Eur J Cancer Clin Oncol, Vol. 20, No. 2, pp. 253–259, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Glycolytic Enzymes from Human Neuroectodermal Tumors of Childhood

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Abstract—*In this study pyruvate kinase, hexokinase and aldolase are investigated in two types of embryonal tumors, neuroblastomas and medulloblastomas; the results are compared with similar studies in gliomas. The activities of hexokinase and pyruvate kinase are significantly decreased in neuroblastomas. In neuroblastoma and medulloblastoma all five forms of pyruvate kinase (K_4, K_3M, K_2M_2, KM_3 and M_4) are present. In contrast, the gliomas investigated are characterized by the presence of mainly K_4 and a little K_3M. In neuroblastomas, medulloblastomas and gliomas, hexokinase type* I *is present; in addition, hexokinase type* II *is present in two medulloblastomas. Aldolase A is the predominant isozyme in all tumors investigated; this is in contrast with normal nervous tissue. It can be concluded that the isozyme characteristics especially of pyruvate kinase from neuroblastomas and medulloblastomas are comparable with similar findings in retinoblastoma; these findings support the hypothesis that these three tumors have a common embryonic origin.*

INTRODUCTION

RECENTLY we presented the results of characterization of the glycolytic enzymes pyruvate kinase, hexokinase and aldolase from normal human retina (both fetal and adult) and retinoblastoma [1]. Retinoblastomas belong to the so-called embryonal neuroectodermal tumors [2]. Other embryonal tumors from neuroectodermal origin are neuroblastoma and medulloblastoma [3]. There is experimental support to the hypothesis that medulloblastoma is a stem cell neoplasm [2], just like neuroblastoma. There are remarkable histological similarities between neuroblastoma and retinoblastoma too, such as the so-called (pseudo-)rosettes [4].

It is well known that, as in other tumors [5–8], in brain tumors alterations in glycolytic enzymes can be found [9], especially in regulator enzymes such as pyruvate kinase and hexokinase. Fetal brain is characterized by the presence of all five forms of pyruvate kinase (K_4, K_3M, K_2M_2, KM_3 and M_4), whereas in poorly differentiated gliomas of adults mainly K_4 and a little K_3M are present [9]. Retinoblastomas are characterized by the presence of all the forms except M_4 [1]. This may be caused by a different embryological origin of these tumors. To give more evidence to this hypothesis, we investigated some glycolytic enzymes in neuroblastomas, medulloblastomas and gliomas of childhood. We compared the data with the results found earlier in another neuroectodermal tumor, i.e. retinoblastoma [1].

Accepted 11 July 1983.
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MATERIALS AND METHODS

Patients

Nine neuroblastomas (three classified as poorly differentiated neuroblastomas and six as more or less ganglioneuroblastomas) of eight patients, four medulloblastomas and eight gliomas of childhood were studied. The data of these patients are summarized in Table 1.

Sample preparation

Neuroblastomas, medulloblastomas and gliomas were stored immediately after surgery at

Table 1. Data of the patients studied

Patient number	Age at diagnosis (yr/months)	Sex (M/F)	Histology
Neuroblastoma			
1*	5/-	M	Neuroblastoma
2*	6/-	M	Neuroblastoma
3	-/3	M	Neuroblastoma
4	-/10	F	Ganglioneuroblastoma
5	1/4	M	Ganglioneuroblastoma
6	10/-	F	Ganglioneuroblastoma
7	1/-	M	Ganglioneuroblastoma
8	-/2	M	Ganglioneuroblastoma
9	6/-	M	Ganglioneuroblastoma
Medulloblastoma			
1	5/3	M	Medulloblastoma (cerebellar)
2	5/4	M	Medulloblastoma (cerebellar)
3	7/1	F	Medulloblastoma (cerebellar)
4	10/9	F	Medulloblastoma (cerebellar)
Glioma			
1	7/2	M	Ependymoma
2	13/2	F	Pilocytic astrocytoma
3	17/8	F	Astrocytoma
4	5/2	M	Ependymoma
5	1/6	F	Ependymoma
6	7/2	M	Ependymoma
7	6/7	M	Optic glioma
8	-/9	F	Anaplastic glioma

Patient 1* and 2* is the same patient operated on two occasions.

-70°C. Tissues were homogenized by mincing one part with five parts of extraction buffer, containing 50 mM Tris-HCl, pH 8.0, 0.1 M KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol and 0.1 M sucrose. The mixture was rapidly minced (maximum duration 1 min in a pottermincer). After centrifugation at 15,000 *g* for 10 min, the clear supernatant was used for the experiments; when the enzyme preparation was stored (at -70°C) sucrose was added up to a final concentration of 0.5 M (storage had no influence on the enzyme activities and isozyme profiles).

Electrophoresis

Pyruvate kinase. The extracted enzymes were diluted to an activity of about 1.0 U/ml in the electrophoresis buffer containing 20 mM Tris-citrate (pH 7.7), 1 mM fructose-1,6-diphosphate, 1 mM disodium EDTA and 0.05 mM dithiothreitol. Electrophoresis and staining for pyruvate kinase activity was carried out as previously described [10].

Scanning of electropherograms

The relative intensities of the bands in the electropherogram were quantitated at 540 nm with a densitometer (Helena Quickscan). The percentage of K- and M-subunits, respectively, were calculated assuming: (a) a subunit distribution as indicated by the suffix in K_4, K_3M, K_2M_2, etc., and (b) equal contribution of K- and M-subunits to the intensity of the stain.

Aldolase. Electrophoresis was performed on cellulose acetate in a 0.04 M sodium-phosphate buffer (pH 7.0). The gels were run at room temperature and 10 V/cm during 1.5 hr. Staining and scanning was performed as previously described [1].

Hexokinase. Electrophoresis on cellulose acetate was carried out at 4°C and 20 V/cm (± 2 mA/strip) during 45 min in a Tris-Veronal buffer (Gelman High-Resolution buffer) of pH 8.8 (I 0.05 mol/l) to which 2 mM glucose, 0.05 mM dithiothreitol and 1 mM EDTA were added. Staining for hexokinase activity was carried out as previously described [1].

Assay for glycolytic enzymes

Glycolytic enzyme activities were determined by the methods of Beutler [3] at 37°C and expressed as U/mg protein. The protein content was determined by the method of Lowry *et al.* [11] with crystalline bovine serum albumin as standard. Alanine inhibition of pyruvate kinase was determined as previously described [10].

Chemicals

Substrates and auxiliary enzymes for determination of enzyme activities were obtained from Boehringer Mannheim, F.R.G. All other chemicals were of the highest purity available.

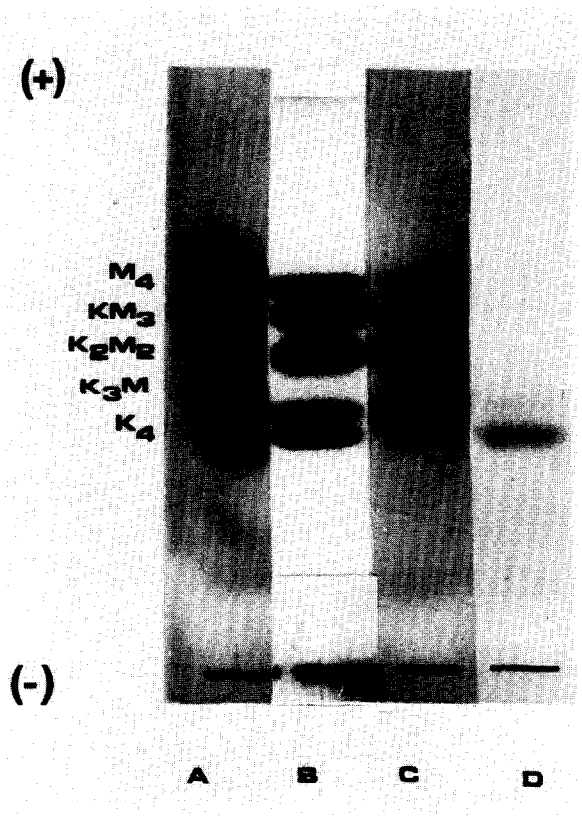

Fig. 1. *Combined electrophoretic pattern of pyruvate kinase extracted from hypophysis (A = reference), neuroblastoma (B), medulloblastoma (C) and glioma (D).*

RESULTS

Enzyme activities

Table 2 summarizes the activities of some glycolytic enzymes from neuroblastomas ($n = 9$), medulloblastomas ($n = 4$) and gliomas of childhood ($n = 9$). Some activities of non-key enzymes of glycolysis in medulloblastomas, i.c. phosphoglucose isomerase, triosephosphate isomerase and enolase, are significantly increased with respect to neuroblastomas and gliomas. The activities of the key enzymes hexokinase and pyruvate kinase are significantly decreased in neuroblastomas. Within the group of neuroblastomas no significant differences are found between the poorly differentiated neuroblastomas ($n = 3$) and the more differentiated ganglioneuroblastomas ($n = 6$). It should be noted that the activities shown in Table 2 concern only the cytosolic enzymes.

Pyruvate kinase. The isozymes of pyruvate kinase and their hybrids are according to Ibsen [12] designated as M_4, KM_3, K_2M_2, K_3M and K_4. Figure 1 shows the electrophoresis of pyruvate kinase from one representative neuroblastoma, one medulloblastoma and one glioma. Neuroblastomas are characterized by the presence of all the five forms of pyruvate kinase (K_4, K_3M, K_2M_2, KM_3 and M_4) and medulloblastomas show all forms also (little K_4). In contrast, gliomas are characterized by mainly K_4 and a little K_3M [13]. The percentages of K subunits are calculated from electropherograms by scanning (see Materials and Methods) and summarized in Table 3. Although neuroblastomas contain less K subunits than medulloblastomas ($P < 0.02$), both are strikingly different from gliomas, in which K is by far the predominant subtype. The residual activities of pyruvate kinase in the presence of alanine are in good agreement with this observation (Table 3).

Neuroblastomas and medulloblastomas show relatively high residual pyruvate kinase activity in the presence of alanine, in contrast to the gliomas.

Hexokinase. Four isozymes of hexokinase are known, designated as I–IV in order of their increasing anodal electrophoretic mobility. The electrophoretic results of neuroblastomas, medulloblastomas and gliomas are summarized in

Table 2. Activity of glycolytic enzymes in neuroblastoma, medulloblastoma and glioma. The activities are expressed as U/mg *protein; the values are mean ± S.D.*

Enzyme	Neuroblastoma $n = 9$	Medulloblastoma $n = 4$	Glioma $n = 8$
Hexokinase	0.036 ± 0.023 (<0.02)	0.077 ± 0.042	0.071 ± 0.041
Phosphoglucose isomerase	1.25 ± 0.54	2.69 ± 0.48 (<0.001)	1.56 ± 0.034
Aldolase	0.069 ± 0.048	0.072 ± 0.013	0.065 ± 0.023
Triosephosphate isomerase	25.0 ± 13.7	73.8 ± 63.5 (<0.05)	26.1 ± 10.2
Glyceraldehyde phosphate-dehydrogenase	1.64 ± 0.95	1.91 ± 1.32	2.04 ± 0.90
Phosphoglycerate kinase	1.93 ± 0.62	3.04 ± 1.61	2.19 ± 0.72
Enolase	0.24 ± 0.10	0.52 ± 0.20 (<0.05)	0.31 ± 0.15
Pyruvate kinase	1.26 ± 0.84 (<0.003)	3.46 ± 1.46	3.02 ± 1.41

The values in parentheses indicate the significance of the differences (P-value, Student's t-test).

Table 3A. The percentage of K *subunits and the residual activity of pyruvate kinase in the presence of alanine; values are mean ± S.D.*

	Neuroblastoma $n = 9$	Medulloblastoma $n = 4$	Glioma $n = 8$
% K subunits	35 ± 11	47 ± 7	85 ± 6
Residual activity of pyruvate kinase in the presence of 4 mM alanine (%)	74 ± 11	53 ± 7	7 ± 4
Electropherogram	K_4, K_3M, K_2M_2, KM_3, M_4	(K_4), K_3M, K_2M_2, KM_3, M_4	K_4, K_3M

Table 3B. Hexokinase and aldolase isozyme distribution in neuroblastoma, medulloblastoma and glioma

	Neuroblastoma $n = 9$	Medulloblastoma $n = 4$	Glioma $n = 8$
Hexokinase	type I	type I + II	type I + II (trace)
Aldolase			
% of A subunits (mean ± S.D.)	88 ± 6	85 ± 15	72 ± 14

Table 3B. Neuroblastomas are characterized by the presence of hexokinase type I; little or no hexokinase type II could be detected. Medulloblastomas show besides hexokinase type I also type II in two of the four tumors investigated (17 and 57%, respectively). The gliomas show a predominance of type I hexokinase and only a trace amount of hexokinase type II.

Aldolase. Three isozymes are known of this tetrameric enzyme. The isozyme A_4 is predominant in adult skeletal muscle and most other tissues while the B_4 isozyme is confined to the liver and kidney. Aldolase C_4 is the principal form in nervous tissue. In this tissue, five hybrids of the A and C isozymes are found (A_4, A_3C, A_2C_2, AC_3, C_4).

In contrast to normal nervous tissue, the main isozyme of aldolase in neuroblastomas and medulloblastomas as well as gliomas is the A_4 type. The percentage of A subunits as calculated from densitograms are summarized in Table 3B.

DISCUSSION

Recently we reported the characterization of pyruvate kinase, hexokinase and aldolase from normal fetal and adult retina and retinoblastoma [1]. In this paper we report the characterization of the same enzymes from neuroblastomas, medulloblastomas and gliomas.

Neuroblastomas and medulloblastomas have much in common with retinoblastomas [4]. All three tumors show histological similarities, e.g. partial or complete rosette formation and more or less necrosis and calcification. All three types of tumors are characteristically childhood tumors and claimed to be of neuroectodermal origin [4]. Because of these common findings we compared the results of the enzymological investigations as found in retinoblastomas [1] with those in neuroblastomas and medulloblastomas. We compared the results in these three neuroectodermal tumors as a whole with those found in gliomas of childhood, e.g. tumors of the supporting tissue of the central nervous system.

From the activities of the glycolytic enzymes shown in Table 2, one may conclude that differences exist between the three kinds of tumors. However, due to the large heterogeneity of the enzyme activities within the individual tumor groups, these differences are probably not of diagnostic value.

Secondly, we characterized the isozyme patterns of pyruvate kinase, hexokinase and aldolase in retinoblastomas, neuroblastomas and medulloblastomas as compared with gliomas of childhood. With respect to pyruvate kinase one may conclude that the observed isozyme pattern in neuroblastomas is similar to that in medulloblastomas and identical to that earlier found in retinoblastomas [1]. In neuroblastomas all potential five forms (K_4, K_3M, K_2M_2, KM_3 and M_4) are present as in medulloblastomas; the M subunits are slightly predominant. This isozyme pattern is different from that in the gliomas of childhood (mainly K_4 and a little K_3M, but no other forms) and from adults [9].

The residual activity of pyruvate kinase in neuroblastomas and medulloblastomas in the presence of alanine is comparable to that in retinoblastomas [1] and in striking contrast with the very low residual activity observed in the gliomas [13].

There is an ontogenic evolution of pyruvate kinase isozymes in brain. Van Veelen *et al.* [13] demonstrated that the K-subunit is present in greater amounts in fetal than in adult human brain. With maturation a shift in isozyme composition occurs to an adult pattern in which M_4 is predominant with little or no K_4 and K_3M. In fetal human brain of 12 and 16 weeks, both type M, type K and the three hybrids can be detected. The same pattern is found in neuroblastomas, retinoblastomas [1] and medulloblastomas. Therefore the pyruvate kinase isozyme distribution in these tumors resembles that found in fetal brain and fetal retina. For neuroblastomas, however, we cannot conclude that the observed isozymes pattern resembles that of the fetal tissues because we are not informed about the pyruvate kinase isozyme pattern of the tissue from which the tumors originate. Nevertheless the isozyme composition of pyruvate kinase in neuroblastomas, medulloblastomas and retinoblastomas is different from that found in gliomas of childhood.

In neuroblastomas and gliomas almost no hexokinase type II is found: type I is largely predominant. In medulloblastomas, type I and II are found, as was shown earlier in retinoblastomas. All three kinds of tumors show about the same isoenzyme pattern of aldolase. In contrast to normal nervous tissues, mainly the A_4 isoenzyme and only small amounts of the other hybrids of the A–C set can be detected. As recently demonstrated [1], in retinoblastomas only A_4 aldolase is present, a pattern resembling the fetal retina. However, it is important to realize that both fetal brain and adult brain contain aldolase A + C mixed multimers, as was demonstrated by Hatzfeld and Schapira [14]. This may explain why the aldolase pattern of gliomas is identical to that of normal brain. Nevertheless the observed isozyme pattern of aldolase in neuroblastomas, medulloblastomas and retinoblastomas [1] is different from that found in gliomas.

It has been proposed by Weber [6, 7] that especially so-called regulator enzymes are altered

in cancer. However, for aldolase, *not* being a regulator enzyme, isozyme changes in many tumors have been reported [5]. Therefore the findings regarding aldolase contradict Weber's concept. In conclusion, it appears that in neuroblastomas and medulloblastomas, the findings common with retinoblastomas in clinical and histological respect may be extended with common findings in certain isozyme patterns. This is especially the case for pyruvate kinase, which is a good marker for neuroectodermal tumors of childhood [15]. The observed electrophoretic pattern may be an expression of the stage of development.

Acknowledgements—We thank Mrs M. de Jong and E. L. Huisman-Backer Dirks for typing the manuscript.

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