Carboxyterminal Propeptide of Type I Procollagen in Cerebrospinal Fluid in Childhood and in Children with Leukemia Undergoing Intrathecal Treatment

Leena Vainionpää, Leila Ristell, Marjatta Lanning, and Juha Ristell

We determined the reference interval for the carboxyterminal propeptide of type I procollagen (PICP), an indicator of the synthesis of type I collagen, in cerebrospinal fluid (CSF) by studying 32 infants and children, ages ≤15 years. The concentration of PICP is age dependent, with particularly high concentrations occurring in children younger than 1.5 years. In older children the concentration is stable (reference interval 20–92 μg/L). We also investigated the possibility that PICP in CSF could reflect local fibroproliferative changes in the arachnoid in a cohort of 42 children with acute lymphoblastic leukemia who were monitored by repeated sampling in connection with intrathecal therapy. Initially, there was no difference in PICP between the children with newly diagnosed leukemia and the controls. PICP concentrations were significantly higher (P < 0.01) during intrathecal methotrexate therapy, with median values above the reference interval. Continuous corticosteroid treatment was associated with a significant decrease in PICP (P < 0.02 and P < 0.01, respectively, in two groups treated according to different protocols), close to the lower limit of the reference interval. Intrathecally administered methotrexate and systemic corticosteroid treatment are known to be associated with the development of arachnoiditis and with general repression of collagen synthesis, respectively. We conclude that PICP in CSF is a sensitive indicator of local fibroproliferation and ongoing collagen synthesis.

Additional Keyphrases: pediatric chemistry · reference interval · arachnoiditis · fibroproliferation · chemotherapy · methotrexate · corticosteroids

Type I collagen, the most abundant collagenous protein in most connective tissues (1), is synthesized in a larger precursor form, type I procollagen, from which the additional sequences are removed by cleavage by two specific proteolytic enzymes (2). At least one of these extensions, the carboxyterminal propeptide of type I procollagen (PICP), can be found in the free form in blood and interstitial fluid (3), where its concentration is considered to reflect the rate of type I collagen synthesis (4). We developed a radioimmunoassay recently for assessing the concentration of PICP in serum (5).

Inflammation in any location is known to trigger a fibroproliferative reaction in the tissue involved (6). Such a reaction involves the proliferation of fibroblastic cells and enhanced expression of extracellular matrix components, including fibronectin and collagen of types I and III (7). These phenomena have also been described as taking place in the pia-arachnoid membranes and between the nerve roots in cases of adhesive arachnoiditis induced by radiographic contrast material (8). There is also a report of a child with leukemia in whom scarring of the subarachnoid space led to an inability to walk (9). This patient had received intrathecal methotrexate and cranial irradiation. Although this type of adhesive arachnoiditis is rare, intrathecal methotrexate therapy is often associated with a clinical syndrome characterized by transient headache, nausea, vomiting, meningism, fever, back pain, and cerebrospinal fluid (CSF) abnormalities (10–13). The frequency of these findings, which suggest the occurrence of clinically manifested arachnoiditis, is 5% to 40% in children being treated for acute lymphoblastic leukemia (ALL); subclinical arachnoiditis, in the form of CSF pleocytosis, may in fact occur in a majority of patients (14).

We undertook the present work to find out whether the assay of PICP can be used in studies of CSF and whether a process leading to arachnoiditis also stimulates the rate of collagen synthesis in the meninges. We first established the reference interval of PICP in CSF in children without any structural lesion of the central nervous system (CNS), and subsequently investigated children with recently diagnosed ALL who were receiving repeated intrathecal injections of methotrexate. We hypothesized that if this drug does induce a fibroproliferative response in the meninges, this treatment should lead to an increase in the PICP concentration that would be measurable in the CSF.

Materials and Methods

Subjects

We determined PICP concentrations in the CSF of two groups of children: first, 18 children, ages 0.3–14.8 years, from whom samples were drawn for diagnostic reasons (10 virus infections, five bacterial infections, one suspected convulsion, one vertigo, and one myasthenia gravis); and second, 14 children, ages 1.0–6.0 years, with a history of one simple febrile convulsion shortly before the lumbar puncture. In all 32 cases, the CSF cell counts and protein and glucose values were normal; children with findings of CNS infections, hemorrhages, or structural lesions were excluded.

We also studied patients—42 children, 17 boys and 25 girls, ages 1.4–15.3 years (median 5.6 years)—admitted to the Department of Pediatrics, University of Oulu,
from March 1986–August 1989 for initial treatment of ALL. Table 1 lists the main clinical characteristics of these patients. None had any neurological disease or CNS leukemia. Four patients were admitted for leukemia relapses 1.2–5.5 years after the completion of earlier therapy. All the patients achieved complete remission, although five had bone marrow or CNS relapses during the treatment (one during the first year and two during the third year). PICP concentrations in the CSF were also determined for two additional patients (ages 6.0 and 9.6 years) at the time of the CNS leukemia relapses.

We divided the patients into three risk groups—standard risk, intermediate risk, and high risk—based on criteria common to all Nordic countries (15). The 21 patients with standard-risk leukemia were treated according to the therapeutic regimens of the Nordic countries and the eight intermediate-risk and 13 high-risk patients according to the Berlin–Frankfurt–Münster (BFM) 83 protocol (16). The first phase, induction therapy, included parenteral vincristine, L-asparaginase (EC 3.5.1.1), doxorubicin or daunorubicin, and daily oral prednisolone for four or five weeks. The intermediate- and high-risk patients also received cyclophosphamide, cytosine arabinoside, and oral 6-mercaptopurine; in addition, the high-risk patients received two doses of intravenous methotrexate and teniposide, together with dexamethasone daily for five days. All patients received repeated doses of intrathecal methotrexate. In the second phase of therapy, all the patients received methotrexate repeatedly, administered intrathecally and at intermediate doses intravenously, and the intermediate- and high-risk patients also received oral 6-mercaptopurine. In the third, re-induction phase, the standard-risk patients received prednisolone daily for one week, and a single dose of vincristine; this regimen was repeated at eight-week intervals. Between these intervals, single doses of methotrexate were administered as above, also at eight-week intervals. Maintenance therapy included oral methotrexate and 6-mercaptopurine. The intermediate- and high-risk patients received dexamethasone daily for four weeks in place of prednisolone, and otherwise, the same drugs as during the induction therapy.

We obtained multiple sequential CSF samples (n = 287) from the 42 children in connection with intrathecal methotrexate therapy, and determined PICP concentrations prospectively from the time of diagnosis of leukemia. All patients received intrathecal therapy repeatedly in accordance with the therapy protocol, but a few CSF samples could not be studied here because the small amount of fluid obtained was needed for other clinically indicated examinations. PICP concentrations at diagnosis were determined for 22 patients. CSF cell counts and glucose and protein concentrations were determined every time a lumbar puncture was performed; the fluid samples for the propeptide assays were stored at −20 to −70 °C.

The investigation was carried out according to the provisions of the Declaration of Helsinki and approved by the Ethical Committee of the Faculty of Medicine at the University of Oulu. The samples were taken only when lumbar punctures were required for drug administration according to the therapy protocol or when otherwise clinically indicated.

**Methods**

PICP was analyzed with a commercially available RIA (Farmos Diagnostica, Oulunsalo, Finland) (5). The sensitivity of the assay is 1.2 μg/L and the intra- and interassay coefficients of variation within the range of PICP concentrations found here are about 3% and 5%, respectively. The main characteristics of the assay are comparable with those of a method for PICP reported elsewhere (17). The molecular size of the PICP antigen was studied by gel filtration as described previously (5).

**Statistical Analyses**

We tested the distributions of the variables statistically for normality. The difference between the PICP values for the patients at diagnosis and for the control children of the same age was tested by Student’s t-test. The values had a normal (gaussian) distribution. However, the distributions of the PICP values of the patients during the second phase, intravenous and intrathecal methotrexate therapy, were skewed; therefore, we described these values as medians and used nonparametric tests for the statistical analyses (Wilcoxon signed-rank test for paired observations and Spearman’s rank-correlation test). Differences were significant at P <0.05. For data management and analysis we used the SAS computer program package (Statistical Analyses System, Inc., Cary, NC).

**Results**

The distribution of PICP concentrations in the CSF of the control children is shown in Figure 1. Quite high concentrations are seen at ages <1.5 years, after which the concentration remains relatively constant at least

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**Table 1. Clinical and Laboratory Data of 42 Children with Acute Lymphoblastic Leukemia**

<table>
<thead>
<tr>
<th>Immunological cell type</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Non-T, non-B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CALLA* positive</td>
<td>33</td>
<td>7.1</td>
<td>1.5–570.0</td>
</tr>
<tr>
<td>CALLA negative</td>
<td>5</td>
<td>78</td>
<td>35–156</td>
</tr>
<tr>
<td>T cell</td>
<td>1</td>
<td>67</td>
<td>9–415</td>
</tr>
<tr>
<td>B cell</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte count, × 10⁶/L</td>
<td>7.1</td>
<td>0–7</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>78</td>
<td>0.15</td>
<td>0.07–0.32</td>
</tr>
<tr>
<td>Platelet count, × 10⁵/L</td>
<td>67</td>
<td>3.4</td>
<td>2.3–6.5</td>
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<tr>
<td>Cerebrospinal fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte count, × 10⁶/L</td>
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<td></td>
<td></td>
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<tr>
<td>Protein concn, g/L</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>3.4</td>
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</table>

*Common acute lymphoblastic leukemia-associated antigen.
up to age 15. A reference interval (mean ± 2 SD) of 20.0–92.4 μg/L, with a normal distribution of values, was obtained for subjects at ages 1.5–14.8 years, calculated for the children without convulsions. In gel filtration, the antigen in the CSF had the same molecular size as standard PICP (not shown), indicating an efficient processing of type I procollagen.

The children with ALL from whom a sample representing the time of diagnosis was available were all ages >1.5 years (Figure 1), and their initial PICP concentrations did not differ significantly from those of the control children (P = 0.059, Student’s t-test), although five of 22 CSF samples exceeded the reference interval.

The changes in PICP values in CSF during chemotherapy for ALL are shown in Figure 2. PICP concentrations were significantly higher during the second phase, methotrexate therapy, than at the time of diagnosis (P <0.01, Wilcoxon signed-rank test), a phenomenon observable during both treatment protocols. Most of the PICP values were well above the upper limit of the reference interval during this phase (Figure 2).

The effect of continuous corticosteroid treatment on PICP in CSF was monitored during the first (induction) phase in the standard-risk patients (Figure 2A) and later, during the third (re-induction) phase, in the intermediate- and high-risk patients (Figure 2B). Both of these treatment phases were associated with significant decreases in the CSF concentration of PICP (P <0.02 and <0.01, respectively; Wilcoxon signed-rank test). A similar decrease was seen in the intermediate- and high-risk patients during the first week of the induction therapy. The lowest median values obtained during these phases were close to the lower limit of the reference interval, indicating a marked repression of type I collagen synthesis. One-week pulses of corticosteroid treatment were given to the standard-risk patients at eight-week intervals during the third therapy phase, with intrathecal and systemic methotrexate in between, also at eight-week intervals. The PICP values observed here were similar to those observed during the second, methotrexate therapy, phase.

The PICP concentration did not correlate with the CSF cell counts or protein concentrations at the time when leukemia was diagnosed or during continuous corticosteroid therapy, but there was a slight correlation between PICP and the number of leukocytes in the CSF during the second phase, intravenous and intrathecal methotrexate therapy (P <0.05, r = 0.38, Spearman’s rank-correlation test). The highest PICP concentrations (>2380 μg/L) were seen in patients with simultaneous pleocytosis (cells ≥ 68 × 10^6/L). Only one patient repeatedly had PICP values >300 μg/L without any pleocytosis (leukocytes ≥5 × 10^6/L) in CSF during the methotrexate therapy phase. None of the patients had any findings that suggested encephalopathy.

The correlation between the PICP in CSF and protein during the second, methotrexate therapy, phase was P <0.02 (r = 0.42). The increase in PICP was generally greater than the increase in total protein (Table 2).

Our material included CSF samples from five patients at the time that CNS leukemia relapse was diagnosed. Their PICP values ranged from 143 to 630 μg/L. One of these children was in the phase of intravenous and intrathecal methotrexate therapy, whereas intrathecal therapy was no longer included in the treatment of the others or all leukemia therapy had already been stopped.

<table>
<thead>
<tr>
<th>Table 2. Median PICP (μg/L):Total Protein (mg/L) Ratios in CSF of Children with ALL</th>
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<tr>
<td></td>
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<tr>
<td>---------------------------------------------</td>
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<tr>
<td>At diagnosis</td>
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<tr>
<td>During continuous corticosteroid therapy</td>
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<tr>
<td>During methotrexate therapy</td>
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<tr>
<td>&lt;sup&gt;a&lt;/sup&gt; Induction therapy.</td>
</tr>
<tr>
<td>&lt;sup&gt;b&lt;/sup&gt; Re-induction therapy.</td>
</tr>
<tr>
<td>&lt;sup&gt;c&lt;/sup&gt; P &lt;0.02  &lt;sup&gt;d&lt;/sup&gt; P &lt;0.05; Wilcoxon signed-rank test for paired observations compared with the ratios before treatment.</td>
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</table>

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Discussion

PICP is released during the extracellular processing of type I collagen, and thus reflects the rate of synthesis of this matrix component in a manner analogous to the relationship between the C-peptide of proinsulin and endogenous insulin production. The interstitial concentration of PICP seems to reflect fibroproliferative activity readily, e.g., in healing surgical wounds (3), whereas its concentration in the circulation is dependent on the growth rate of the individual, with infants and small children having by far the highest values (18, 19). There is evidence that most of the PICP in blood is normally derived from metabolism in the bones, where type I collagen constitutes >90% of the organic matrix.

PICP is a globular protein with a molecular mass of ~100,000 Da, which cannot be expected to cross the blood–brain barrier. Thus its presence in the CSF is most probably derived from local collagen synthesis. The age dependency resembles that of PICP in blood, most probably reflecting the growth rate of surrounding collagen-rich tissues such as the meninges.

Most drugs for the systemic treatment of ALL do not penetrate the blood–brain barrier (20). Corticosteroids are an important exception, as are cytotoxic arabinoside and methotrexate to a limited extent. Additional intrathecal administration of the latter is nevertheless required to attain a therapeutically sufficient concentration in the CSF. Thus, we assumed that only the systemic corticosteroids and intrathecal methotrexate, among the drugs given to our patients with ALL, could affect the CSF.

Our finding of a relatively uniformly and specifically increased PICP concentration in the CSF during intrathecal methotrexate therapy suggests that at least some degree of arachnoiditis invariably develops after this therapy (Table 2). Also, the slight correlations observed between PICP and pleocytosis or the total protein content of the fluid agree with this. Although clinical or even chemical signs of arachnoiditis were not present in all the patients, there seems to have been enough irritation to cause a transient fibroproliferative response. Methotrexate can also affect the nervous tissue (21, 22), but the present series did not contain any cases of encephalopathy.

Corticosteroids have long been known to prevent fibroproliferative reactions and to reduce collagen synthesis (23). In our patients, continuous systemic treatment with corticosteroids was always associated with a low absolute (Figure 2) and relative (Table 2) concentration of PICP in the CSF, irrespective of the disease phase, whereas intermittent treatment between intrathecal methotrexate administration had no such effect. The PICP concentration in blood is also known to react to corticosteroid therapy within 12 h of onset and to be restored within a couple of days after cessation (I. Elomaa et al., University of Helsinki, unpublished). Some have suggested that corticosteroids lessen the severity of arachnoiditis (24, 25), and our observations agree with this. It is worth considering administering these drugs simultaneously with the intrathecal injections during phases of methotrexate therapy.

The results indicate that the concentration of PICP in the CSF acts as a sensitive marker of local collagen synthesis and fibroproliferation, processes that can be started and stopped by methotrexate and corticosteroids, respectively. PICP is nonspecific with respect to the cause of the process, as evidenced by the fact that CNS leukaemia may also increase the concentration of PICP in the CSF. Price and Johnson (26) detected arachnoid fibrosis in association with CNS leukaemia. The clinical importance of an ongoing fibroproliferative response naturally depends on the length of time in which it is active and on whether the connective tissue deposited remains at the site or is efficiently removed.

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References

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