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ISOTACHOPHORESIS AS A USEFUL TOOL FOR MONITORING NEUROLOGICAL COMPLICATIONS OF ACUTE LEUKAEMIA IN CHILDREN

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SUMMARY

Cerebrospinal fluid proteins from 42 children with acute lymphoblastic leukaemia were analysed by isotachophoresis. The isotachopherograms of cerebrospinal fluid taken from patients undergoing central nervous system prophylaxis with neurological complications showed an increase of several peaks (albumin, prealbumin, and an unidentified peak), and changes in the globulin zone, compared with those from patients who had completed central nervous system prophylaxis for at least six months. The most striking finding was that these alterations were not associated with any other biochemical changes in the cerebrospinal fluid, as assayed by routine analysis. Isotachophoresis may be useful in the monitoring of therapy in children affected with acute lyphoblastic leukaemia.

INTRODUCTION

Leukaemia of the central nervous system (CNS) is the most common form of extramedullary relapse. Clinical features, the cerebrospinal fluid (CSF) cell count, the presence of blast cells and biochemical changes, have to be considered in an assessment of a diagnosis of meningeal leukaemia. Furthermore, increased

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attention is being given to the importance of biochemical changes in the detection of the potential toxicity and side-effects of antileukaemia therapy.

Analytical studies of CSF glucose, enzymes, fibrinogen, etc. have not lead to satisfactory conclusions. Electrophoretic fractionation of CSF proteins has been shown to be important in the diagnosis in various diseases of CNS [1]. Recently, isotachophoresis (ITP) has been demonstrated to be useful in the diagnosis of nervous diseases, such as multiple sclerosis [2, 3]. ITP has a concentrating effect, owing to a discontinuous electrolyte system where the leading ion (electrolyte) has a higher net mobility than the terminating ion (electrolyte). All molecules with a proper charge and intermediate mobility added to the system, will be concentrated between the leading and terminating ions [4]. The purpose of this study was to determine whether it was possible to detect characteristic abnormalities in the isotachophoretic pattern of CSF, in the presence of CNS complications, during the course of acute lymphoblastic leukaemia (ALL), and their therapeutic approach.

MATERIALS AND METHODS

The following reagents were used: hydroxypropylmethyl cellulose (HPMC, Down Chemical); 2-morpholinoethanesulphonic acid (MES, Merck); 2-amino-2-methyl-1,3-propanediol (AMMEDIOL, Merck); 6-aminohexanoic acid (Merck); barium hydroxide (Merck); ampholine pH 7–11 (LKB); glycine (Canalco); valine (Merck); β-alanine (Merck).

Patients

One hundred CSF samples were obtained from 42 patients who had ALL. The patients were categorized into five groups.

Group I consisted of 21 patients who had completed CNS prophylaxis for at least six months. Among these, ten patients were off therapy. These patients have been treated with "Roma 72" [5] and 7601 [6] protocols (Table I).

Group II consisted of twenty patients undergoing CNS prophylaxis. CSF was obtained during prophylactic treatment that was carried out according to 7902 or 7903A protocols [6] (Table I). The former was used for the low-risk leukaemia, the latter for the high-risk leukaemia.

Group III consisted of two patients with clinically overt signs and symptoms of somnolence syndrome. They had received cranial irradiation (1800 rads) and multiple doses of intrathecal methotrexate (12 mg per week, for six weeks) for CNS prophylaxis.

Group IV consisted of six patients with meningeal leukaemia. These patients were not receiving therapy for active meningeal leukaemia when CSF was obtained. All had received cranial irradiation and intrathecal methotrexate as prophylaxis. All presented leukaemic pleocytosis at the time of the study.

Group V consisted of two patients with clinically overt signs and symptoms of leukoencephalopathy. They had received cranial irradiation and multiple doses of intrathecal methotrexate for CNS prophylaxis. One of them had presented the somnolence syndrome before the onset of leukoencephalopathy and the other one had had the somnolence syndrome and a measles infection two months before.

TABLE I

SUMMARY OF THERAPY REGIMES

Abbreviations: *n* = number of patients, VCR = vincristine; PDN = prednisone; i.t. MTX = intrathecal methotrexate; RT = radiotherapy; 6-MP = 6-mercaptopurine; ASP = asparaginase; TG = thioguanine; ARA-C = cytosine arabinoside, MTX = methotrexate; ADR = adriamycin; i.t. ARA-C = intrathecal cytosine arabinoside; DRB = daunorubicin; CPM = cyclophosphamide; HUR = hydroxyurea; BCNU = bis(β-chloroethyl)nitrosourea.

Protocol	*n*	Induction	Consolidation	CNS prophylaxis	Maintenance
ROMA 72	13	VCR + PDN i.t. MTX		i.t. MTX RT 2400 r	MTX + 6-MP VCR + PDN
7601	9	VCR + PDN i.t. MTX	ASP	i.t. MTX RT 2400 r 6-MP	MTX + PDN
7902*	10	VCR + PDN i.t. MTX	ASP ARA-C + TG ADR	RT 1800 r	MTX + 6-MP VCR + PDN
7903**	10	VCR + PDN + ARA-C + MTX i.t. MTX + i.t. ARA-C	ASP ARA-C + TG ADR	i.t. MTX + i.t. ARA-C	TG + CPM HUR + DRB BTX + BCNU ARA-C + VCR i.t. MTX

*Used for low-risk leukaemia.
**Used for high-risk leukaemia.

Serum preparation

Blood was taken from the children affected by ALL. Serum was prepared from venous blood after clotting (2 h at room temperature) and centrifugation for 10 min at 1500 *g* at 4°C. Serum samples were immediately frozen (−20°C), until the assays were performed. After 1 h of incubation at 22°C, the serum, for ITP analysis, was diluted 1:10 with distilled water.

CSF preparation

CSF (1 ml) was obtained from patients by lumbar puncture at the time of routine clinical examination or immediately before scheduled intrathecal injection of antileukaemic drugs. CSF from patients affected by meningeal leukaemia was centrifuged at 1500 *g* at 4°C for 10 min before ITP assay. From a single lumbar puncture, two samples of CSF were taken: the first was used for routine analysis and the second for ITP analysis. All CSF samples were routinely analysed by cell count in a Nageotte chamber without dilution with Turk solution, by blast cell investigation using a cytocentrifuge for glucose, by the colorimetric method reported by Werner et al. [7] for total protein, by a colorimetric method reported by Weichselbaum [8]. The presence of haemoglobin and bilirubin was ruled out by spectrophotometric analysis of the CSF. All samples that did not contain red cells and haematic pigments were immediately frozen at −20°C until ITP was performed.

Isotachophoresis

The apparatus used in this investigation was the LKB 2127 Tachophor (LKB, Bromma, Sweden). Separation was carried out in a PTFE capillary tube (23 cm × 0.5 mm I.D.), kept at a constant temperature of 12°C. The apparatus was equipped with a UV detector set at 280 nm. The leading

electrolyte was 5 m*M* MES—10 m*M* AMMEDIOL—0.5% HPMC, and the pH 9.1. No adjustment to the pH was carried out. The terminating electrolyte was 10 m*M* 6-aminohexanoic acid—10 m*M* AMMEDIOL, adjusted to pH 10.8 with barium hydroxide. The initial current setting on the instrument was 200 μA, maintained until a potential of 15 kV had been reached. The current was then reduced to 40 μA. During detection, under a constant current of 40 μA, the voltage rose from 5 to 16 kV. The UV gain was 2. The separation time was less than 50 min. The samples were 9 μl of unconcentrated CSF plus 2 μl of spacer solution and 3 μl of serum (already diluted 1:10 with distilled water) plus 2 μl of spacer solution. To detect the sample compounds efficiently, spacer substances are often needed [9]. Spacer solutions were: 1.6 mg of glycine plus 1.6 mg of valine plus 1.44 mg of β-alanine plus 0.3 ml of ampholine (pH 7—9) plus 0.18 ml of ampholine (pH 9—11) [10].

RESULTS AND DISCUSSION

Unconcentrated CSF from children in Group I showed the albumin peak and an unidentified protein that migrated very rapidly (Fig. 1). The latter, which is also found in normal CSF specimens [11], was present in greater amounts in the samples from patients undergoing CNS prophylaxis (Fig. 2A) and somnolence syndrome (Fig. 3). This increase, also reported to occur in CSF from patients with chronic meningoencephalomyelitis, seems to reflect CNS damage [11].

ITP of CSF from patients undergoing CNS prophylaxis according to the 7902 protocol (Table I) also showed an increase in the albumin content and the presence of peaks migrating before glycine. Only immunoglobulin (Ig)

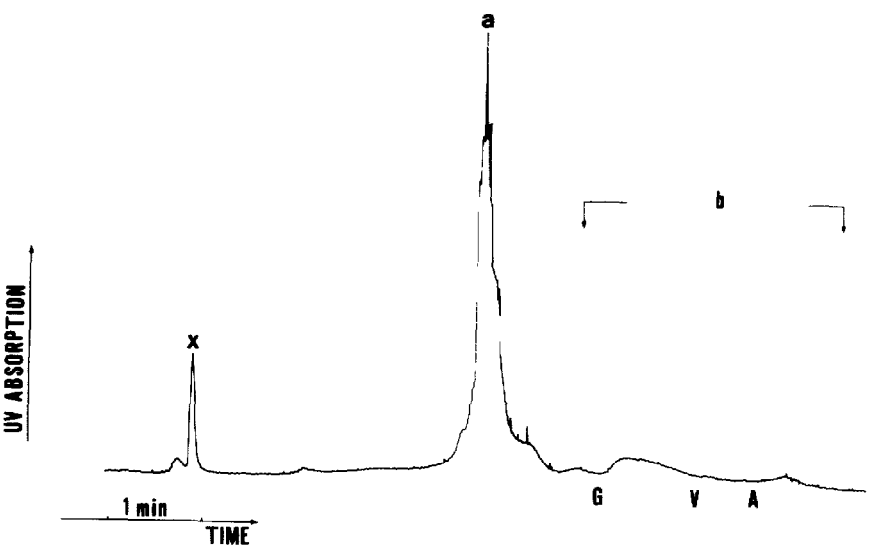

Fig. 1. Isotachopherogram showing the separation of CSF proteins, in the presence of spacer solution, from ALL patients off therapy. Peaks: X = unidentified; a = albumin; b = globulin zone; G = glycine; V = valine; A = β-alanine.

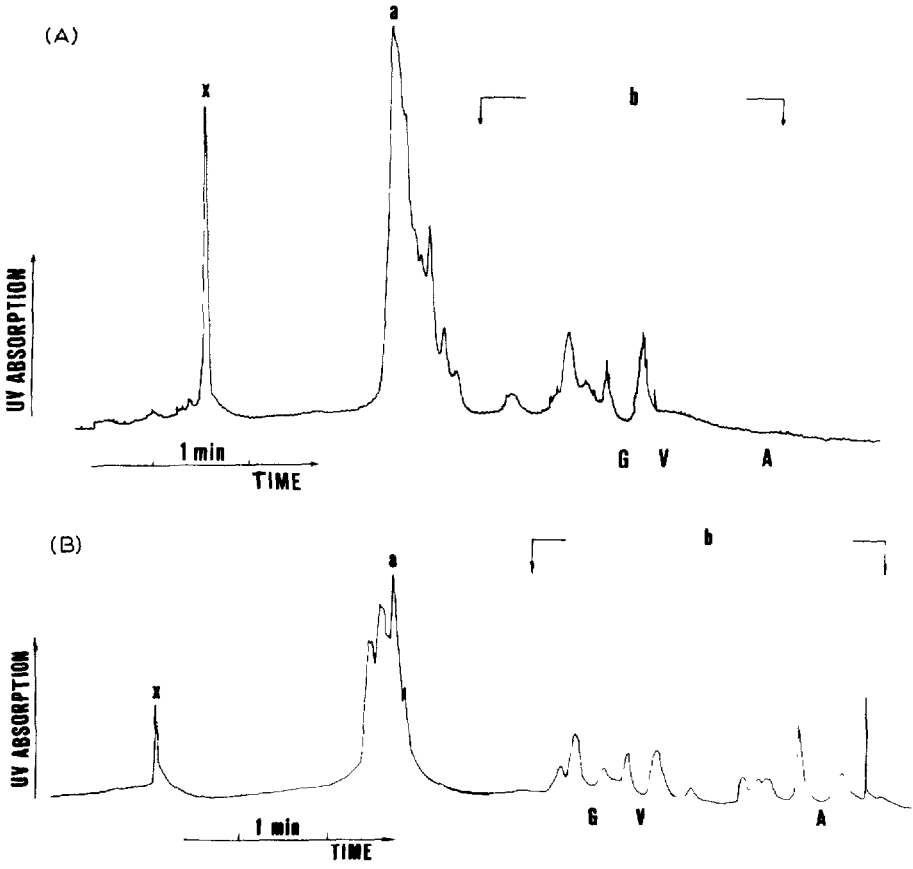

Fig. 2. ITP patterns of CSF from patients undergoing CNS prophylaxis, according to (A) protocol 7902, (B) protocol 7903A. For abbreviations, see Fig. 1.

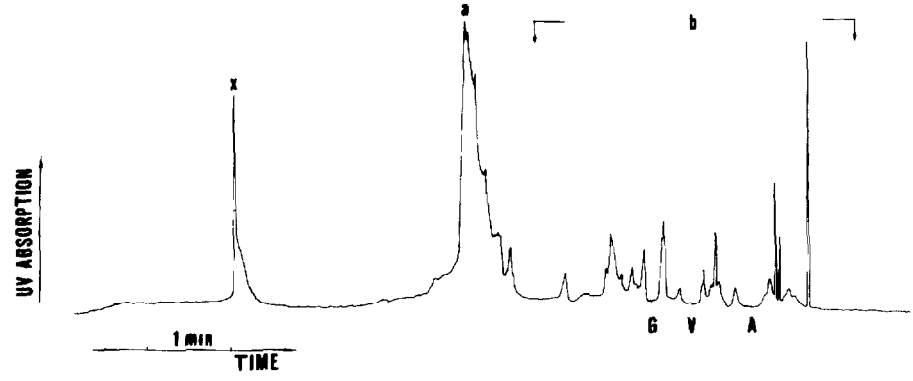

Fig. 3. ITP pattern of CSF from patients affected with somnolence syndrome. For abbreviations, see Fig. 1.

A appeared after the spacer [12] (Fig. 2A). This pattern suggests damage to the blood—brain barrier.

CSF from Group II patients, treated with the 7903A protocol (Table I), showed a change in the globulin zone after the amino acid valine (Fig. 2B). More marked alterations in this zone were observed in CSF from Group III patients (somnolence syndrome) (Fig. 3). The use of β-alanine allowed a separation into fast- and slow-moving globulins [13]. CSF specimens from patients with somnolence syndrome contained an increased percentage of slow-migrating IgG, corresponding to an IgG oligoclonal band in the high-alkaline region. It has been reported that this indicates an increased intrathecal

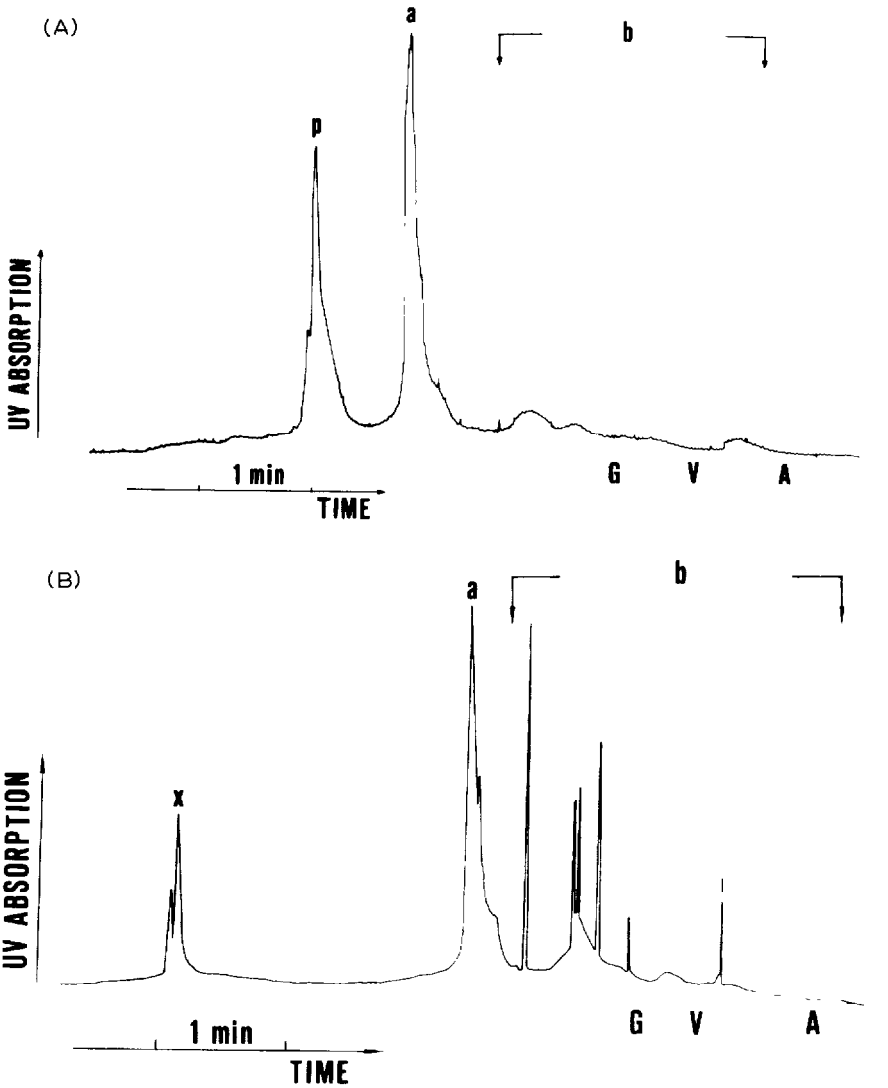

Fig. 4. (A) ITP trace of CSF from ALL patients affected with meningeal leukaemia, showing the presence of the prealbumin peak (p). (B) ITP trace of CSF from ALL patients showing an increase of the peak migrating before glycine. For abbreviations, see Fig. 1.

synthesis of IgG [2, 13]. Oligoclonal immunoglobulins have been found in several neurological disorders, accompanied by inflammatory reactions within the CNS, in patients with multiple sclerosis, and in cases of acute aseptic meningitis [14—18]. It has been reported that the somnolence syndrome may be an early indicator of permanent neurological damage, relative to the dosage of CNS irradiation [9, 20]. Our finding suggests that immunological processes, possibly following CNS prophylaxis, are involved in the pathogenesis of this syndrome [21].

ITP patterns of CSF from patients affected with meningeal leukaemia (Group IV) (Fig. 4A) showed the presence of prealbumin [22], which was not found in the serum. Similar patterns occurred in CSF from subjects with leuko-encephalopathy (Group V) (Fig. 5). In some Group IV patients more peaks were observed in the region between albumin and glycine (Fig. 4B). It is known that such an increase is found in the CSF of patients with neurological disease, perhaps owing to local synthesis [23]. It must be remembered that important acute-phase reactants migrate in the region between albumin and glycine, and that routine analysis of CSF from all patients was in the normal range. The six patients affected with meningeal leukaemia presented no CSF changes, except leukaemic pleocytosis.

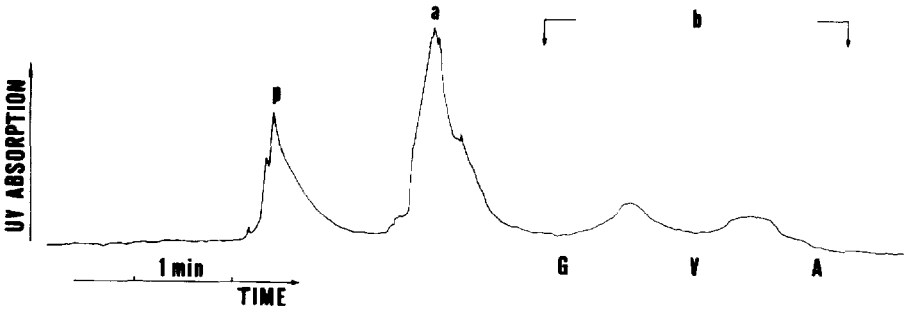

Fig. 5. ITP trace of CSF from ALL patients affected with leukoencephalopathy: note the enlarged prealbumin peak (p). For abbreviations, see Fig. 1.

In conclusion, ITP analysis could be a useful tool in the diagnosis of some CNS complications during ALL. In particular, it seems useful in the monitoring of CNS prophylaxis by intrathecal injections of antileukaemic drugs and/or irradiation [24]. It must be stressed that the presence of peaks other than albumin, in unconcentrated CSF, suggests pathological processes. ITP seems to be important in the investigation of possible markers of CNS damage (due to ALL per se or to the therapy), and of the pathogenesis of CNS complications. The advantages of analytical ITP are that very small samples of unconcentrated CSF can be examined in a short time (less than 50 min) and the results are immediately obtained on a recorder. Low- and high-molecular-weight compounds can be analysed. The method gives high resolution, is reproducible and easy to perform.

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