Serum Copper Concentration and Hepatic Enzyme Induction during Long-Term Therapy with Anticonvulsants

J. Carlos Tutor, M. Pilar Fernandez, and J. Manuel Paz

We evaluated hepatic enzyme induction by measuring urinary D-glucaric acid and serum \( \gamma \)-glutamyltransferase in a group of 40 adult epileptics of both sexes who were receiving long-term treatment with phenobarbital and (or) phenytoin. Total concentrations of copper and ceruloplasmin in their serum and the oxidase activity of ceruloplasmin were significantly greater than in the control group. However, non-ceruloplasmin copper and specific oxidase activity of the ceruloplasmin (activity per gram) were unchanged. A highly significant relationship was found between \( \gamma \)-glutamyltransferase and (a) copper \((r = 0.682, p < 0.001)\), (b) ceruloplasmin \((r = 0.523, p \approx 0.001)\), and (c) the oxidase activity of the ceruloplasmin \((r = 0.598, p < 0.001)\). There is also a significant correlation of hemopexin with ceruloplasmin \((r = 0.531, p \approx 0.001)\) and the oxidase activity of the ceruloplasmin \((r = 0.598, p < 0.001)\). These results suggest that hypercupremia in patients undergoing long-term anticonvulsant therapy is a direct result of hepatic enzyme induction caused by the drugs that induce synthesis of ceruloplasmin.

Additional Keyphrases: ceruloplasmin - hemopexin - hypercupremia - anticonvulsant drugs - \( \gamma \)-glutamyltransferase - D-glucaric acid - reference interval

Although not in total agreement, results of various workers support the hypothesis of an altered metabolism of copper in epileptics, but do not explain its mechanism. Plum and Hansen \((1)\) found a slight but significant increase in copper concentrations in serum but no increase in oxidase activity of ceruloplasmin. Similar results were reported by Canelas et al. \((2)\), who attributed the increase of serum copper to the non-ceruloplasmin fraction. Brunia \((3)\) confirmed the finding of hypercupremia in epileptic patients receiving anticonvulsant therapy and demonstrated that diet had no effect. In a later paper Brunia and Buyze \((4)\) suggested that the increase in serum copper could be an effect of the epilepsy itself. Cantu and Schwab \((5)\), on the other hand, found that patients being treated with phenytoin had a significant increase in oxidase activity of ceruloplasmin but not in serum copper. Merlo and Fernández \((6)\) reported higher values for both serum copper and the oxidase activity of ceruloplasmin, which, rather than being the result of epileptic seizures, were due to a cerebral lesion or to phenytoin acting in an unknown manner. Taylor and Higgins \((7)\) and Vasiladias and Sahawneh \((8)\) speculated that phenytoin, through an unknown mechanism, might increase concentrations of copper and ceruloplasmin in serum but not in correlation with the concentration of the circulating drug. Mora et al. \((9)\) also found increases in ceruloplasmin and its oxidase activity in a group of patients with grand mal who were being treated with phenytoin. Seager \((10)\), working with a group of 16 epileptic children, established that after six months of treatment with phenytoin the ceruloplasmin concentra-
plasmin was determined at 30 °C with o-dianisidine as substrate in an ABA-100 analyzer (19). In a day-to-day precision study the CV was 0.88% for an average activity of 175.50 U/L. Hemopexin was measured by radial immunodiffusion in agar gel with monospecific antiserum, with use of M-Partigen plates and serum standards from the Behring Institute. In a day-to-day precision study the CV was 3.5% for an average concentration of 810.3 mg/L.

Also, in all patients, we measured serum bilirubin, aspartate aminotransferase (EC 2.6.1.1), 5'-nucleotidase (EC 3.1.3.5), lactate dehydrogenase (EC 1.1.1.27), and alkaline phosphatase (EC 3.1.3.1). For the last we also studied thermal inactivation at 56 °C and used electrophoresis on cellulose acetate to identify the isoenzymes in samples showing high activity.

In the statistical study we used parametric tests (Student’s t-test for difference of means and the Pearson coefficient for correlations) when distributions satisfied the Kolmogorov-Smirnov normality test. However, for urinary D-glucaric acid and serum GGT the best normalization was achieved with logarithmic transformation. The significance of the correlation coefficients was determined by previous normalization of r by conversion to z.

Results

Values for serum bilirubin, lactate dehydrogenase, and 5'-nucleotidase in the patients were all within the normal reference interval for this laboratory. There was a slight increase of aspartate aminotransferase in one patient and alkaline phosphatase was higher in 45% of the group, but these increases were produced by the bone isoenzyme.

Average excretion of urinary D-glucaric acid was 640.33 μmol/g of creatinine; in 97.5% of the patients the amount exceeded the upper limit of the normal reference interval, which had been calculated by previous logarithmic transformation to be 57.77 μmol/g of creatinine. The average activity of serum GGT was 126.9 U/L for the men, 143.0 U/L for the women. In 82.5% of the patients the activity exceeded the upper limit of the normal range for this laboratory, 45 U/L for men and 35 U/L for women. There was no significant correlation between serum GGT and urinary D-glucaric acid (r = 0.220, 0.2 > p > 0.1).

The average values for serum copper and some related variables are given in Table 1 for the patients and the control group. Total serum copper, ceruloplasmin, and oxidase activity of ceruloplasmin were significantly higher in the patient group, but there was no statistically significant difference between the two groups in the values for non-ceruloplasmin copper and specific oxidase activity of ceruloplasmin.

For the control group the correlation of serum copper concentration with ceruloplasmin concentration was 0.929, with its oxidase activity, 0.895; for the patients, the corresponding correlations were 0.796 and 0.797; and for both groups combined, 0.921 and 0.917. We also found a significant correlation of ceruloplasmin with oxidase activity in both the patient groups (r = 0.892) and the control group (r = 0.934).

Serum GGT in the patient group correlated highly significantly with serum copper, with ceruloplasmin, and with the oxidase activity of ceruloplasmin (Figure 1). There was no significant correlation between urinary D-glucaric acid and any of these variables.

The average concentration of hemopexin in the patient group was 897.8 (SEM 22.7) mg/L, slightly more than the average for the control group: 826.8 (SEM 21.1) mg/L. The difference of means between the groups was statistically significant (p = 0.025). Among the patients there was a significant correlation of hemopexin with both the concentration (r = 0.531, p = 0.001) and the oxidase activity (r = 0.598, p < 0.001) of ceruloplasmin.

Discussion

The biochemical tests we used revealed no signs of hepatocellular damage or the cholestatic process among the patients we studied. The increase of alkaline phosphatase at the expense of its bone isoenzyme has already been described (20, 21) and is related to alterations in the metabolism of vitamin D produced by long-term treatment with anticonvulsant drugs.

The results found for serum GGT and urinary D-glucaric acid suggest the existence of a serious hepatic enzyme induction produced by the drugs administered. Some authors have reported a significant correlation between urinary D-

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**Table 1. Serum Copper and Related Variables in Controls and Epileptic Patients (Mean ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 36)</th>
<th>Patient group (n = 40)</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Serum copper, μg/L</td>
<td>1048.6 ± 29.9</td>
<td>1477.5 ± 38.4</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Ceruloplasmin, mg/L</td>
<td>294.2 ± 10.4</td>
<td>438.1 ± 13.0</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Oxidase activity, U/L</td>
<td>111.6 ± 3.6</td>
<td>169.3 ± 4.8</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Specific oxidase activity, U/g</td>
<td>376.2 ± 4.6</td>
<td>387.3 ± 5.4</td>
<td>0.2 &gt; p &gt; 0.1</td>
</tr>
<tr>
<td>Non-ceruloplasmin copper, μg/L</td>
<td>105.1 ± 12.5</td>
<td>76.2 ± 25.6</td>
<td>p = 0.3</td>
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**Fig. 1. Relationship between GGT and serum copper (left), ceruloplasmin (middle), and oxidase activity of ceruloplasmin (right)**

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glucaric acid and serum GGT in patients who are being treated with anticonvulsants (22, 23, 28); however, other authors have not found this correlation (24), which agrees with the findings we present here as well as with previous data obtained in our laboratory (21). Although there are several methods for measuring enzyme induction, none is entirely satisfactory. From the low correlation between these methods it can be inferred that they respond in various degrees to different kinds of enzyme induction. Consequently, a set of indicators should be used to characterize with more precision the effects of a drug on enzyme systems (25).

The results given in Table 1 show that in the group of patients studied there was a significant increase in serum copper and in both the concentration of ceruloplasmin and its oxidase activity. The concentration of non-ceruloplasmin copper as well as the correlation of serum copper with ceruloplasmin and oxidase activity of ceruloplasmin indicate that in these patients the hypercupremia is attributable to ceruloplasmin copper. According to Weil-Malherbe (26) all forms of stress produce an increase in serum ceruloplasmin. However, Brunia and Buyse (4) consider it very unlikely that stress or muscular activity during an epileptic fit could explain the increase in serum copper they found in a group of epileptics. The patients we studied were generally well-controlled and had no fits, at least in the weeks before the specimens were taken, and under normal circumstances the biological half-life of ceruloplasmin is 4.2 to 5.2 days (27).

Isolated increase of serum GGT activity in epileptics treated with anticonvulsant drugs has been interpreted to reflect drug-induced enhancement of liver enzymes rather than liver damage (28-30). Serum GGT activity can serve as a convenient marker of hepatic enzyme induction (31), and in patients treated with phenobarbital there is a significant correlation between the activity of this enzyme in serum and the [14C]-aminopyrine breath test (32). The highly significant correlation of GGT with ceruloplasmin and related variables suggests that the hypercupremia seen in the patients studied is a direct result of enzyme induction, produced when the administered drugs increase the hepatic synthesis of ceruloplasmin, for which specific oxidase activity is unchanged.

Serum GGT activity does not increase with physical exercise (33) and values for it in epileptics do not correlate with frequency of fits or the date of the last convulsive attack before the sample was taken (29, 30). Epileptics who do not receive anticonvulsive treatment have normal activity of GGT (28, 34), although this activity is high in non-epileptic patients who are treated with these drugs for other reasons (29, 30). The significant correlation of ceruloplasmin with GGT suggests a similar behavior of both enzymes.

In our patients there was a significant correlation of ceruloplasmin with hemopexin, although in comparison with the control group the average increase of this protein (8.6%) was far more modest than that of ceruloplasmin (48.9%). Hemopexin cannot be considered as an acute-phase reacting protein (35). In healthy volunteers who were injected with the potent inflammatory agent etiocholanolone, the concentration of hemopexin remained unchanged (36), and previous studies (37) have shown no increase in hemopexin as a result of trauma in humans. However, the induction of hemopexin by agents known to enhance the synthesis of hepatic microsomal drug-metabolizing enzymes has been reported previously in rabbits (35, 38).

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References
Homogeneous Enzyme Immunoassay for Tobramycin Evaluated and Compared with a Radioimmunoassay

Jannie Woo, Mary A. Longley, and Donald C. Cannon

We evaluated a commercially available homogeneous enzyme immunoassay (EMIT, Syva Co.) for tobramycin against a reference radioimmunoassay (RIA) method. Between-assay precision (CV) was 2.9% at 6.2 mg/L and 3.0% for values in the range of 1.0–7.6 mg/L. Accuracy based on a recovery experiment (1.0–13.0 mg/L) yielded an analytical recovery of 88–112%. A correlation study with 75 sera from patients on tobramycin therapy showed that EMIT = 0.984 RIA − 0.0608, r = 0.993. Neither the EMIT nor the RIA procedure was affected by the presence of gentamicin, amikacin, and vancomycin. Absorbance data from the EMIT system calculated with the conventional RIA logit-log algorithm correlate well with results generated by the Syva data-handling system (logit-log = 1.077 Syva − 0.318, r = 0.998). A reagent stability study indicated that the EMIT reagents, once reconstituted, remain stable for at least 17 days when stored at refrigerated temperatures, or 11 days if stored at room temperature, thus enabling frequent “stat” assays without the need to prepare a calibration curve each time.

Additional Keyphrases: drug assay · antibiotics · aminoglycosides · data handling

Tobramycin is an aminoglycoside antibiotic used to treat infections caused by a wide spectrum of Gram-negative organisms, including those of the lower respiratory tract, central nervous system, urinary tract, skin, and bone (1). Studies on humans as well as animals indicate that both nephrotoxicity and ototoxicity associated with aminoglycosides are less with tobramycin than with gentamicin (2, 3). Furthermore, some microorganisms, including Pseudomonas species, are less prone to develop resistance to tobramycin (4, 5).

There are at least three reasons for measuring the serum concentrations of tobramycin. Like most other aminoglycosides, tobramycin is excreted primarily by the kidneys (6, 7). The concentration of tobramycin in serum is therefore very much dependent on renal function, and patients with impaired glomerular filtration rate require appropriate dosage adjustments. Secondly, several studies indicate that the estimation of the half-life of aminoglycosides on the basis of sex, age, body weight, serum creatinine, urea nitrogen, and hematocrit is far from satisfactory (1). Hence optimal dose-regimen and dosage intervals must be determined individually to account for variations in drug clearance. Lastly, because of the narrow therapeutic range (peak-trough = 10–2 mg/L) (8), determination of tobramycin concentrations in serum is necessary to assess the risks of nephrotoxicity and ototoxicity.

Techniques commonly used for monitoring serum concentrations of tobramycin include microbiological assay (9), radioenzymatic assay (10), radioimmunoassay (RIA) (11, 12), gas chromatography (13), and “high-pressure” liquid chromatography (14). Of these, the method of choice has been RIA because of its greater sensitivity and specificity (15). Enzyme immunoassay (16, 17), in which the radioisotope is replaced by an enzyme, has been widely applied to drug analysis in the last few years. This technique is particularly useful in therapeutic drug monitoring, where exquisite sensitivity is ordinarily not a stringent prerequisite and speed of reporting is essential. We have evaluated a homogeneous enzyme immu-