Neuron specific enolase (NSE) and thymidine kinase (TK) as markers in biological fluids of brain tumor patients


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We studied the activity of two enzymes NSE and TK in the biological fluids of 104 patients with nervous system diseases, who fell into 4 groups. 20 subjects out of 35 in the tumor group had glial tumors. We fixed a cut-off value of NSE and TK activity at the 95th percentile of the control group, both in serum and in CSF. The aim of our investigation was to assess the reliability of TK and NSE assays in separating brain tumors from other neurological diseases. In our patients, most of the TK activity above the cut-off value was found in the tumor group. Serum TK seems to be a useful marker for following up cerebral tumors after surgery, but NSE is less useful for this purpose.

Key-Words: Thymidine kinase — neuron specific enolase — markers — brain tumor

Introduction

Extensive investigation has been done to establish the reliability of biochemical markers in detecting damage within the CNS, or in detecting neoplastic processes in the CNS. The clinical significance of enzymes in CNS in detecting neurological abnormalities is still poorly defined; at present no specific biochemical markers of damage are known. We studied activity of two enzymes, NSE and TK. They have both been detected in the biological fluids of patients with CNS diseases. These specific enzymes are released into biological fluids when nervous tissue is damaged and so might be possible markers for injury of the nervous system [17, 18, 12, 4, 14]. Enolase is dimeric cytoplasmic enzyme of molecular weight 90000 which catalyses the interconversion of 2-phospho-d-glycerate and phosphoenolpyruvate in the glycolytic pathway. The enzyme has three immunologically distinct subunits and is widely distributed in mammalian tissue. Neuron specific enolase (NSE) is mainly localized in neuronal and neuroendocrine cells of central and peripheral NS. Normally the enzyme is detectable histochemically in neurons and APUD (amine precursor uptake and decarboxylation) system cells [21, 13, 2, 33, 27, 7, 8]. It is doubtful that NSE is a neuron specific protein, since it is also present in neoplastic cells that are not of neuronal lineage nor of the APUD system. Immunohistochemical demonstrations suggest finding NSE also in glial tumors. The enzyme was found in cultured cell lines of anaplastic glioma and astrocytomas of rat and human origin [24]. NSE was also found in primary glial tumor [25, 23, 18], or together with glial fibrillary acid protein (GFAP) within some glial cells [26]. Therefore we know that NSE occurs even in brain glial tumors. As regards TK activity, we know that different isoenzymes of TK appear in different kinds of cells. The cytosolar enzyme TK-F is present only in proliferating cells, including tumor cells where it represents most of TK ac-
tivity [1]. TK is an enzyme of the pyrimidine metabolism, in which it catalyses the phosphorylation of thymidine that is essential for the incorporation of thymidine into DNA. TK activity is increased mostly in patients with hematological and viral diseases, as well as in vitamin B12 deficiency [3, 10, 5, 9, 6]. But it can be measured in CSF of patients with kinds of primary and secondary brain tumors also [4]. The highest values were found in the most rapidly growing tumors [15] CSF samples taken before and after treatment showed that TK activity rapidly declined after intrathecal chemotherapy and irradiation as well as after surgery [15].

The aim of our investigation was to assess the reliability of NSE and TK assays in the CSF and serum in separating brain tumors of non-neuronal lineage from other neurological diseases.

We limited our investigation to tumor patients already treated surgically.

Patients and method

CSF and blood samples were collected from 104 inpatients who underwent lumbar puncture for diagnostic purposes and entered this study. We have evaluated the NSE and TK activity in serum and in CSF in four groups of patients age and sex matched.

The patients were grouped as follows:

**Group 1**) healthy subjects: 29 cases in all, mostly undergoing myelography for suspected lumbar disc disease. We may consider these patients as healthy in respect of CNS diseases, because TK activity was barely detectable. They served to establish an upper normal limit of TK activity.

**Group 2**) patients with cerebral tumors: 35 cases in all, 30 with primary and 3 with secondary cerebral tumor, and 2 with disseminated systemic malignancy without known brain metastases. There were 20 bearing glial tumors of which: 4 were differentiated, 7 were anaplastic astrocytomas and 9 were glioblastomas. All tumor patients had been treated surgically prior to the study.

**Group 3**) patients with inflammatory diseases: 25 cases in all, mostly demyelinating: 10 with multiple sclerosis, 7 with Guillain Barré syndrome, 2 with myelitis, 1 with encephalitis and the others were miscellaneous.

**Group 4**) patients with degenerative diseases: 15 cases in all: 5 with ALS (amyotrophic lateral sclerosis), 3 with olivo-ponto-cerebellar atrophy, 3 with dementia, 2 with acquired hydrocephalus, and the others were miscellaneous. None of our patients had hematologic disease, such as anemia or granulocytosis. Concurrent viral disease was ruled out.

TK ASSAY - CSF and serum TK levels were assessed by a sensitive radioenzymatic assay (Prolifigen TK-REA, AB Sangtec medical, Bromma Sweden). Briefly, specimens were added to i²¹² labelled substrata for TK (iododeoxyuridine). After a 4 hr incubation, the reaction was stopped by a separator tablet with high affinity for the phosphorylated product. Then the separator was washed, and the associated radioactivity measured in a gamma-counter. TK activity was calculated by linear regression from a standard calibration line and expressed as units/l. Assay detection limit was 5 U/l.

NSE ASSAY - A high sensitive double antibody radioimmunoassay (NSE RIA Pharmacia diagnostic AB Uppsala Sweden) was performed on CSF and serum specimens. NSE levels are expressed as ng/ml. Assay detection limit was 12.5 pg/ml.

Enzyme levels are expressed as mean ± SD. Statistical analysis was carried out with the Mann Whitney U and Wilcoxon Rank Sun W test. Enzyme efficiencies in discriminating between groups were assessed by the Chi Square test.

Results

To evaluate our data we fixed a cut off value at the 95th percentile of the control group (CSF TK and NSE, 2.020 U/l and 17.19 ng/ml respectively; Serum TK and NSE, 6.92 U/l and 12.7 ng/ml, respectively). Our observed serum 95th percentile levels are slightly higher than those indicated by previous studies [20, 16] (i.e. 5 U/l TK and 12.5 ng/ml NSE). Thus our criteria for determining pathological values are more restrictive.

Levels greater than the cut-off value were considered pathological; they are at least two SD above the mean of controls. Linear regression analysis was applied to assess fitting with a normal distribution of the observed TK and NSE levels in the control group. According to cumulative probability plots, CSF (Fig. 1A) and serum (Fig. 1B) TK values follow a normal distribution in the control group, since the multiple standardized correlation coefficient (R) is highly significant (0.940 and 0.953 respectively; p < 0.0001). If data grouping had a main role in determining the results, an asymmetry about the modal value would have been fairly clearly shown by the plot.

In tumor patients, the enzyme levels fall perfectly on the regression line, in relation to percent of the cumulative probability; and these patients exhibit the same pattern over a wide range of values. When average TK or NSE levels in serum and CSF were considered, a significant difference was noted only in TK activity between tumor patients and these with all other pathologies combined (12.08 ± 22.5 vs. 4.54 ± 2.85, respectively; p < 0.0001).

No statistically significant difference was found.