Difficulties in assessing the effect of strychnine on the outcome of non-ketotic hyperglycinaemia. Observations on sisters with a mild T-protein defect

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Abstract. Sisters with a mild variant of non-ketotic hyperglycinaemia resulting from a defect in the T-protein of the glycine cleavage system had different clinical outcomes. The older sister was ascertained at 6 months of age because of mental retardation. She received only brief treatment with sodium benzoate from 11-15 months and at 15 years of age is profoundly retarded and has epilepsy. The younger sister was diagnosed 36h after birth, was treated with strychnine, sodium benzoate and arginine from the neonatal period and at 27 months of age is only moderately retarded and free of seizures. The possible role of strychnine in the improved outcome is discussed.

Key words: Non-ketotic hyperglycinaemia – Strychnine – Glycine cleavage system

Introduction

Non-ketotic hyperglycinaemia (NKH) is a recessively inherited disorder of the glycine cleavage system [17]. This is a complex mitochondrial enzyme system composed of four proteins: P-protein (a pyridoxal phosphate-dependent glycine decarboxylase), H-protein (a lipoic acid containing protein), T-protein (a tetrahydrofolate requiring enzyme) and L-protein (a lipoamide dehydrogenase). Recently, patients have been described with defects in the P- and T-proteins [12]. A defect in the glycine cleavage system leads to increased amounts of glycine in the cerebrospinal fluid (CSF), plasma and urine.

NKH usually presents in the newborn period with an overwhelming illness characterized by coma, respiratory failure, hypotonia and seizures. If the infant survives, severe mental retardation and seizures are the rule. Milder forms of NKH occur and lead to presentation later in the first year with developmental delay, seizures, hypotonia and failure to thrive [5, 17]. A truly mild juvenile form has been reported to cause only mild mental retardation, expressive language deficit and hyperactivity [6]. Occasionally, affected individuals have presented with features of a neurodegenerative disorder [4, 22, 24]. The clinical features of NKH have been attributed to its effects on myelination and to the effect of high glycine levels on glycine receptors in the brain and spinal cord where glycine is an inhibitory neurotransmitter.

To date, no effective form of treatment has been found for the severe form of NKH. In particular, strychnine has been tried because it blocks the binding of glycine to its receptor in the central nervous system. Experience with this treatment suggests that strychnine does not alter significantly the outcome of the severe form of NKH although it may cause minor improvements in muscle tone and responsiveness [3, 5, 23, 25, 26].

Strychnine has also been used to treat several children with mild variants of NKH [5]. The clearest indication of potential benefit comes from a child treated from 6 months of age [5, 11]. This child showed immediate and significant improvement and continuing developmental progress over a period of 5 years. An untreated older sibling with NKH was microcephalic, severely mentally retarded, hypotonic and had epilepsy.

We report and discuss different clinical outcomes in two sisters with a mild variant of NKH, the younger girl having been treated with strychnine from the neonatal period.

Case reports

Patient 1, a girl, was the first child of unrelated Australian parents. She was born at term weighing 3.83 kg after a normal pregnancy, labour and delivery and was well at birth.

She was investigated at 6 months of age because of delayed development. Smiling had appeared at 4 months of age. Examination revealed a relatively unresponsive girl with very poor head control and truncal hypotonia. Limb tone was mildly increased and the deep tendon reflexes were hyperactive. Head circumference was 44.5 cm (90th percentile). Multifocal myoclonic jerks were present but were easily controlled by anticonvulsants.

Glycinuria was detected on high voltage electrophoresis of urine. When measured on two occasions glycine excretion in
the urine was 3.93 and 3.33 mmol/day and fasting serum glycine was 493 and 387 μmol/l (normal 125–318 μmol/l). An electroencephalogram was diffusely abnormal with a prominent excess of slow activity and frank epileptiform discharges. Pneumoencephalography suggested cerebral atrophy. The following investigations gave normal results: haemoglobin, white cell count, acid-base, sodium, potassium, chloride, calcium, glucose, creatinine, ammonia, cerebrospinal fluid protein, cell count and glucose, skull X-ray and a bone age.

The child was thought to have NKH. She was treated with sodium benzoate from 11–15 months of age without observable improvement in her performance or in her seizures. Psychological assessment at 5 years of age showed her to be functioning at the 6–12 month level.

She was re-investigated at 9 years of age. Cerebrospinal fluid glycine was 42 μmol/l (normal 6.6 ± 1.8 μmol/l), fasting plasma glycine was 447 μmol/l (normal 160–304 μmol/l), the CSF glycine/plasma glycine ratio was 0.09 and excretion of glycine in the urine was 6.34 mmol/day. The renal clearance of glycine was normal. These results confirmed the diagnosis of NKH.

The patient was given a trial of strychnine at 9½ years of age. Strychnine (0.8 mg/kg per day) given for 6 weeks produced no improvement. Higher doses led to abdominal cramps and spasms of the trunk, limb and neck muscles.

An operative liver biopsy was performed when the girl was 15 years old to define the biochemical defect. Assay of the glycine cleavage system and its individual components was performed by the method described in Hayasaka et al. [12] and demonstrated a defect in the T-protein. T-protein activity was 0.3 μmol product/g protein per h (control 30.2–77.9). Activity of the overall glycine cleavage system was 1.0 μmol product/g protein per h (control 3.8–9.5), of the P-protein was 5.7 μmol product/g protein per h (control 4.5–5.7) and of the H-protein was 23.8 μmol product/g protein per h (control 14.6–22.0). The histology of the liver was normal at both light and electron microscopy. Cerebrospinal fluid glycine was 35.3 μmol/l and plasma glycine was 904 μmol/l when measured at the time of liver biopsy.

Patient 2 is the younger sister of patient 1. A brother born between the two girls is normal at 14 years of age. Patient 2 was born at term after a normal pregnancy, labour and delivery weighing 3990 g and with Apgar scores of 9 at 1 min and 10 at 5 min. Thirty-six hours after birth she was clinically normal but CSF glycine was 72 μmol/l (normal 6.6 ± 1.8 μmol/l), plasma glycine was 739 μmol/l (normal 106–254 μmol/l) and a moderate glocinuria was present. The CSF glycine/plasma glycine ratio was 0.1. No abnormality was detected on gas-liquid chromatography of urine. These results demonstrated that the child also had NKH. She was mildly hypotonic, lethargic, sleepy and fed poorly in the second half of the first week of life but thereafter became more alert and fed normally.

She was admitted to the Royal Children's Hospital at 9 days of age and was normal on routine clinical examination. Head circumference was 36.7 cm. CSF and plasma glycine levels had fallen spontaneously (Table 1). Hoping to prevent the severe mental retardation and epilepsy experienced by the sister, treatment was begun with strychnine 1 mg/kg per day and arginine 500 mg/kg per day. Arginine was used in the hope that it would be conjugated with glycine in the central nervous system to form creatinine, thus lowering free glycine levels. Six days after treatment was started CSF glycine levels had fallen further (Table 1). Sodium benzoate 250 mg/kg per day was added and 6 days later the CSF glycine level was little different from that prior to starting sodium benzoate. Subsequent measurements of CSF, plasma and urine glycine and the CSF glycine/plasma glycine ratio are set out in Table 1. Strychnine has been continued at a dose of 1 mg/kg per day while the dose of sodium benzoate has been adjusted according to the level of free benzoate in the urine and is currently 90 mg/kg per day.

Development appeared normal during the first year of life. At 5 weeks she smiled responsively, at 10 weeks held a toy, at 9 months reached out, at 6 months began to babble, at 7 months was rolling over, sitting briefly and drinking from a cup and at 9 months was crawling. Developmental delay became apparent during the second year of life. She walked at 21 months and when formally assessed by a psychologist at 27 months was found to be functioning at the 12–15 month level in most areas of development.

Growth has been normal with weight following the 90th percentile, length the 75th percentile and head circumference the 98th percentile.

The activity of the glycine cleavage system was measured at 20 months of age in liver obtained by needle biopsy. This followed delineation of the enzyme defect in the older sister. Insufficient liver was available for assay of the T-protein but the overall activity of the glycine cleavage system was 1.4 μmol of product/g protein per h (control 4.5–5.7).

### Table 1. Glycine levels in CSF, blood and urine at different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>CSF glycine</th>
<th>Plasma glycine</th>
<th>CSF/Plasma glycine ratio</th>
<th>Urine glycine</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 h</td>
<td>Nil</td>
<td>72.0</td>
<td>739</td>
<td>0.1</td>
<td>↑↑</td>
<td></td>
</tr>
<tr>
<td>9 d</td>
<td>Nil</td>
<td>34.4</td>
<td>354</td>
<td>0.1</td>
<td>↑↑</td>
<td></td>
</tr>
<tr>
<td>15 d</td>
<td>S, A for 6 days</td>
<td>20.9</td>
<td>498</td>
<td>0.04</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>S, A, B for 6 days</td>
<td>25.8</td>
<td>321</td>
<td>0.08</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>10 w</td>
<td>S, A, B</td>
<td>14.8</td>
<td>315</td>
<td>0.05</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>4 m</td>
<td>S, A, B</td>
<td>15.0</td>
<td>215</td>
<td>0.07</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>14 m</td>
<td>S, A, B</td>
<td>21.6</td>
<td>360</td>
<td>0.06</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>20 m</td>
<td>S, A, B</td>
<td>17.9</td>
<td>225</td>
<td>0.08</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>22 m</td>
<td>S, A, B</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>659</td>
<td></td>
</tr>
<tr>
<td>26 m</td>
<td>S, A, B</td>
<td>23.2</td>
<td>434</td>
<td>0.05</td>
<td>2892</td>
<td></td>
</tr>
</tbody>
</table>

* Amino acid analysis was performed using a Biotronics LC5000 automated amino acid analyser
  S = strychnine, A = arginine, B = benzoate; ND = not done
  Dickinson and Hamilton [8]
  Pohlandt [21]
  Applegarth et al. [2]
  Perry et al. [20]
  Parvey et al. [19]