Abnormal alkaline phosphatase of hepatic type in cerebrospinal fluid of a patient with intracranial metastasis from lung cancer

T Hoshino, K Kumasaka, K Kawano, I Koyama, Y Arai-Fujimori, F Yamagishi, Y Sakagishi, T Komoda

Abstract
High alkaline phosphatase (ALP) activity was found in the cerebrospinal fluid of a patient with intracranial metastases from adenocarcinoma of the lung. On agarose gel electrophoresis of the major ALP isoenzyme found in the cerebrospinal fluid, its mobility was different from those of the usual serum ALP isoenzymes. This abnormal mobility might be due to the linked glycan phosphatidylglycerol anchor in the ALP molecule, as the mobility became the same as that of the common liver type ALP after treatment with phosphatidylglycerol specific phospholipase. The immunochemical antigenicity of the cerebrospinal fluid ALP was identical with that of the common serum liver type ALP, but its sugar moiety was similar to the membranous liver-type ALP rather than the serum liver type ALP. The molecular size of the cerebrospinal fluid ALP was 140 kD, 12 less than the common serum liver type ALP, suggesting that the ALP in the patient's cerebrospinal fluid was derived from the intracranial metastatic carcinoma.

Methods
Serum and cerebrospinal fluid samples from the patient were kept at -80°C until use. ALP activity was determined using a Hitachi model-736 autoanalyzer (Hitachi Co. Ltd., Tokyo, Japan) at 37°C using an assay kit (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) with 9-8 mM 4-nitrophenylphosphate and 0-16 mM MgCl₂ in 0-81 M diethanolamine–HCl buffer (pH 10-0).

For agarose gel electrophoresis, Gel 8 for ALP isozymes and an ALP high resolution staining kit were obtained from Ciba Corning Diagnostics (Palo Alto, California, USA). Procedures for electrophoresis and ALP staining of the gel plate were performed according to the manufacturers' instructions. To remove the glycan-phosphatidylglycerol (GPI) anchor moiety in the ALP molecule, the ALP sample was treated at room temperature for 3 hours with 0-1 U/ml of phosphatidylglycerol specific phospholipase C (PIPLC, EC 3.1.4.10, Funakoshi Pharmaceutical Co., Tokyo, Japan), as reported before. The resulting aliquot specimen was used as a sample for the electrophoresis.

To determine ALP antigenicity, the ALP sample (9 µl) was pre-incubated for 1 hour at room temperature with 1 µl of monoclonal antibodies against liver, bone, term placental, and adult intestinal ALP. Subsequently, the resulting ALP isozyme-anti-ALP antibody complex in the incubation mixture was electrophoretically isolated.

Twenty microlitres of the ALP sample were incubated overnight at 4°C with 2 µl of monoclonal antibodies against liver, bone, term placental, and adult intestinal ALP. The mixture was then incubated with protein A (BDH Chemicals, Ltd, Dorset) and left to stand for 1 hour at room temperature, and centrifuged at 2000 × g for 5 minutes. The supernatant fluid was assayed for ALP activity. A control assay was carried out using saline in place of the monoclonal antibodies.

The sugar chain heterogeneity of the ALP isozymes was isolated by Concavalin A,
phytohaemagglutinin E, pea seed agglutinin and wheat germ agglutinin affinity chromatographies according to the method described before. The yields of the enzyme activity from the respective lectin columns were more than 90%.

For determination of molecular size, the enzyme sample was treated for 3 hours with 0·1 U/ml PIPLC and 0·1 U/ml neuraminidase. The resulting aliquot was run on 5% polyacrylamide gel with 0·1% sodium dodecyl sulphate (SDS) under non-reducing conditions. The enzyme active band was detected by 5-bromo-3-indolyl phosphate p-toluidine salt containing 10 μM of Zn(CH₃COO)₂ and 1 mM MgCl₂. The molecular size was estimated from relative mobility using standard molecular size markers (Pharmacia, Uppsala, Sweden).

**Results**

The serum ALP activity of the patient ranged from 289 to 598 U/l (our reference range: 80–287 U/l). ALP activity in the cerebrospinal fluid of this patient ranged from 102–287 U/l, outside the reference range (<5 U/l) derived from the data obtained in 100 patients with meningitis or other non-neoplastic diseases and calculated using non-parametric statistics.

The figure shows that the ALP isozymes in the patient’s cerebrospinal fluid on agarose gel electrophoresis were found at the α2β-globulin region with a tailing phenomenon. Treatment of cerebrospinal fluid with PIPLC shifted the mobility to that of the common liver type ALP.

In the electrophoretic separation of ALP isozymes preincubated with their respective monoclonal antibodies, the abnormal ALP isozyme in the patient’s serum and cerebrospinal fluid reacted mainly with the monoclonal antibody against liver ALP isozyme. Furthermore, the results of immunoprecipitation indicated that the major type of ALP in the patient’s serum and cerebrospinal fluid was liver type ALP. The percentage of liver type ALP in the patient’s cerebrospinal fluid was similar to that normally found in liver tissue (table).

From its apparent molecular size, the ALP in the patient’s cerebrospinal fluid was estimated to be 140 kilodaltons equal to that of human duodenal ALP, and unlike human liver (152) and bone (160) ALP.

**Discussion**

As the ALP in the cerebrospinal fluid of the present case exhibited abnormal isozyme patterns on agarose gel electrophoresis, the organic origin of the patient’s ALP could not be identified by its electrophoretic mobility compared with the control ALP isozyme markers (figure). Following treatment with PIPLC, the ALP from the patient’s cerebrospinal fluid returned to normal values and shifted to the common liver type ALP, suggesting that the abnormal ALP on the isozyme pattern was attributable to the existence of a GPI anchor in ALP. This abnormal ALP in the cerebrospinal fluid is thought to be derived from live or necrotising cancer cells which can secrete or liberate the ALP molecules, with the GPI anchor being released directly into the cerebrospinal fluid. It is intriguing to speculate whether phospholipase D, which is usually present in healthy serum, might not occur in the cerebrospinal fluid of this patient.

With immunological identification made possible with monoclonal antibodies, it was confirmed that the ALP in the patient’s cerebrospinal fluid was liver-type ALP, and the major ALP isozyme estimated by immunoprecipitation was also the same as that in liver. Furthermore, the sugar moiety of ALP from the patient’s cerebrospinal fluid, as determined by serial lectin affinity chromatography, was also similar to that of liver tissues (data not shown).

Several tumour associated ALPs have been described. These are particularly found with breast and gastrointestinal carcinomas, but there has been one report of a serum liver type ALP in a patient with malignant schwannoma of a peripheral nerve. The ALP in this was derived from the liver rather than the schwannoma.

The reason for the discrepancy in molecular size between the ALP samples from the patient’s cerebrospinal fluid and liver tissues may derive from an alternative processing of ALP with respect to protein or sugar chain concentrations as reported for the tumour associated ALPs expressed ectopically in certain tumours. Murakami et al reported...
that liver-type ALP was produced in a patient with meningioma, but they did not find an abnormal ALP value. In addition, we did not find--abnormal ALP values in 100 patients with non-neoplastic intracranial diseases.

Taken together, our data suggest that the abnormal ALP found in the cerebrospinal fluid from this patient was liver-type ALP produced by intracranial cancer cells which had metastasised from an alveolar lung adenocarcinoma.

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Cholecystitis, cholelithiasis, and ganglioneuromatosis of the gall bladder: an unusual presentation of MEN type 2b

R Chetty, S P Clark

Abstract
A 40 year old man with multiple endocrine neoplasia type 2b (MEN 2b) presented with cholecystitis caused by gall stones. Twenty four years earlier, he had had a partial thyroidecotmy for a cold nodule. At his initial presentation, MEN 2b with medullary carcinoma of the thyroid had not been made. This was diagnosed while investigating his gall bladder symptoms and he was found to have asymptomatic residual medullary thyroid carcinoma and bilateral adrenal phaeochromocytomas. The cholecystectomy specimen contained several mixed calculi and extensive ganglioneuromatosi with large, prominent nerves containing ganglion cells in the gall bladder wall.

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Neuronal hyperplasia or ganglioneuromatosis are well recognised components of multiple endocrine neoplasia type 2b (MEN 2b)\(^2\) and neurofibromatosis.\(^3\) Ganglioneuromatosis of the gastrointestinal tract often precedes the appearance of medullary thyroid carcinoma and phaeochromocytoma in MEN 2b.\(^4\) Within the context of MEN 2b, presentation related to gall bladder disease is rare. A case of MEN 2b is presented in which the patient complained primarily of symptoms related to gall bladder disease.

Case report
The patient was a 40 year old man who at the age of 16 years had had a partial thyroidecotmy for a cold nodule. This was interpreted to be a Hürthle cell carcinoma at the time. Twenty four years later, he complained of right upper quadrant pain, dyspepsia, and flatulence. Examination showed him to be hypertensive (although he did not complain of hypertension) and to have right upper quadrant tenderness. He was also noted to have a lump in his residual thyroid and bilateral loin masses. A computed tomogram revealed an enlarged gall bladder filled with calculi, a tumour in the residual thyroid, and bilateral adrenal medullary tumours. Subsequent examination showed mucosal neuromas of the lips, tongue, larynx and cornea. Furthermore, the patient was noted to have a marfanoid feature. MEN 2b was diagnosed and a detailed review of the patient’s history disclosed a positive family history with the patient’s mother having bilateral pheocho-