Anticopper efficacy of captopril and sodium dimercaptosulphonate in patients with Wilson's disease

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Summary

The aim of this study was to explore and compare initial treatment effects of captopril (Tensiomin) and sodium dimercaptosulphonate (DMPS) on a relatively large series of Wilson's disease inpatients. Two important markers of anticopper efficacy: serum sulphhydryl and 24h urinary copper levels in the patients were evaluated before and after treatment. The patients were randomly subdivided into 4 groups to allow statistical analysis (ANOVA) of the values recorded. The protocol was an open-label study of all the patients treated for 8 weeks (i.e., all the patients except those in the no-drug group), and a further six-month follow-up (post hospitalization) of the 14 patients administered captopril. Several copper-related variables were studied to evaluate the effect of the drugs on copper, and several biochemical and clinical variables were studied to evaluate potential toxic effects. Captopril was found to have a significant anticopper effect and did not markedly raise serum sulphhydryl levels within this limited patient sample; the anticopper efficacy of captopril was, however, found to be markedly lower than that of DMPS; DMPS was found to raise the patients' serum sulphhydryl and urinary copper levels. Evaluation of data from individual patients revealed evidence of a toxic side effect in only 1 patient, treated with DMPS, who exhibited transiently raised serum alanine aminotransferases, while no serious adverse events, upstanding syncope, irritating cough and leukopenia induced by captopril were noted. The results obtained in this four-group sample suggest that captopril might be a mild anticopper agent for Wilson’s disease, possibly relieving the hepatic portal hypertension, but that DMPS has a greater field of anticopper efficiency than captopril. The authors also discuss recent experience of the overall treatment in China.

KEY WORDS: serum sulphhydryl, Tensiomin, sodium dimercapto - sulphonate, urinary copper excretion, Wilson’s disease.

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Introduction

Wilson’s disease is a recessive autosomal inherited disorder (1,2) that in Asia (Japan, Korea, the Middle East and China) is not considered particularly rare. In mainland China over 3000 Wilson's disease patients have been admitted as inpatients to the University Hospital of Anhui College of T.C.M., Hefei. Wilson's disease may present dramatically: a healthy child may suddenly develop acute fulminant hepatic failure; or a child may suffer an abrupt and rapid deterioration in performance, followed by drooling and difficulty in controlling speech and writing. The disease, which is due to copper toxicity, can be treated so specifically with medication that its progression can be halted. The first potent anticopper: penicillamine (PCA) was identified by JM Walshe in 1953 (3-5) and has since been applied in the treatment of Wilson’s disease (hepatolenticular degeneration). When searching for the best anticopper drugs, a few key considerations are always taken into account, such as anticopper efficiency, hepato-protection, side effects, different disease types/forms, and whether the drug is suitable for initial or for maintenance therapy. In mainland China, not only PCA, but also dimercaptosuccinic acid (DMSA), sodium dimercaptosuccinate (DMS) and sodium dimercaptosulphonate (DMPS), and medicinal Chinese herbs, have all been used successfully (3). In the USA, there has been a tendency, since the 1980s, to use triethylene tetramine dihydrochloride (TETA) in place of PCA (6). To our knowledge, there is currently no other report of captopril (Tensiomin) as a possible anticopper agent that also affords the liver protection. This study was an attempt, using the ANOVA method, to identify the anticopper effects of captopril and DMPS in inpatients with Wilson’s disease.

Materials and Methods

General materials

A series of twenty-eight patients with Wilson's disease (18 males and 10 females, aged 14-20 years, mean 15.29±3.96) were enrolled in the study. The research protocol was approved by the patients, their relatives, and the hospital ethics committee. The following criteria were used to select the patient sample from among Wilson’s disease cases admitted to the Institute of Neurology University Hospital, Anhui College of TCM in 1999 and 2000:

1. presence of extrapyramidal symptoms and signs;
2. presence of characteristic corneal Kayser-Fleischer ring upon observation with slit lamp;

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3. serum ceruloplasmin (CP) <200 mg/l, and copper oxidase <0.21 units (measured by optical density);
4. Urinary copper >100 µg (1.56 µmol)/24 hr.

Twenty-eight patients completely fulfilled the above criteria (4). Any doubtful cases and possible heterozygotes were excluded. In accordance with the Factorial Design, these 28 cases were randomly subdivided into 4 groups, each numbering 7 cases: Group A treated with captopril, Group B treated with DMPS, Group C treated with captopril plus DMPS, and Group D not submitted to any anticopper treatment. Serum sulphhydril levels in 32 healthy volunteers (20 males and 12 females, aged 17-23 years, mean 18.51±2.12) were also investigated.

**Treatment methods**

None of the Wilson’s disease patients had previously undergone therapy with an anticopper agent. Throughout the research treatment, all the patients followed a strict low-copper diet. Group A received captopril orally, 1 mg/kg/day, the total daily amount divided into three separate doses, corresponding to a maximum (for adults) of 25 mg tid; in Group B, DMPS was administered intravenously, 20 mg/kg/day, diluted with 500 ml glucose solution, 5%; Group C received both captopril and DMPS simultaneously, according to the doses and methods described for Groups A and B; for two weeks, Group D did not receive any specific anticopper agent. The duration of the treatment course in Groups A, B and C was 8 weeks. The fourteen patients belonging to Groups A and C were conservatively observed for a further six-month follow-up period in order to collect data on the continuous administration of captopril and captopril-plus, and to monitor the possible occurrence of side effects after hospitalisation. The symptoms and signs of all the patients were recorded, as were adverse events. Before treatment, and once a week after treatment, the serum sulphhydril and excrated urinary copper levels of Groups A, B and C were measured, and peripheral blood count, liver function, kidney function and EKG were performed; the serum sulphhydril and excrated urinary copper levels of the Group D patients were only evaluated twice, with an interval of one week (for moral considerations, the patients in Group D were not left untreated any longer than two weeks, moreover baseline values are already assumed to be relatively stable at two weeks).

**Laboratory methods and statistical analysis**

Copper and zinc elements were measured by means of WFX-II atomic absorption spectrum analysis. Serum sulphhydril was tested by spectrophotometer 7211-XP72, the main reagents containing sodium nitrite, sulphhydrilamid, dihydrochloride N-(1-naphthalin)-ethanediamine, potassium cyanide, and mercury chloride. The colour of the terminal reactive product is a beautiful rose-red, which means that it can be measured by photoelectricity analysis with a wavelength of 550 nm. Statistical analysis of the data was performed using: ANOVA (analysis of variance), linear regression from the SPSS.10 statistical package, and t-test.

**Results**

**Urinary copper excretion**

The post-treatment urinary copper levels, derived from the mean values of the treated patients’ urinary copper levels in the second, fourth, sixth and eighth weeks of treatment, were subtracted from pre-treatment urinary copper excretion values, giving the values (differences) shown in Table 1. The urinary copper differences recorded in the patients belonging to the four groups were submitted to statistical analysis with ANOVA. In Group A (captopril only), the ANOVA analysis resulted in Fₐ (Fₐ value based on Group A) = 12.93>7.82, p<0.01, corresponding to a markedly significant copper-reducing effect. In Group B (DMPS only) the result was Fₐ (Fₐ value based on Group B) = 200.22>7.82, p<0.01, indicating

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urinary copper (µmol/24 hr)</th>
<th>Serum Sulphydryl (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>-0.06</td>
<td>-1.3</td>
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<tr>
<td></td>
<td>-0.07</td>
<td>0.4</td>
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<tr>
<td></td>
<td>0.47</td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td>-0.35</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>-0.08</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>-0.06</td>
<td>-1.0</td>
</tr>
<tr>
<td>CAPTOPRIL</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td>3.23</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>-0.3</td>
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<tr>
<td></td>
<td>1.55</td>
<td>0.6</td>
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<td></td>
<td>1.01</td>
<td>0.8</td>
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<tr>
<td></td>
<td>2.50</td>
<td>-1.0</td>
</tr>
<tr>
<td>DMPS</td>
<td>7.86</td>
<td>19.6</td>
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<tr>
<td></td>
<td>8.67</td>
<td>7.6</td>
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</tr>
<tr>
<td></td>
<td>5.02</td>
<td>9.1</td>
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<tr>
<td>CAPTOPRIL+ DMPS</td>
<td>7.9</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>15.6</td>
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<td>7.54</td>
<td>7.8</td>
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</table>

Abbreviations: DMPS=sodium dimercaptosulphonate
that DMPS has a very markedly significant anticopper effect. The \( F_{AB} = 15.47 > 7.82, p < 0.01 \), also indicated that the anticopper effect of DMPS was significantly more potent than that of captopril. When both captopril and DMPS were administered simultaneously (Group C), no significant cross-effect was found, as reflected in the \( F \) value (cross-effect) <4.32, \( p > 0.05 \).

**Serum sulphphydryl**

The mean serum sulphphydryl level prior to treatment was 29.5 mg/l (±4.2) in the 28 cases, and 30.1 mg/l (±3.5) in the control group (no.=32), with no significant difference emerging between the two groups on t-test. The difference values given in Table 1 were obtained by subtracting serum sulphphydryl levels recorded after the eight-week course of anticopper treatment from pre-treatment serum sulphphydryl values. ANOVA was again applied, giving the following results: In Group A, \( F_A = 3.25 < 4.32, p > 0.05 \), suggesting that fixed doses of captopril did not increase the serum sulphphydryl of patients with Wilson’s disease. The result obtained in Group B, \( F_B = 101.15 > 7.82, p < 0.01 \), on the other hand, suggested that DMPS did increase significantly the serum sulphphydryl of patients with Wilson’s disease. When both were used simultaneously (Group C), no significant cross-effect was found: \( F \) (cross-effect) <4.32, \( p > 0.05 \).

**Linear regression between the serum sulphphydryl and urinary copper differences recorded in Group B**

By linear regression (SPSS statistical software package), the relationship of the serum sulphphydryl and urinary copper differences in Group B was investigated, so \( F=6.566 > 5.99, p < 0.05 \). It showed a significant linear regression between the raised urinary copper and raised serum sulphphydryl in the patients of Group B, treated with DMPS.

**Side effects and adverse effects.**

Peripheral blood counting revealed no marked decreases in white/red blood cells and blood platelets in Groups A, B, C during the 8-week treatment course, and liver and kidney functions showed no deterioration except for transiently raised serum alanine aminotransferases in one Group B patient. None of the fourteen patients administered captopril (Groups A and C) showed any side effects or adverse events such as irritating cough, upstanding syncope or leukenopa during the 8-week study; neither were such effects noted in the further six-month follow-up data on the cases continuously administered captopril or captopril-plus.

**Discussion**

Although the fundamental pathogenic defect in Wilson’s disease has its source in the hepatobiliary system, the consequences of the defect express themselves at multiple organ and system level: indeed, expressions may be neurological, psychiatric, hepatic, or even osteo-muscular (especially in Asians, as first noted by Indian doctors) (7), while some patients may be asymptomatic (6). Hepatic problems, an initial feature in over 50% of patients, can present as asymptomatic enlargement of both liver and spleen. Hepatic dysfunction generally falls into one of several subtypes (3,4):

1. acute hepatitis. As a common secondary form (25%), typically characterized by jaundice, anorexia and fatigue, this subtype may be mistaken for viral hepatitis or infectious mono-nucleosis;
2. acute fulminant hepatitis. Presenting as rapidly progressive liver failure, encephalopathy and coagulopathy with severe erythrocyte anaemia, this subtype is habitually described as the abdominal form of Wilson’s disease and has an extremely high mortality rate;
3. chronic active hepatitis, occurring in 10-30% of cases;
4. progressive cirrhosis with a post-necrotic picture as the most common hepatic manifestation, typically associated with slowly progressive liver failure and splenomegaly, ascites, oesophageal varices, and encephalopathy. The jaundice in this condition may have different sources: cellular, haemotic, or congenital (usually juvenile gallstones). In 40% of patients the first-noted clinical features are neurological symptoms: tremor, dysarthria, dystonia, chorea, athetosis and seizures; according to most reports, psychiatric and neurosis-like symptoms were clinical features in 20%-50% of cases (5,6). Symptoms of endocrine disturbances in Wilson’s disease, for example sexual disorders – these may take the form of impotence in males and menopause disorders or sexual hyperactivity in females – have often been observed in our clinic.

Urinary copper excretion rises and typically exceeds 100 µg or 1.56 µmol/ day in patients with Wilson’s disease; accordingly urinary copper content is often used as a practical diagnostic or/and anti-copper index. In this paper, the post-treatment urinary copper values were derived from the mean urinary copper excretion values (for the second, fourth, sixth and eighth weeks of treatment) of the patients administered anticopper treatments. This was on the basis of the reasonable assumption that urinary copper excretion peaks in the second week of anticopper treatment, and that urinary copper excretion levels may decrease gradually after the fourth week and possibly reach their lowest level during the eighth week (3.8). The average level of serum sulphphydryl in our patients was 29.5 mg/l, slightly, but not significantly, lower than the level recorded in normal controls.

The following interventions might be considered in the management of Wilson’s disease (6):

1. metabolic intervention: dietary control (high zinc and lower copper), chelate agents (e.g., PCA and DMSA), and product replacement (e.g., replacement of zinc-binding factor in patients with acrodermatitis enteropathica);
2. gene product therapy: cofactor replenishment, organ transplantation, enzyme replacement;
3. gene therapy; all are designed to overcome or alleviate disease by a procedure in which genes, gene segments or oligonucleotides are introduced into the cells of an affected individual. The genetic material
may be transferred directly into cells of the affected individual, or cells may be removed from the patient and the genetic material inserted into them. Because the molecular basis of Wilson’s disease can vary widely, the gene treatment strategies represent the greatest challenge. Presently liver transplantation, by replacing the healthy cells and genes, may be considered an organ-level “gene therapy”;

4. preventive measures: heterozygote screening, and prenatal diagnosis and treatment. Wilson’s disease is a recessive-type disorder of trace element metabolism resulting from the absence or dysfunction of a copper transporting P-type ATPase (ATP7B) gene with many exons and introns. Approximately 150 mutations of the ATP7B gene have been identified to date. This ATP7B transporter has two functions: transport of copper into the plasma CP protein, and elimination of copper through the bile. Disturbed export of copper into bile results in accumulation of copper in the liver and secondarily in other multiple organs such as the brain. Interestingly, the locus of the Wilson’s disease gene is located on 1q14.1-21.4, whereas the CP gene is located on 3p23-25 and the metallothionein gene on chromosome 16.

It now seems that anticopper therapy may constitute a first key step, although surgical methods, too, are actively used. In China more than 10 patients with Wilson’s disease have undergone successful liver transplantation, and about 200 patients successful splenectomy (3,8). However, we would emphasize the need to bear in mind the fact that any surgical operation may deteriorate the neurological symptoms (6). Stereotactic neurosurgery has sometimes been performed targeting extrapyramidal system symptoms in the neurological subtypes, especially in some countries of Eastern Europe, as well as in China (up to 10 cases operated). In the 1950s, Liang Y and Ding GS (Shanghai) first invented DMSA, primarily with the aim of protecting military personnel against chemical poisoning (9). We have observed the efficiency and side effects of DMSA since the 1980s, and deemed it to produce milder adverse effects in comparison with PCA, while having comparable anticopper efficacy (3,8). In 1998, we proposed captopril as a candidate anticopper agent, for the following reasons: captopril has one sulphhydryl radical per molecule, similar to PCA; captopril itself, a kind of inhibitor of angiotensin converting enzyme (ACE) (10), could possibly alleviate the hepatic portal hypertension safely, so may partly replace propranolol, as the latter might be considered to induce and even deteriorate hepatic coma, particularly in the pre-stage of hepatic encephalopathy.

Generally, captopril is a stable and moderate anti-hypertension agent, however it may occasionally cause leukopenia and persistent coughing. Interestingly, captopril and PCA are semi-immunosuppressants, that is alternative therapies for rheumatoid arthritis.

Our clinical data suggest that: captopril may be a mild anticopper agent that promotes the excretion of urinary copper in patients with Wilson’s disease. On the other hand, it produced a non statistically significant increase in serum sulphhydryl, a result that may be attributable to the small size of the sample and the captopril doses. Another preliminary result is that captopril did not inhibit the raising of serum sulphhydryl by DMPS. Raised urinary copper excretion has been demonstrated in patients with rheumatoid arthritis treated with captopril (8).

Adverse effects, such as allergy, upstanding syncope, irritating cough and leukopenia, were not noted in this study.

Research in this field has shown that DMPS is possibly a more potent anticopper agent than DMSA and PCA (11). Practical problems in this disease, include the collection of sufficiently large inpatient samples, and the integration of other anti-copper agents, but it is a field offering plenty of new challenges to clinical researchers. For example, both captopril and DMPS have such a similarly efficient sulphhydryl radical to chelate the copper ion that they may be mutually competitive within the human body, and might therefore inhibit each other. This research did not show any significantly marked cross-effects in their effects on sulphhydryl levels and urinary copper levels. Tetramethylolmolybdate (TM) cannot be used for maintenance therapy. Rarely, adverse effects such as transient arrest of bone marrow (haematological toxicity), have been reported (12-14). Our observation of a Chinese case treated with TM showed the drug to have a surprisingly potent anticopper efficacy, raising the patient’s urinary copper levels more than 10-fold in the clinical week compared with baseline. DMS, which has mild side effects, such as slight haemolytic anaemia and skin purpura, has an anticopper effect that is approximately twice that of PCA (8). DMSA can be orally managed, even though, as regards its chemical structure, it is similar to DMS, which is only managed by injection. Some American patients with Wilson’s disease were treated by DMSA in California. PCA has also been used as one of the most potent anticopper agents, however attention must be paid to the development of allergic rashes, fever, leukopenia, and thrombocytopenia. Also highlighted in the literature is Hepatolithentic Degeneration Relief Decoction or Gandou Decoction (8,11), a recipe based on traditional Chinese medicinal herbs, which were identified to promote the secretion of biliary copper (the main pathway via which humans discharge superfluous copper into the stool) and supply hepato-protection.

Schilsky 1994 (15) collected 55 patients with Wilson’s disease presenting fulminant liver failure and requiring urgent liver transplantation, and found post-operative one-year survival in 79%. We observed that the main mortal risks were hepatic coma and the upper gastrointestinal bleeding in the visceral type, and serious infections such as pneumonia and septicaemia in the neuropsychiatric type (3). These mortality rates are similar to Brewer’s (16). It is emphasized that liver-protection is also a vital treatment. Captopril may protect the tissues in Wilson’s disease against the free radicals induced by lipid peroxidation and oxidative stress, resulting from the higher serum copper ion and lower serum vitamin E (3,17-19) levels, as well as promote liver regeneration (20); it also attenuates the progression of rat and mice hepatic fibrosis (21,22) and reduces human portal hypertension (9,23).

In summary, captopril might be a mild anticopper and liver-protecting agent, however further studies are needed in order to establish the appropriate doses. DMPS as a much more potent anticopper agent, may significantly raise sulphhydryl and markedly elevate uri-
nary copper levels. Progress in molecular medicine is now overwhelming, and it is probable that in the coming decades gene therapy will emerge as a practical approach to the management of Wilson’s disease (24,25).

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References