Tissue zinc status and drug elimination in patients with chronic liver disease

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(Received 23 October 1989; accepted 30 January 1990)

SUMMARY
1. The zinc status and drug-metabolizing ability of 15 patients with histologically diagnosed hepatic cirrhosis were studied. Zinc status was assessed using both serum and leucocyte zinc concentrations, and drug-metabolizing ability was assessed by antipyrine kinetics.
2. Patients with cirrhosis were found to have lower serum and leucocyte zinc concentrations when compared with a healthy control group.
3. Leucocyte zinc content and antipyrine clearance were correlated. Those patients with the lowest leucocyte zinc content had the greatest impairment of drug metabolism. Antipyrine elimination and serum zinc concentrations were not correlated.
4. Leucocyte zinc concentrations and antipyrine clearance were not influenced by the severity of liver dysfunction, as assessed by using the Child Turcotte classification.
5. These results suggest that tissue zinc depletion in some patients with hepatic cirrhosis may explain in part the impaired capacity to metabolize drugs.

Key words: antipyrine clearance, drug metabolism, hepatic cirrhosis, leucocyte zinc, zinc status.

INTRODUCTION
Zinc-deprived animals have delayed elimination of some drugs [1], raising the possibility that zinc is required for the normal activity of drug-metabolizing enzyme systems.

Intracellular zinc depletion occurs in patients with hepatic cirrhosis. This is probably caused by a combination of decreased dietary intake, malabsorption and increased loss via the gastrointestinal tract and in the urine [2, 3]. A recent study suggests that zinc deficiency in liver tissue occurs not only in hepatic cirrhosis but also in less advanced alcoholic and non-alcoholic liver disease such as chronic active hepatitis [4].

Three major factors determine the rate of drug elimination by the liver: delivery by liver blood flow, binding of the compound to plasma proteins and, most importantly, the activity of hepatic drug metabolism enzymes [5-8]. The hepatic cytochrome P-450-dependent microsomal mono-oxygenases are responsible for the biotransformation of a wide variety of drugs. The activities of these enzymes have been shown to be impaired in liver tissue from patients with alcoholic cirrhosis [9]. In this study, we investigate the possibility that intracellular zinc deficiency in patients with hepatic cirrhosis may influence hepatic microsomal enzyme activity and hence delay drug elimination.

The assessment of zinc status is difficult as zinc is predominantly an intracellular ion. We therefore measured both serum and leucocyte zinc concentrations as the latter has been shown to represent most closely the main intracellular sites of zinc storage [10]. Antipyrine was chosen as the marker of hepatic oxidative drug-metabolizing ability because of its well recognized properties which include almost complete hepatic metabolism with a low hepatic extraction ratio independent of liver blood flow, negligible renal elimination and insignificant plasma protein binding [11, 12].

METHODS
Fifteen patients (10 male, mean age 52 years) took part in the study which was approved by the hospital Ethics Committee. All patients had histologically diagnosed hepatic cirrhosis; in 11 patients liver disease was secondary to chronic alcohol intake, two patients had chronic active hepatitis, one patient had haemochromatosis and one had primary biliary cirrhosis. All patients were in a clinically stable condition without associated cardiac or renal pathology; none was on concomitant
medication known to interfere with drug metabolism (two
were on diuretics, one was on prednisolone 5 mg daily).
All patients were hospitalized at the time of study and did
not ingest alcohol for at least 7 days before the study. The
severity of liver disease was assessed by using the Child
Turcotte classification, where a combination of clinical
features, e.g. nutritional status, encephalopathy, ascites
and laboratory measurements (plasma albumin and bil-
irubin), are used [5].

Zinc levels were measured in both serum and leuco-
cytes by atomic absorption spectrophotometry. At 08.00
hours, a fasting blood sample (50 ml) was taken with
minimal stasis into trace-element-free vacutainers
containing preservative-free sodium heparin (50 i.u./ml).
Erythrocytes prepared from an aliquot of whole blood
were washed in saline (150 mmol/l NaCl). Centrifugation
at 800 g resulted in sedimentation of the cells. Dextran
sedimentation (dextran/blood ratio, 1:4) yielded mixed
leucocytes. The mixed leucocyte population was
separated into mononuclear and polymorphonuclear
fractions by centrifugation on Ficoll–Paque. The remain-
ing erythrocytes were eliminated by hypotonic lysis. Cells
were counted under ultraviolet light using a fluorescent
dye (ethidium bromide 16 mg/l and Acridine Orange
4 mg/l). The purity and viability of the preparations were
checked. The leucocytes were centrifuged to sediment the
cells. Cell pellets in pre-weighed glass tubes were dried at
100 °C for 16 h and then weighed to the nearest 0.05 mg
within 2 h of removal from the oven. The dried pellets
were extracted with 0.3 ml of mol/l HCl for 20 h before
analysis for trace elements by atomic absorption spectro-
photometry. Care was taken throughout to avoid contami-
nation of the leucocytes in the cirrhotic patients was
compared with that of 15 healthy age- and sex-matched
control subjects.

Antipyrine clearance, an index of oxidative drug
metabolism, was determined from the systemic clearance
of a single intravenous dose of antipyrine (10 mg/kg).
Blood samples were taken from an indwelling cannula at
0, 3, 6, 9, 12 and 24 h, and antipyrine concentrations
were determined by high-pressure liquid chromatog-
raphy. Antipyrine clearance was calculated from the
intravenous dose ($D$) and the area under the plasma con-
centration versus time curve (AUC; calculated by the
trapezoidal rule), according to the equation:

$$\text{Antipyrine clearance} = \frac{D}{\text{AUC}}$$

Statistical analysis was performed using non-parametric
tests. The Wilcoxon rank sum test on unpaired data (the
two-sample test) was used as a test of significance of
difference between mean values. Correlation coefficients
were calculated by least square linear regression analysis.

RESULTS

Both serum and leucocyte zinc levels were found to be
significantly ($P<0.05$) reduced in patients with hepatic
cirrhosis compared with the control group (Table 1). The

mean ($\pm$ SEM) antipyrine clearance of the chronic liver
disease patients was $31.2 \pm 3.6$ ml/min. A significant
positive correlation was noted between leucocyte zinc
levels and antipyrine clearance in the patients with
hepatic cirrhosis (Fig. 1). No such relationship was noted
with antipyrine half-life and no significant correlation
between serum zinc concentrations and antipyrine
clearance was found. Leucocyte zinc concentrations and
antipyrine clearance were not influenced by the severity
of liver dysfunction (Fig. 2).

DISCUSSION

Zinc is an essential trace element in man and its
deficiency is a recognized feature of chronic liver disease.
Zinc is an essential component of over 200 metallo-
enzymes and thus plays a significant role in many
enzymatic processes [13]. Experimental animal data sug-
gest that zinc is essential for the normal activities of some
drug-metabolizing enzyme systems. Studies of
microsomal enzyme activities in zinc-depleted rats
demonstrate a reduction in the rate of metabolism of a
number of drugs including aminopyrine and $p$-nitro-
benzoic acid [1]. A prolongation of the activities in vivo
of certain drugs, e.g. barbiturates, is also seen in these zinc-
deficient rats. Correction of the zinc deficiency state
restored normal rates of drug metabolism, suggesting a
possible role for zinc in drug metabolism [1].

Table 1. Zinc concentrations in 15 patients with hepatic
cirrhosis and in 13 age- and sex-matched control subjects

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<tr>
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<th>Control subjects</th>
<th>Cirrhotic patients</th>
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<tbody>
<tr>
<td>Leucocyte zinc (µg/g)</td>
<td>61.2 ± 1.5</td>
<td>52.1 ± 1.2*</td>
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<tr>
<td>Serum zinc (µmol/l)</td>
<td>15.1 ± 0.7</td>
<td>10.9 ± 0.3*</td>
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Fig. 1. Leucocyte zinc versus antipyrine clearance in patients with hepatic cirrhosis. $n=15$; $r=0.68$; $P<0.01$. 

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The mean values of both parameters were not significantly different between zinc status, represented by serum zinc, and antipyrine elimination half-life. Furthermore, it must be recognized that zinc is found predominantly intracellularly (99%) and therefore a more accurate assessment of zinc status would be obtained from measurement of tissue zinc levels. Leucocytes provide an easily accessible tissue for the determination of zinc status. Leucocyte zinc has been shown to reflect the main intracellular sites of zinc storage, e.g. muscle tissue zinc, both in chronic liver disease patients and in normal control subjects. Leucocyte zinc also fluctuates less than serum zinc levels in response to a wide range of conditions, including stress, acute and chronic infections and alcohol intake.

Our results show no significant relationship between serum zinc levels and antipyrine clearance. The variability of serum zinc levels may also contribute to this finding. The relationship between leucocyte zinc levels and antipyrine clearance was, however, significant. We found that the lower the zinc status, the greater the impairment in antipyrine clearance (Fig. 1). This supports the findings of experimental animal studies which have suggested a facilitatory role for zinc in drug metabolism. Despite the fact that these two variables are correlated, it is possible that both zinc status and antipyrine clearance are independent markers for liver disease.

In this study leucocyte zinc concentration and antipyrine clearance were not influenced by the severity of liver disease, as assessed by using the Child Turcotte classification. This is consistent with a previous study in which antipyrine elimination could not easily distinguish between patients with and without the various complications of cirrhosis [15]. Furthermore, associations are not necessarily causally related. The effect of zinc supplementation on impaired drug metabolism in cirrhosis requires study.

REFERENCES