THIAMINE PYROPHOSPHATE EFFECT AND NORMALIZED ERYTHROCYTE TRANSKETOLASE ACTIVITY RATIO IN WERNICKE–KORSAKOFF PATIENTS AND ACUTE ALCOHOLICS UNDERGOING DETOXIFICATION

H. K. ROOPRAI, O. E. PRATT†, G. K. SHAW‡ and A. D. THOMSON

Department of Neuropathology, Institute of Psychiatry, London SE5 8AF, †Elmdene Alcoholic Unit, Bexley Hospital, Bexley, Kent, DA5 2BW and ‡Gastroenterology & Liver Unit, Greenwich District Hospital, London SE10 9HE, UK

(Received 19 February 1996; in revised form 4 July 1996; accepted 11 July 1996)

Abstract — Thiamine deficiency may be assessed clinically by an abnormally low specific erythrocyte transketolase activity and/or by an abnormally large activation by thiamine diphosphate in vitro (or ‘TPP effect’). In the present investigation, we report erythrocyte transketolase activation by TPP in acute alcoholics and Wernicke–Korsakoff patients undergoing detoxification. A new age-dependent parameter was used to improve the reliability of transketolase activity as an indicator of marginal thiamine deficiency. Thus, normalized transketolase activity ratio (NTKZ), primary activation ratio (PAR) and further activation ratio (FAR) were measured in 29 acute alcoholics and 12 Wernicke–Korsakoff patients upon admission, and also on 47 control subjects. It was possible to follow up 14 of the 29 acute alcoholics after 7 days of treatment. Twenty-one per cent of the acute alcoholics and 33% of the Wernicke–Korsakoff patients, on admission to the detoxification Unit, had NTKZ values beyond the defined critical conditions for thiamine deficiency, whereas 7% of the former and 25% of the latter had PAR values beyond these critical conditions. Furthermore, all three parameters were significantly different in the Wernicke–Korsakoff patients compared to the other two groups. The pattern of improvement of the different parameters on follow-up varied considerably and is difficult to explain, as only the NTKZ was statistically significant. Hence, only eight out of 14 acute alcoholics showed improvement in NTKZ, seven showed improvement of PAR and six showed improvement of FAR after treatment. Five patients showed improvement of both NTKZ and PAR and none of the patients showed improvement of all three parameters. We conclude that our findings confirm previous reports and that this modified transketolase activation test improves its reliability as an indicator of marginal thiamine deficiency.

INTRODUCTION

Wernicke’s encephalopathy and Korsakoff’s psychosis are now generally accepted as being two facets of the same disease entity: Wernicke–Korsakoff syndrome (WKS). Although most commonly associated with chronic alcoholism, this rare disorder may result from nutritional disturbances, particularly thiamine deficiency (Centerwall and Criqui, 1978; Hoyumpa, 1980).

The early detection of thiamine deficiency in WKS patients poses a severe diagnostic problem. Even though the incidence of diagnosed Wernicke–Korsakoff syndrome is low, being seen in ~2% of all brains at post-mortem (Cravioto et al., 1961; Harper, 1979, 1983; Victor et al., 1989), cognitive disorders are observed in a large proportion of all alcoholic patients. It is therefore important to improve the methods of detection of thiamine deficiency to avoid potentially preventable brain damage.

Following the early findings that thiamine diphosphate (TPP) acted as a coenzyme for transketolase (EC 2.2.1.1), Brin (1962) and Dreyfus (1962) were independently able to show that the assay of erythrocyte transketolase with and without the addition in vitro of thiamine pyrophosphate (i.e. the ‘TPP effect’) could be used as an indicator of marginal thiamine deficiency. Subsequently this assay, with various modifica-
Thiamine deficiency may be assessed either by an abnormally low specific activity of erythrocyte transketolase and/or by an abnormally large activation by TPP in vitro. In practice, however, there appears to be little to choose between the two assessment criteria. The degree of erythrocyte transketolase activity and the TPP effect have often been reported in alcoholics (Dreyfus, 1962; Wood et al., 1977; Camilo et al., 1981; Leigh et al., 1981; Jeyasingham et al., 1987a; Nixon et al., 1990), including those undergoing detoxification or thiamine treatment (Jeyasingham et al., 1987b; Price et al., 1991; Nordentoft et al., 1993).

In a preliminary study to the present investigation, we reported that the reliability of the specific transketolase activity as an indicator of marginal thiamine deficiency would be improved if the results were expressed as a percentage of the mean normal value corrected for age, with values <60% of the age adjusted mean indicating thiamine deficiency (Rooprai et al., 1990). We report here the erythrocyte transketolase activity and TPP effect in a group of acute alcoholics and Wernicke-Korsakoff patients undergoing detoxification, using the new (age-dependent) parameter described previously.

SUBJECTS AND METHODS

Subjects

There were three groups of subjects, a control group and two groups of alcoholics. One group, designated 'acute alcoholics', consisted of chronic alcohol abusers who were admitted to Elmdene Alcohol Treatment Unit at Bexley Hospital for detoxification. They comprised 29 patients (24 males and 5 females) and 14 males in this group were followed up after a week's treatment. All patients had hepatomegaly, but liver biopsies were not performed. Laboratory investigations and abdominal ultrasound examinations did not indicate cirrhosis or portal hypertension and none of the patients were thought to have Wernicke's encephalopathy clinically.

The second group, labelled 'Wernicke-Korsakoff', were also chronic alcohol abusers with WKS diagnosed clinically on neurological examination by an experienced psychiatrist (Dr Shaw) using criteria described by Victor et al. (1989). In all cases, blood was drawn before vitamins had been administered. There was no indication of oral vitamin supplementation and these patients usually had malabsorption of oral thiamine. There were 6 males and 6 females. The mean (±SD) ages of acute alcoholics and of the Wernicke-Korsakoff patients were 47 ± 18 years and 52 ± 11 years, respectively. The control group included 47 subjects (30 males and 17 females) with a mean age of 50 ± 15 years. Some of these subjects were staff members, whereas others were non-alcoholic patients from the Maudsley Hospital, free of any conditions likely to be associated with malnutrition.

Sampling of blood

Venous blood (20 ml) samples were taken from both groups of alcoholic patients on admission, before any treatment, and, when possible, after a week of treatment which consisted of multiple vitamin therapy, Parentorite (including 250 mg of thiamine daily). An assessment of their hepatic function was also carried out at Bexley Hospital and none of the patients showed any biochemical evidence suggestive of hepatic failure or cirrhosis. The blood samples were collected in heparinized containers, chilled and used for biochemical assay within 48 h.

Preparation of erythrocyte haemolysates

Blood was centrifuged (at 4°C) for 10 min at 1000 g and then as much supernatant, buffy coat and fat as possible were removed. The cells were washed three times with physiological saline (0.15 M NaCl). A portion (100 µl) of the erythrocytes was suspended in 1.9 ml of 0.1 M Tris–HCl buffer, pH 8.0 (at room temperature) and frozen rapidly using liquid nitrogen. A portion (200 µl) of this suspension was used for estimating transketolase activity.

Estimation of transketolase activity

This was determined by a modification of the method of Smeets et al. (1971). A solution of 1 mM NADH (Na-nicotinamide adenine dinucleotide, reduced form, Sigma Grade III) was freshly prepared in 100 mM Tris buffer, pH 8.0. Glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) 10 U/ml and triosephosphate isomerase (EC 5.3.1.1) 1 U/ml (GDH + TPI) was also freshly prepared in the same buffer. The 2 ml reaction mixture consisted of the following reagents: 600 µl of 0.1 M Tris
Table 1. Critical conditions in transketolase function parameters to exclude 5% of the control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 47)</th>
<th>Acute alcoholics (n = 29)</th>
<th>Wernicke-Korsakoff (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Beyond limit</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beyond limit</td>
</tr>
<tr>
<td>NTKZ &gt; 0.64</td>
<td>1.000 ± 0.186</td>
<td>2 (4%)</td>
<td>0.898 ± 0.237</td>
</tr>
<tr>
<td>PAR &lt; 1.18</td>
<td>1.052 ± 0.087</td>
<td>1 (2%)</td>
<td>1.051 ± 0.113</td>
</tr>
<tr>
<td>FAR &lt; 1.06</td>
<td>0.921 ± 0.075</td>
<td>2 (4%)</td>
<td>0.937 ± 0.134</td>
</tr>
</tbody>
</table>

The data include results from acute alcoholics admitted for detoxification and Wernicke-Korsakoff patients thought to be brain-damaged. For each parameter in turn is shown the proportion of the samples beyond the threshold of normality according to the conditions specified with the corresponding percentage in parentheses. NTKZ = normalized transketolase activity ratio (with respect to age); PAR = primary activation ratio; FAR = further activation ratio.

Table 2. Probabilities of occurrence, as calculated by the Fisher exact method from the data, for Wernicke-Korsakoff patients compared to the other two groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>χ² value</th>
<th>d.f.</th>
<th>P value</th>
<th>Fisher exact P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTKZ</td>
<td>10.33</td>
<td>2</td>
<td>0.006</td>
<td>0.03</td>
</tr>
<tr>
<td>PAR</td>
<td>8.37</td>
<td>2</td>
<td>0.015</td>
<td>0.03</td>
</tr>
<tr>
<td>FAR</td>
<td>12.89</td>
<td>2</td>
<td>0.002</td>
<td>0.004</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 1.

buffer (pH 8.0); 200 μl of 0.1 M ribose-5-phosphate; 200 μl of 0.008 M xylulose-5-phosphate; 200 μl of 12 mM magnesium chloride; 200 μl of GDH + TPI prepared as described above; 200 μl of the haemolsate prepared as described above, 200 μl of either thiamine diphasphate (3 or 30 mM) or Tris buffer and 200 μl of 1 mM NADH (freshly prepared). The buffer, sugar and magnesium chloride were placed in a small glass test-tube and preincubated for 10–15 min at 37°C before adding the GDH + TPI and erythrocyte haemolsate. The reaction was initiated by the addition of NADH. The mixture was transferred to a 5 mm silica cell and placed in an SP800 spectrophotometer (Pye Unicam, England). The chart recorder was started immediately and the change in optical density at 340 nm was recorded at 37°C, between 15 and 30 min. The reference cell contained Tris buffer only. Assays of all samples were performed in duplicate. Haemoglobin was measured in a portion of the haemolsate with a commercial kit (Sigma Ltd) based upon conversion to methaemoglobin (Stadie, 1920).

Data analysis

For each blood sample, the following parameters and ratio were calculated:

- **Specific transketolase activity (STZ).** This was the enzyme activity (without addition of TDP) expressed in units/g of haemoglobin.

- **Primary activation ratio (PAR).** This was the ratio of the activity in the presence of 0.3 mM TDP to that in the absence of added TDP.

- **Further activation ratio (FAR).** This was the ratio of the activity in the presence of 3 mM TDP to that in the presence of 0.3 mM TDP.

- **Normalized transketolase activity ratio (NTKZ).** This is a new parameter derived from the predicted STZ value with reference to age (Rooprai et al., 1990) and is given by the ratio calculated from the equation:

  Predicted STZ = 0.6066 – 0.002045 × age.

  Thus

  \[
  NTKZ = \frac{STZ}{0.6066 – (0.002045 \times age)}.
  \]

Selection criteria

A critical condition was selected by reference only to the data from the control group so as to exclude 5% of the current results from this group within the normal range, to determine the limits of
Table 3. Changes in various erythrocyte transketolase activity parameters in 14 acute alcoholics followed up after thiamine treatment

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>NTKZ</th>
<th>PAR</th>
<th>FAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>No change</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>No change</td>
</tr>
</tbody>
</table>

+ Denotes improvement after treatment with thiamine supplements, whereas – indicates no improvement after treatment.

For abbreviations, see Table 1.

normality for each of the three parameters (NTKZ, PAR and FAR). FAR is an alternative way of trying to detect forms of the apo-transketolase with an abnormally low affinity for TPP. These critical conditions thus selected to define the 'normal' are consistent with the consensus of published work on the activation of erythrocyte transketolase (Pratt et al., 1985; Jeyasingham et al., 1987a,b) and were calculated as follows.

In a population of normal (Z) distribution, $1.65 \times SD$ (standard deviation) leads to a tail that gives a probability of 5% of the data to be excluded from normal. If this value is subtracted from the mean of each group of patients then anything below this value has <5% probability of being significant.

Thus, mean NTKZ (control group) – ($1.65 \times SD$)

$$= 1.00 - (1.65 \times 0.216)$$

$$= 0.64.$$  

As $1.00 = 100\%$, the cut-off point (0.64) is 64%. Values <64% of normalized enzyme activity ratio are indicative of possible thiamine deficiency (see Table 2).

**Statistical analysis**

All analyses were carried out using SPSS/PC+ version 5.0. The significance of possible differences between the values of NTKZ, PAR and FAR in the three groups was initially tested by one-way analysis of variance (ANOVA), followed by Scheffé’s test for paired comparisons. Additionally, the significance of the differences between on-admission values of the parameters and their respective post-treatment values was assessed by paired Student’s t-test, in the 14 of the 29 acute alcoholics who were followed-up.

**RESULTS**

**Incidence of abnormal findings**

The distributions of NTKZ, PAR and FAR of the three groups are shown in Figures 1, 2 and 3, respectively. These scatter-diagrams show that the pattern of distribution for each parameter in each group is different. Both the acute alcoholics and the Wernicke–Korsakoff patients showed lower mean values of normalized transketolase activity ratio (NTKZ1) compared to the control group, on
admission (Fig. 1). Furthermore, the distribution for the Wernicke-Korsakoff patients appeared to be bimodal for NTKZ. For each group of patients in turn, the measurements from the blood samples were used to see whether the three parameters (NTKZ, PAR and FAR) fulfilled the critical conditions selected. It can be seen that for all the parameters in the acute alcoholic and Wernicke-Korsakoff patient groups, the proportion of 'abnormal' (i.e. thiamine deficient) values exceeded that in the control group. Table 1 shows the means (±SD) of the appropriate parameter for each group of patients as well as the proportion of each group which appears to be thiamine deficient. It can be seen that with the exception of the control group, appreciable proportions of the rest of the parameters in the other groups are beyond the threshold limits. In the Wernicke-Korsakoff group, 33% of NTKZ, 25% of PAR and 42% of FAR were beyond the threshold values. The corresponding values for the acute alcoholics were less (21%, 7% and 17% respectively). Statistically, the initial one-way ANOVA followed by Scheffe's test for paired comparisons demonstrated significant differences for NTKZ and FAR in the Wernicke-Korsakoff group compared to the other two groups (P < 0.05). Further analysis using $\chi^2$-tests indicated significant differences for NTKZ, PAR and FAR. A Fisher exact test for the differences between Wernicke-Korsakoff patients and the other two groups was also significant for all three parameters (Table 2).
Changes in various transketolase activity parameters in 14 acute alcoholics during detoxification

In 14 of the 29 acute alcoholic patients admitted for detoxification, it was possible to do a follow-up study on erythrocyte transketolase after treatment with thiamine supplements. Figures 4, 5 and 6 show the NTKZ, PAR and FAR, respectively, of each of these patients, before and after treatment. Figure 4 shows that eight of the 14 patients (57%) had an improvement in NTKZ after treatment, and one other patient showed no change. It is of interest to note that two of these patients with NTKZ values below the threshold level (i.e. thiamine-deficient) showed considerable improvement to near normal mean values. Of the remaining five patients, three showed only small decreases in activity to well above the threshold level. Figure 5 shows that seven of the 14 patients (50%) showed an improvement of the primary activation ratio, after treatment. Of these, only one patient whose PAR was above the threshold limit showed a considerable drop in this ratio. However, three of the other patients whose PAR values were higher than the mean of the control group, before treatment, showed an increase in this ratio beyond the cut-off point, after treatment. The changes in the further activation ratio of these 14 patients (Fig. 6) do not correspond to the changes seen in their primary activation ratio as shown in Table 3, such that the first patient, who showed an improvement of FAR (being beyond the threshold limit, on admission), did not show a similar change in PAR. Six of the 14 patients (43%) showed improvement and one patient showed no change after treatment. Statistical analysis using paired t-test showed that there was significance in the NTKZ mean values only between the on-admission values and their respective post-treatment values in the 14 acute alcoholics who were followed up (P < 0.05).

DISCUSSION

The high primary activation ratio and low normalized transketolase activity ratio values found in the present investigation in a considerable proportion of the alcoholics are indicative of
thiamine deficiency and confirm frequently observed findings by other workers (Wood et al., 1977; Jeyasingham et al., 1987b). Only 21% of the acute alcoholics and 33% of the Wernicke-Korsakoff patients on admission to the detoxification unit had NTKZ values beyond the critical condition. This is not an unexpected finding, since individuals in such a group are liable to suffer from a very poor diet resulting from poverty caused by a low earning capacity and malnutrition caused by alcohol itself since it accounts for at least 50% of their diet (Morgan, 1982).

Previously, we have shown that human erythrocyte transketolase can be resolved into two components (Pratt et al., 1985). One component has its TPP coenzyme firmly bound, while the other variant of the enzyme is a smaller molecule which is inactive without added TPP, for which it has a comparatively reduced affinity. It was suggested that the low molecular weight variant represents a damaged form of the enzyme normally present in small amounts but formed in larger proportions in vivo in abnormal conditions like chronic alcoholism and thiamine deficiency. The increased rate of breakdown of transketolase in vitro after conversion to the apo-form (Jeyasingham et al., 1986) supported the assumption that such degradation of the enzyme is more likely to occur in thiamine deficiency when part of the transketolase remains in the apo-form.

The parameter PAR measures the proportion of transketolase that is in the apo-form and readily reactivated. The present study showed that 7% of the acute alcoholic and 25% of the Wernicke-Korsakoff patients, on admission, had PAR beyond the defined critical conditions. However, a previous survey using similar criteria (Jeyasingham et al., 1987a) showed a higher proportion of the alcoholic patients to be thiamine-deficient, i.e. 28% of the former and 50% of the latter group had PAR values beyond this critical limit.

It is apparent from the follow-up study of acute alcoholics that those patients who showed improvement in PAR alone or improvement in both PAR and NTKZ did not necessarily show a similar change in FAR. However, significant difference was only seen in one parameter (NTKZ) when comparing on-admission with post-treatment values. The latter finding is in agreement with those reported by Nordentoft et al.
Fig. 6. Diagram to show further activation ratio in 14 acute alcoholic patients who were followed up.

The open bars represent the ratio on admission and the closed bars that after 7 days of treatment. Each bar is the mean of two determinations. The horizontal line represents the mean of the control group, and the dotted line the cut-off point (critical condition for thiamine deficiency).

(1993), who showed that erythrocyte transketolase activity increased in the alcoholic patients after intensive thiamine treatment and that the changes in the ‘TPP effect’ were not significant. These findings taken together seem to imply that the PAR and FAR parameters are independent and that thiamine deficiency affects the two parameters differently. The low erythrocyte transketolase activity in some thiamine-deficient patients before treatment is probably limited by the formation of fresh red blood cells. Since relatively few newly formed red blood cells will be released from the bone marrow during the 7 day treatment period, the return towards normal values could be due to the recombination of the apo-transketolase found in vivo with the thiamine supplement during detoxification.

Not all patients with lesions in the Wernicke area can be identified ante mortem on clinical criteria (Harper et al., 1986) and it is therefore possible that some of the thiamine-deficient patients in the ‘acute group’ would have also suffered damage in the Wernicke area although no definite clinical diagnosis could be made. However, it seems reasonable to assume that the ‘Wernicke group’ patients all had damage and were more severely affected. Although individuals in this latter group may have some normal thiamine activation ratios, when NTKZ, PAR and FAR are combined, virtually all of the patients showed significant abnormalities in one of these parameters. It is also possible that other thiamine-dependent enzyme systems may contribute to brain damage.

In conclusion, NTKZ, PAR and FAR values for the Wernicke–Korsakoff patients, on admission for detoxification, were significantly different from the acute alcoholics and the controls. In addition, the only parameter that was significantly different in the acute alcoholics was the NTKZ. Even though correction of the transketolase activities for patient age improves the reliability, it is still useful to measure the activation ratios, if only because the information provided by FAR is largely independent of that provided by NTKZ and PAR. The latter two are indicative of marginal deficiency, whereas abnormal FAR values appear to give an indication of something more complex than simple nutritional lack and may help identify those individuals with more chronic and subtle damage to the enzyme, possibly those who are liable to suffer from Wernicke’s encephalopathy.
Acknowledgement — The authors are most grateful to the Brain Damage Research Trust for their financial support of this work and to Dr Graham Dunn for statistical analysis of the data.

REFERENCES