 1994). Chemokine production by Kupffer cells is also modulated by ethanol bing (Bukara et al., 2000; Bautista, 2001).

The effect of binge drinking in serum NOx levels has not been studied before. Our findings indicate that after acute ethanol administration healthy controls and patients with histologically proven chronic viral hepatitis demonstrate an initial significant increase of serum NOx levels that is evident at 12 h after ethanol ingestion. In view of the experimental evidence mentioned before (Oshita et al., 1994; McCuskey et al., 1995), this effect might protect liver microcirculation from a deleterious ethanol effect.

The initial increase of serum NOx levels was abolished in our cirrhotic patients both of alcoholic and viral aetiology. This might be due to the high basal levels observed in cirrhosis compared to healthy controls.

It is possible that the source organ of nitric oxide overproduction is already maximally stimulated and cannot respond with a further increase of NOx production. Higher NOx levels after binge drinking in patients with chronic hepatitis compared to cirrhotics are in disagreement to this hypothesis. However, another interpretation of these data would be that the source organ, although damaged, can still produce NOx to the maximum of this capacity.

In chronic viral hepatitis, basal NOx levels were increased compared to healthy controls but did not reach those found in cirrhotics. The role of nitric oxide production in chronic viral hepatitis is still unknown. In contrast to our study, lower levels of serum NOx have been reported in patients with chronic viral hepatitis (Amaro et al., 1997). On the other side, there is evidence of a virus-induced upregulation of inducible nitric oxide synthase expression (Majano et al., 1998). HBV X protein has been reported to transactivate the promoter of the inducible isoform of nitric oxide synthase (Amaro et al., 1999). Interestingly, in some patients with HCV infection, although liver tissue iNOs is increased, this was not accompanied by significantly elevated serum NO levels (Mihm et al., 1997).

The origin of NO overproduction in our healthy controls and viral hepatitis patients after binge drinking is not clear. The 12 h time interval required for this increase indicates a NO synthase induction. Whether this induction takes place in the hepatocyte, the Kupffer cell, the peripheral monocytes or the peripheral vasculature requires further studies. Kupffer cell, however, is a strong candidate. In an experimental model of week-end type alcohol binging, activation of Kupffer cells was demonstrated with an increased production of TNFα and superoxide anions (Abril et al., 1999).

In conclusion, our data showed that binge drinking in patients with chronic viral hepatitis causes a significant increase of serum NOx 12 h after alcohol ingestion. This increase subsides at pre-drinking levels after 24 h. Similar behaviour of serum NOx levels was observed in healthy controls. In contrast, serum NOx levels of cirrhotic patients, irrespective of aetiology, are not influenced by binge drinking and remain persistently elevated.

REFERENCES


