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Significant Lower Carboxylesterase Specific Activity in Meningiomas and Gliomas and Derived Primary Cell Culture Indicate Reduction in Anticancer Drug Metabolism

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Authors' contributions

This work was carried out, analyzed and written by author PM and received little guidance and collaborations from other authors and all authors have approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Carboxylesterases (CE) convert carboxylic esters to alcohols and carboxylic acids. CE is a protective factor in the brain cells categorized as phase-I drug-metabolizing enzymes. Therefore we aim to find out the difference CE specific activity for Brain tumors as compared to the normal brain to understand the drug metabolism efficiency.
Study Design: Total Protein and Carboxylesterase assays were performed for Meningiomas and gliomas and derived primary cell culture.
Place and Duration of Study: Sample: Department of Neurosurgery (Neuro center) and Department of Neuropathology, Experiments performed: Departments of Neurochemistry and Neurovirology, NIMHANS, Bangalore, between January 2001 and October 2005.

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Methodology: CE spectrophotometric assays were studied for 30 meningiomas and 52 gliomas in 82 males, while 45 meningiomas and 29 gliomas in 74 females respectively and derived cell culture. The brain tumor protein band pattern was studied by electrophoresis.
Results: The brain tissue extracts for SDS PAGE displayed a high intense single protein (not purified) band of 60 kDa in brain tumors as compared to normal brain.
The similar CE-specific activity between the meningiomas 20.96±5.071 (n=50) and gliomas 20.77±4.4644 nmol/min/mg (n=61) respectively, exhibited significantly lower CE activity as compared to normal Brain (n=106) 52.355 ± 11.15 nmol/min/mg of protein and *p*-value was less than 0.0001 extremely statistically significant. Hence CE activities are significantly lower in all grades of parent Brain tumors as compared to normal brain. Primary cell cultures with respective passages expressed lower CE activities than parent tumors respectively.
Conclusions: The current results indicate the reason for the failure of anticancer drug metabolism efficiency for both meningiomas and gliomas due to one of the reasons for exhibiting lower CE-specific activity. The chance of solving the drug metabolism provided by the current study helps advanced molecular biochemistry in designing drug conjugates for efficient metabolism by CE-specific activity and monitoring the chemotherapy for anticancer drug therapeutics in the future.

Keywords: Carboxylesterase; specific activity; meningiomas; gliomas; drug metabolism.

ABBREVIATIONS

CE	*: Carboxylesterases*
BSA	*: Bovine Serum Albumin solution*
MEM (SIGMA)	*: Minimal essential medium*
FCS	*: Fetal calf serum*
v/v	*: Volume/Volume*
Mg	*: Milligram*
TVG	*: (2% trypsin, 0.2% versene and 10% glucose)*
TBS	*: tris buffered saline*
PBS	*: phosphate-buffered saline*
PMSF	*: poly methyl sulphonyl fluoride*
PAGE	*:Polyacrylamide gel electrophoresis*
EM	*: Electron Microscope*
GBM	*: Glioblastoma Multiforme*

1. INTRODUCTION

Carboxylesterases (CE) are hydrolases that belong to Class III Enzyme classification (EC 3.1.1.1) and specifically belong to the serine hydrolase family [1] and members of the esterase class of proteins [2]. These enzymes catalyze the conversion of carboxylic esters to the corresponding alcohols and carboxylic acids. CE is also capable of hydrolyzing aryl ester compounds and is categorized as phase-I drug-metabolizing enzymes [3].

CE is widely distributed and has been purified from membrane-rich fractions of mammalian cells and tissues. The mammalian CE is localized in the endoplasmic reticulum and cytosol of lysosomal fractions while the highest levels are present in microsomes of the liver respectively. A high abundance of CE in the liver is linked to certain cellular structural functions, particularly in directing protein targeting [4].

Human carboxylesterase1 (hCE1) is primarily expressed in the liver and tends to hydrolyze more small compact molecules, such as oseltamivir. CE1 is found only in the liver, whereas CE2 is found in the intestine, and CE3 is found in brain cells [5].

Multiple forms of CE in human tissues are identified and four different types of CE have been recognized in human brain extracts [6]. In the central nervous system, the CE component of a blood-brain barrier system may function as a protective factor against foreign chemicals in their glial cells, neuronal cells, capillary endothelial cells, and the antibody to serve as a marker for invading macrophages from the systemic circulation [7-9].

Carboxylesterase activity can be influenced by interactions of a variety of compounds either directly or at the level of enzyme regulation. For eg. As drug elimination decreases and the incidence of drug-drug interactions increases when two or more drugs compete for hydrolysis by the same carboxylesterase isozyme [10]. Tissue-specific expression of CE activity determined the location and intensity of certain drugs [11]. Since a significant number of drugs are metabolized by CE for any alteration in the activity of this enzyme has an important role in clinical implications.

Pharmacologists explore the relevance of CES to human diseases or assign the contribution of certain CES in xenobiotic metabolism were

reported. It will also facilitate medicinal chemistry efforts to design prodrugs activated by a given CES isoform or to develop potent and selective modulators of CES for potential biomedical applications [12].

For example, carboxylesterases rapidly convert the anticancer drug irinotecan to 7-ethyl-10-hydroxycamptothecin, a potent inhibitor of topoisomerase I [13]. Irinotecan has shown activity against colorectal, oesophageal, gastric, non-small-cell, and small-cell lung cancers, leukemia, lymphomas, and even central nervous system malignant gliomas [14]. Glioma cells are also capable of converting CPT-11 to SN-38 by intrinsic tumor carboxylesterases [15]. The intratumorally administered CPT-11 can be effectively converted to SN-38 for targeting drug delivery by extending the survival time of animals bearing malignant gliomas [16].

The CE drug metabolism information from another report on Neuroblastoma-bearing mice that received repeat treatment with intravenous hCE1m6-neural stem cells (NSCs) and irinotecan showed significantly lower tumor burden (1.4-fold, p = 0.0093) and increased long-term survival compared with mice treated only with drug alone. These studies support the continued development of NSCs mediated gene therapy for improved clinical outcomes in neuroblastoma patients [17].

Therefore these findings support the importance of cytochemical and CE drug metabolism investigations in brain tumor research. However, CE-specific activity is not being addressed in all types of benign and malignant brain tumors which is significant to understand the role of CE in drug metabolism for efficient therapeutics. Given this, the present study was undertaken to quantify CE further in different WHO-grade brain tumors for both benign and malignant nature by comparing with normal autopsy human brain [18] to determine the specific activity of CE. The efforts for solving the tumor problem is provided by the current study promotes applications in the future by determining tumor CE-specific activity for drug metabolism efficiency.

2. MATERIALS AND METHODS

2.1 Autopsy Brain and Brain Tumor Specimen

The autopsy fresh normal brain tissue samples procured from the Department of Neuropathology, NIMHANS, (National brain tissue repository) are available for research purposes. A total of 27 fresh brain tissue samples are collected from this repository research studies after obtaining institutional ethics committee approval.

The Brain tumors biopsy samples were collected immediately after the surgery. HOD Neurosurgery, Neuro centre NIMHANS permitted for procuring brain tumor samples. A total of 228 Brain tumors meningiomas and gliomas samples were collected. (Supplementary Table1). Brain tumor diagnosis results are provided from the Dept. of Neuropathology, NIMHANS (Table 1) Bangalore.

2.2 Brain Tissue Processing

The brain tumor samples collected, cleared of blood clots, if any, and rinsed with normal saline and net weight of the tissue noted (Dept of Neurochemistry, NIMHANS). Subsequently, the tissue specimens subjected to homogenization in tissue extraction Tris-buffered saline buffer (TBS containing 1% (v/v) Triton-X 100) with 0.5mM PMSF using a glass homogenizer.

The tissue to buffer ratio was maintained at 1:31 w/v (ice) for homogenization. Homogenates centrifuged (16,000 rpm/30 minutes) at 4^0C (Sorvall centrifuges, Bangalore, Karnataka, Bangalore). The supernatants were collected for biochemical analysis and stored at 4^0C until the enzyme activity was assessed.

2.3 Protein Estimation

Was carried out according to Lowry's [19, 20] method using Bovine serum Albumin solution (BSA) as a standard.

2.4 Carboxylesterase

Gomori's method [21] and later modified by Van Asperen [22]. Enzyme reaction initiated by adding 900µl of 5mM α-naphthyl acetate in Phosphate assay buffer (pH 7.0) to pre-incubated 100µl tissue extract and again incubated for 15 minutes at 27^0C. Subsequently, the reaction stopped by the addition of 500µl DBLS reagent and enzyme activity measured by the wavelength at 600 nm of Spectrophotometer (Shimadzu, Bangalore, Karnataka, India).

2.5 Brain Tumor Cell Culture

2.5.1 Primary explants cell culture [23] (Patient-derived explants)

The brain tumors were collected from patients subjected to explants culture. The chopped tissues were transferred to Petri dishes supplemented with an additional volume of MEM with fetal calf serum (Sigma, USA). The six well plates were transferred to a CO_2 incubator (Thermo scientific, Bangalore, India, Karnataka) set at 37^0C. Media changed frequently after every 3 days during maintenance of cell culture (Dept of Neurovirology, NIMHANS).

2.5.2 Subculture

The primary cells were rinsed with TVG (2% trypsin, 0.2% versene and 10% glucose) and detached from the substratum of the petri dish containing MEM with 10 % (v/v) FCS. The half the portion of cell suspension subjected to new Petri dishes for further growth and placed in a CO_2 incubator. The remaining portion of the cell suspension was processed for biochemical studies**.**

2.5.3 Extraction of enzymes from the Brain tumor primary cell culture (Monolayer cells)

The brain tumor cells were cleaned with phosphate-buffered saline (1X PBS) pH=7.4 and incubated in IX PBS for 1-2 minutes. The culture cells were homogenized with tris buffered saline (TBS- 50 mM Tris HCl and 145 mM NaCl, pH 7.4) with 1% Triton X 100 (1:31w/v) and 0.5mM PMSF in ethanol. Cells with TBS centrifuged at 10,000 rpm for 30 minutes at 4^0C. The centrifuged supernatant was collected for biochemical studies to estimate the Protein and CE enzyme assays in respective cell culture subsequent passages from primary cell culture and further followed the similar protocol of parent brain tumors.

2.6 Electrophoresis [24]

2.6.1 SDS PAGE

The brain tissue supernatant and primary cell culture samples containing 20 µg protein electrophoresed in SDS polyacrylamide gel (10%) at a constant voltage of 50 Volts for 10 minutes, followed by 150 Volts for 1 hour. The power stopped when the marker dye reached 1-2 mm above the lower edge of the plate. The gel was carefully transferred to the staining solution with Coomassie Brilliant Blue R-250 at room temperature for 1 hour and de-stained with glacial acetic acid till the background become clear.

2.6.2 Native PAGE electrophoresis

(Biorad, Bangalore, Karnataka, India)—Native protein gel also subjected for protein expression without SDS for Brain tumor samples as compared to normal brain.

2.7 Statistical Analysis

The mean and standard deviation calculated for carboxylesterase concerning different grades and types of brain tumors by comparing with the normal brain using the 't-test calculator entering the data of groups one and two (Quick Calcs, Graphpad Software). The *p*-value was calculated for brain tumor carboxylesterases by comparing with a average activity of normal post-mortem human brain.

3. RESULTS

The salient socio-demographic features and epidemiological characteristics for patients' brain tumor samples are mentioned in Supplementary (155nos. Table 1). A total of 74 meningiomas and 81 glioma tissues were subjected to carboxylesterase assay. Amongst them, more meningioma cases were encountered in females (n=44) as compared to males (n=30). This difference obtained much more prominent in cases of fibrous meningioma (Male: Female =5:14) and transitional meningioma (Male: Female = 3:10). On the contrary, male cases were found to be more in gliomas (n=52) as compared to females (n=29). These differences are much more prominent particularly in cases of Anaplastic astrocytoma (Male: Female = 14:6) and Anaplastic Oligodendroglioma (Male: Female = 10:3).

The results of Hydrolytic enzyme CE activities in brain tumors are mentioned in Table 1 (Fig. 3 to Fig. 6 and Supplementary Table 2A, 2B, 3A, and 3B). The specific activity of an enzyme is defined as the enzyme total activity to total protein and expressed in terms of nanomoles of product formed per minute per milligram of protein.

3.1 Total Protein Content in Brain Tumor Tissue Samples

The total protein content was obtained for all grades of benign and malignant brain tumors

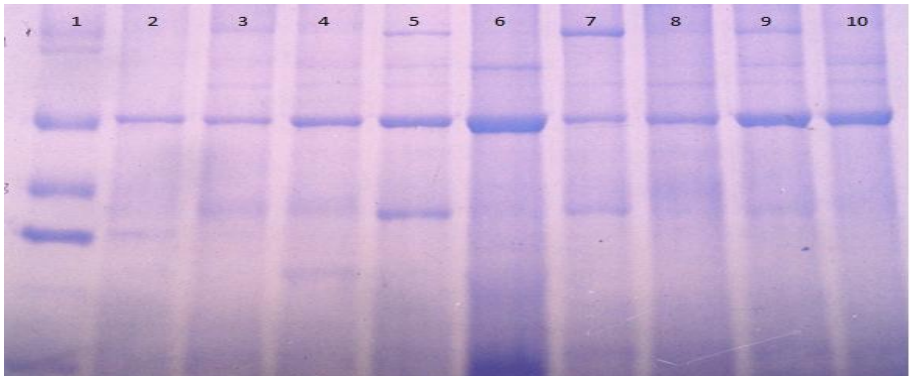
which compared with the normal human brain. More protein content was reported for brain tumors when compared to the gray and white matter of normal human brains respectively, which measured as mg/g of wet tissue. Meningiomas showed an average of 40-50 mg protein and gliomas exhibited 50-60 mg/g of wet tissue respectively.

3.1.1 Protein expression in SDS page electrophoresis

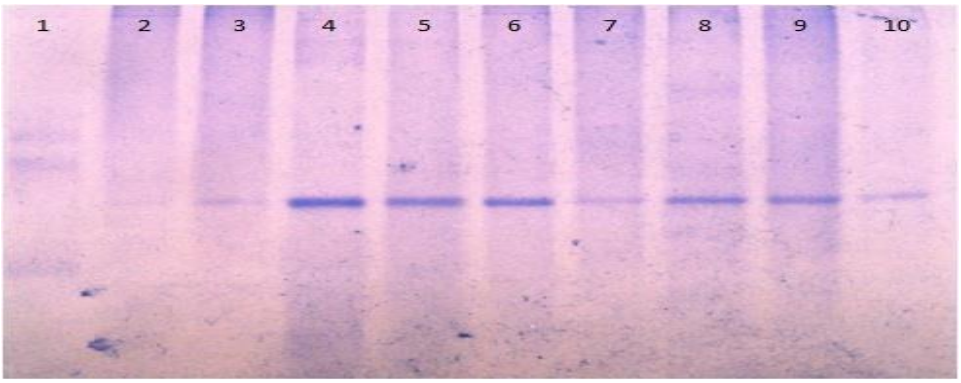
3.1.1.1 SDS PAGE protein expression in Meningiomas and gliomas

The SDS-PAGE gel electrophoresis displayed protein band patterns in both normal brain regions of the human and brain tumors tissue supernatant samples. The brain tissue extracts displayed prominent bands in the sixth lane of Anaplastic oligodendroglioma and for other lanes (Fig,1 set I 5th, 7th, 8, and 9thLanes) and one band in case of Neuroendocrine carcinoma olfactory neuroblastoma as compared to the normal brain.

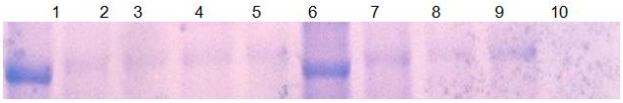
The tissue extracts of grey matter in the normal human brain displayed an intense band ~60 kDa protein than white matter (Fig 1Set II). The same protein (~60kDa) expressed a more intense band in almost all brain tumors in gel, whereas more intense protein bands were also expressed for five samples (Fig.1 Set II A lane 4, 5, 6, 8, and 9) like glioblastoma multiforme left frontal, Malignant astrocytoma G-IV left front temporal region, Meningothelial meningioma parasagittal posterior frontal, Transitional meningioma G-I left tempo parietal, Anaplastic meningioma, as compared to normal brain protein.



Set I Lanes: 1).Marker 2).Hippocampus 38/M 3).Frontal (white) 15/M 4).Frontal (gray) 40/M 5). Anaplastic oligodendroglioma (L) Temporal 50/F 6). Anaplastic oligodendroglioma (L) temporal 50/F 7). Anaplastic oligodendroglioma 53/M 8). Anaplastic oligodendroglioma 32/M 9). Anaplastic oligodendroglioma 45/M 10). Neuroendocrine carcinoma olfactory neuroblastoma 40/M



Set II. Lanes 1) Marker, 2) Frontal (white) 50/M, 3) Frontal (gray) 50/M, 4) Glioblastoma multiforme left frontal 50/M, 5) Malignant astrocytoma G-IV left front temporal region, 6) Meningothelial meningioma parasagittal posterior frontal attached to area 61/M, 7) A Typical meningioma frontal 40/F, 8) Transitional meningioma G-I left tempo parietal, 9) Anaplastic meningioma 45/M, 10) A Typical meningioma G-I left tempo parietal 20/M. (M –Male patient, F-Female patient)



Set III. Lanes: 1) A Typical meningioma G-II tempo parietal 50/M parent tumor, 2) Passage-1, 3) Passage-2, 4) Passage-3, 5) Passage-4, 6) Transitional meningioma G-I left tempo parietal 20/M parent tumor, 7) Passage-1, 8) Passage-2, 9) Passage-3, 10) Passage-4

Fig. 1. SDS PAGE electrophoresis protein expression for benign and malignant Brain tumors and Meningioma primary cell lines

3.1.1.2 SDS PAGE Protein expression in meningioma derived primary cell lines

Meningioma parent tumors of both Grades-I of Transitional meningioma G-I left temporoparietal 20/M and A Typical meningioma G-II tempo parietal 50/M parent tumor expressed thick intense protein band as compared to their respective four subsequent subculture passages which expressed lower protein content (Fig.1 Set III).

3.1.2 Protein Expression in Native PAGE electrophoresis for meningioma derived primary cell lines

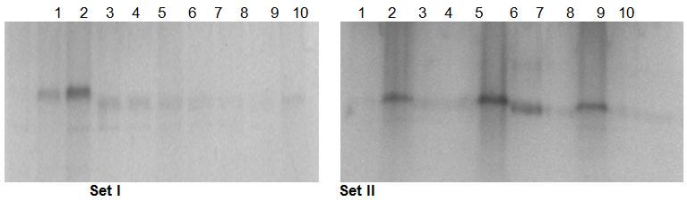
Set I gel displayed an intense protein band for the brain hippocampus as compared to frontal white matter and the same protein band expressed more in parent tumors of fibrous meningioma G-I MCF base, however, the band intensity decreased gradually in their subsequent passages (Fig.2 set I).

Set II gel displayed an intense protein band in parent tumors of meningothelial meningioma WHO G-I right front parietal and transitional meningioma G-I left tempo parietal but not in their passages-1 and 2. Whereas meningioma G-I right C.P angle and their passage-1 also displayed an intense protein band but not for passage-2 (Fig.2 Set II).

3.2 The Specific Activity of Carboxylesterase (CE) in Brain Tumor Samples

Carboxylesterase specific activity is determined for Brain tumors by comparing with Autopsy normal human brain. The CE-specific activity average value of normal brain for a white and gray matter of frontal, parietal, occipital, temporal respectively and tissues of the thalamus, cerebellum, and hippocampus expressed in nmol/min/mg protein.

Meningiomas (n=50) exhibited significantly lower CE activity 20.96±5.071 and gliomas (n=61) showed 20.77±4.4644 nmol/min/mg of protein respectively as compared to normal brain (n=106) 52.355 ± 11.15 nmol/min/mg of protein (Table 1). The two-tailed *p*-value was less than 0.0001 for all meningiomas and gliomas studied. By conventional criteria, this difference is considered to be extremely statistically significant.



Set I Lanes-- **1.**Frontal (white) 26/F; **2.** Hippocampus 42 /F; **3.** Fibrous meningioma G-I MCF base 30/F; **4.**Passage-1; **5.** Passage-2; **6.** Passage-3; **7.** Passage-4; **8.** Passage-5; **9.** Passage-6; **10.** Passage-7

Set II Lanes-- **1.**Thalamus; **2.** Meningothelial meningioma WHO G-I right front parietal; **3.**Passage-1; **4.**Passage-2; **5.**Meningioma G-I right C.P angle; **6.**Passage-1; 7**.**Passage-2; 8**.** Transitional meningioma G-I left tempo parietal 20/M; **9.**Passage-1; **10.**Passage-2.

Fig. 2. Native PAGE Electrophoresis Protein Expression in benign meningiomas and Meningioma primary cell lines

Table 1. Comparison of Average CE specific activities of the Meningiomas and gliomas with normal human brain

Sl No.	Brain Samples	CE Specific Activity (Std. deviation)	Std error	*p*-value
1	Normal brain (n)	106		
	Mean ± SD	52.355 ± 11.15		
2	Meningiomas (n)	50	1.654	0.0001
	Mean ± SD	20.96 ± 5.071		
3	Gliomas (n)	61	1.493	0.0001
	Mean ± SD	20.77 ±4.46		

SA=Specific activity nmol/min/mg of protein. CE=Carboxyl esterase

3.2.1 The specific activity of Carboxylesterase in different grades of meningiomas

The comparative average specific activities of CE are estimated in the different grades of meningiomas presented in Fig. 3A (suppl. Table 2A). Among various types of meningiomas studied, atypical meningiomas G-II exhibited the highest CE specific activity (25.3474 ±7.7647 nmol/min./mg of protein) nevertheless this was significantly lower than a normal brain. The lowest levels of CE were noted in angiomatous meningioma G-I (11.79 ± 3.018 nmol/min./mg of protein). Further, there were no significant differences in the specific activity for CE among the various grades of meningiomas.

3.2.2 The specific activity of Carboxylesterase for meningiomas derived primary cell lines

The CE activity of cell lines varies among the passages they have undergone, but the higher specific activity exhibited in parent tumors than their cell lines. However higher activity in passages of a few cell lines significantly lower than the normal brain (Fig 3B and C).

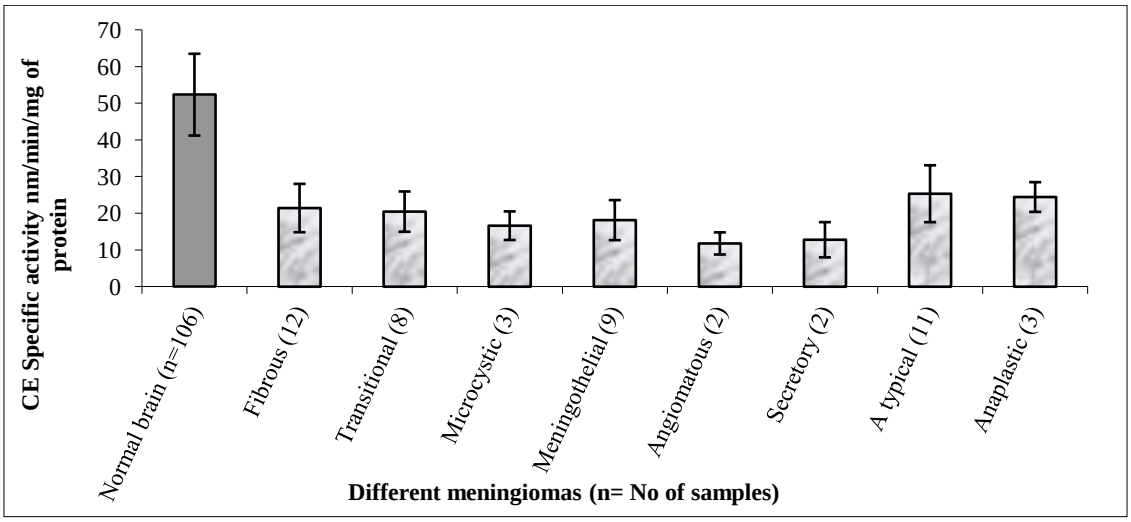
Some of the meningioma cell lines expressed lower enzyme activity concerning parent tumor, except for meningothelial base frontal (Fig 3B), atypical transitional and fibroblastic meningiomas (Fig 3C), which were expressed higher activity in passage-1, later the CE-specific activity decreased for the next passages and varies for other passages. While Transitional meningioma G-I (L) Tempo parietal (passage-4,5), Transitional meningioma G-I ® frontal (passage-6), A Typical meningioma G-II tempo parietal (passage-4,5) expressed higher CE specific activities as compared to their parent tumor.

3.2.3 The specific activity of Carboxylesterase in different grades of gliomas

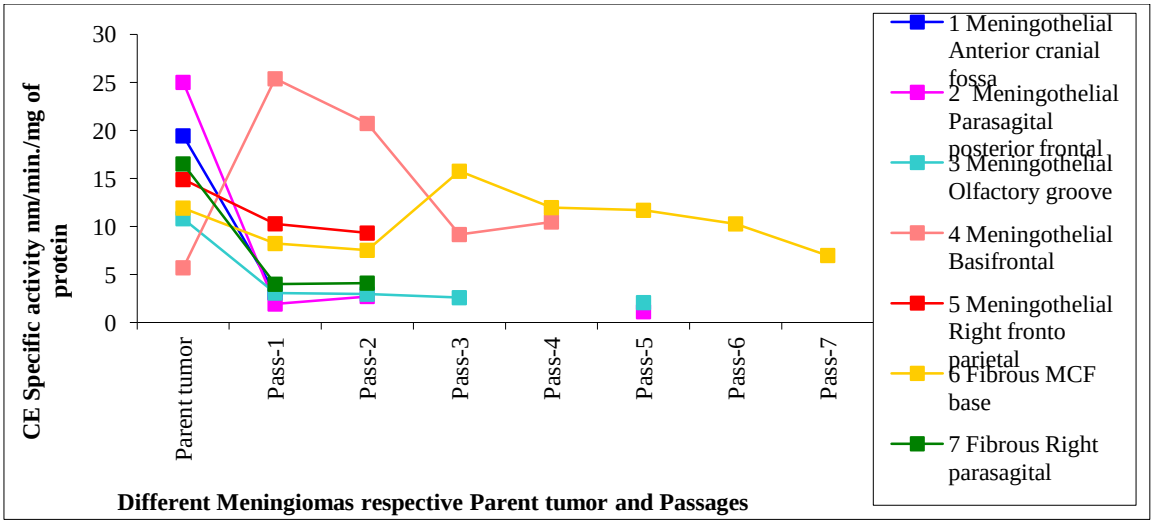
Among the various types of gliomas studied, glioblastoma multiforme G-IV and Malignant Astrocytoma G-III exhibited the highest CE activity 25.585 ± 6.92 and 24.1268±8.0313 respectively and lowest in anaplastic oligoastrocytoma G-III 12.166± 5.1304 nmol/min./mg of protein (Fig 4A, suppl. Table 2B) which were significantly lower as compared to the normal human brain.

3.2.4 The specific activity of Carboxylesterase in gliomas primary cell lines:

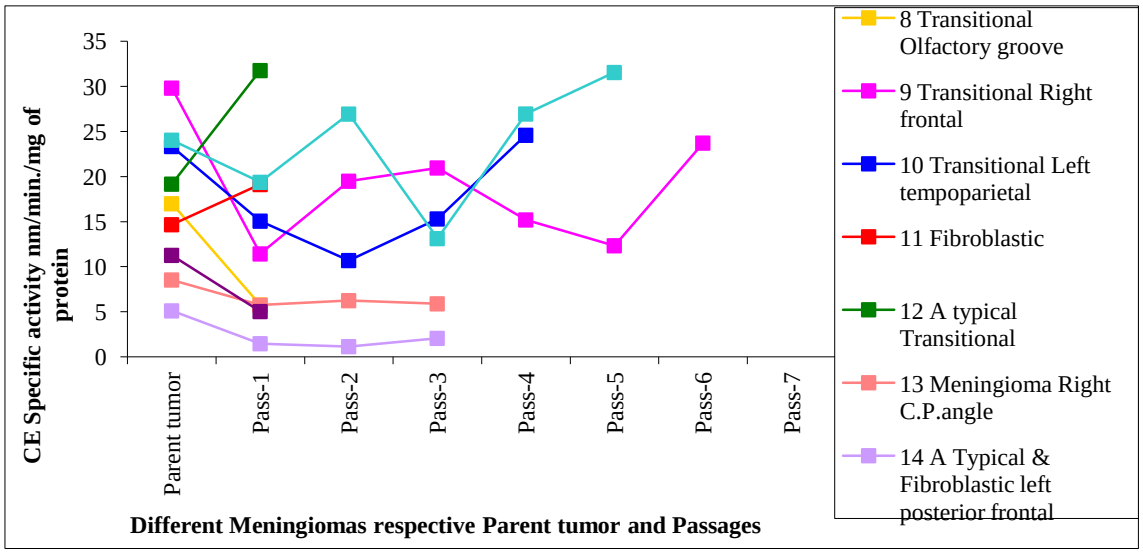
All glioma parent tumors and their derived primary cell lines expressed significantly lower CE activity as compared to normal brain. However higher CE activity was expressed in passage-1 for anaplastic astrocytoma right tempo-parietal area (Fig 4B) as compared to parent tumor but expressed lower CE activity than a normal brain. Anaplastic Astrocytoma G-III R Tempo parietal area (passage-2) and Ewing's sarcoma – skull base Metastasis (passage-3) expressed quite higher CE activities.



A



B



C

Fig. 3.Specific activity of Carboxylesterase in different grades of A Parent Meninigiomas B and C Derived primary cell culture

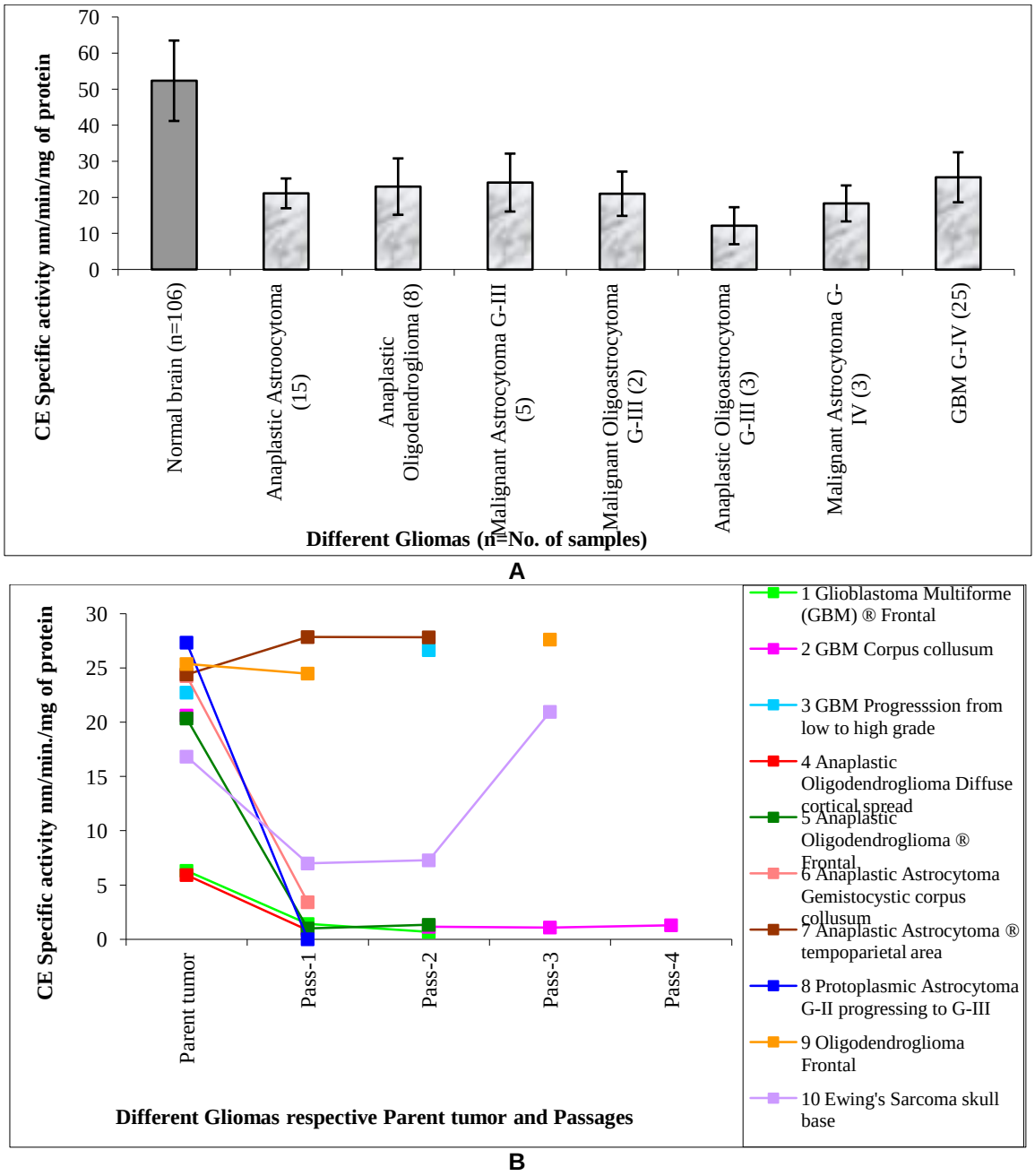


Fig 4. Specific activity of Carboxylesterase in different grades of A Parent Gliomas B Derived primary cell culture

4. DISCUSSION

The Carboxyl esterase specific activity determined for all WHO grades brain tumors (meningiomas and gliomas) and in their derived primary cell culture.

4.1 Total Protein of Brain Tumors

The protein content of Meningiomas exhibited an average value 40-50 mg and gliomas showed 50-60 mg/g wet tissue respectively due to higher proliferation of malignant cells than normal brain cells.

The SDS PAGE gel displayed a single intense protein band of molecular weight ~60, 000 Da. The single dark intense protein band expressed more in Brain tumor samples as compared to the normal brain which displayed like a purified protein band. However, SDS PAGE single band

obtained for brain tissue extracts homogenized and centrifuged supernatant samples which were properly standardized. Hence this crude brain tumor extract in gel displayed a novel single band for one protein ~60,000Da. The brain tissue extracts displayed prominent bands in the sixth lane of Anaplastic oligodendroglioma and for other brain tumors (Fig,1 set I for 5th, 7th, 8, and 9thLanes) due to highly proliferated cells correlates with meningiomas G–I expressed in Set II. The tumors like GBM left frontal, Malignant astrocytoma G-IV left front temporal, Meningothelial meningioma parasagittal posterior frontal, Transitional meningioma G-I left tempo parietal, Anaplastic meningioma displayed single intense band as compared to normal brain (Fig 1and set II). While A Typical meningiomas (Fig 1and Set II of 7th and 10th lanes) expressed a less intense single protein band.

SDS PAGE gel displayed single intense protein band 60KDa in Brain tumor samples similar to earlier reports on Gel chromatography on SephadexG-200 of concentrated samples of brain extract gave two completely separated fractions exhibiting CE specific activity. One with a molecular weight of about 60.000Da contained the bulk of acetyl ester-splitting enzymes [5].

Native PAGE protein expression results are similar to the SDS PAGE band pattern. Set I gel displayed a single intense protein band for fibrous meningioma G-I MCF base and band intensity decreased gradually in their subsequent passages may be due to the viability of cells in primary cell culture (Fig.2 set I).

Set II gel displayed an intense protein band for meningothelial meningioma WHO G-I right front parietal and transitional meningioma G-I left tempo parietal except for their passages-1 and 2. Whereas meningioma G-I right C.P angle and their passage-1 also displayed an intense protein band except for cell culture passage-2 due to reduction in cell growth.

4.2 Importance of CE for Anticancer Drug Metabolism

It is known that Carboxylesterase is a ubiquitous enzyme responsible for the hydrolysis of numerous clinically useful anticancer drugs. Since ester moieties are frequently included in molecules to improve their water solubility and bioavailability, *de facto* they become substrates for CEs [25].

Carboxylesterase-based metabolism is used for the activation of ester prodrugs. For example, irinotecan is rapidly converted by CE to 7-ethyl-10-hydroxycamptothecin, a potent inhibitor of topoisomerase I. Irinotecan is widely used as an anticancer drug for a wide range of malignancies. Whereas some of the patients respond to it poorly, presumably due to lower levels or polymorphistic variants of Carboxylesterase [26].

Since brain tumors showed reduced activity of Carboxylesterase in our study, which turns out lower effect on drug metabolism. In supporting to our data, previous studies have also shown that decreased intracellular CE activity in human ovarian cancer cells caused reduction in the conversion of KW -2189 to its active form in the cell. The decreased conversion of CPT-11 to SN-38 in HAC2/0.1 cells might be explained by lower specific activity of CE [27]. Because of less intrinsic tumor CE activity, CPT-11 cannot be degraded to SN-38 an active metabolite (7-ethyl –10-hydroxy camptothecin) toxic to cancer cells.

4.3 Anticancer Drug Metabolism by CE in Brain Tumors

Recent clinical trials have shown major antitumor activity in recurrent glioblastoma when adding the antiangiogenesis drug bevacizumab with CPT-11. The combination of targeted agents to topoisomerase I inhibitors represent a novel and promising approach for new treatment strategies for primary malignant brain tumors [28].

CPT-11 (irinotecan) has been investigated as a treatment for malignant brain tumors. However, limitations of CPT-11 therapy include low levels of the drug entering brain tumor sites and systemic toxicities associated with higher doses.

These studies demonstrate the feasibility of NSC-mediated delivery of CE to glioma and lay the foundation for translational studies of this therapeutic paradigm to improve clinical outcome and quality of life in patients with malignant brain tumors [29].

The survival of tumor-bearing animals was significantly prolonged by the treatment with F3.CE and CPT-11. This strategy could be considered as an effective treatment regimen for lung cancer brain metastases. [30]. Hence currently by knowing CE-specific activity helps in designing the drugs with conjugates to target efficient anticancer drug metabolism.

4.4 Specific Activity of CE in Parent Brain tumors Meningiomas and Gliomas

Total activity of CE performed with alpha naphthyl acetate and time curve expressed as nanomoles per minute. While specific activity will be in terms of nanomoles per minute per milligram of protein. Thus the novel studies for CE-specific activity for all types of brain tumors (Suppl. Table 2A, 2B) and their derived primary cell culture (suppl. Table 3A and 3B) considered in current research [31,32] for chemotherapeutic applications.

Indeed, a significant difference in CE activity was noted between brain tumors and normal brain tissue. However, CE activity exhibited similarity between meningiomas and gliomas, the finest results and very much necessary for understanding drug metabolism that is been reported here for the first time. Lower and similar average specific activities were obtained for meningiomas and gliomas (Table 1) as compared to normal brain. However in brain tumors' some peculiar patterns are particularly useful for biological interpretation of tissue differentiation and cell metabolism [27].

Certainly, the present study is due to lower CE activity of brain tumors which in turn has a poor effect on anticancer drug metabolism for brain tumors. However, none of the earlier studies have shown for all brain tumors about CE-specific activities.

The brain tumors pathology relation for enzyme CE activity varies with morphological features and grades of the tumor. The lowest levels of CE were noted in angiomatous meningioma G-I (11.79 ± 3.018 nmol/min./mg of protein), which is different from other meningiomas by their morphological features with many small or large vascular channels that may predominate over its meningothelial elements.

4.5 Comparative Study of CE Expression in Meningiomas and Glioma Primary Cell Culture

The Brain tumor primary cell lines obtained up to passages of 1 to 7 in meningiomas and 1 to 4 in Gliomas respectively. The majority of primary cell cultures expressed lower CE as compared to their parent tumors and the normal human brain.

Most of the meningioma cell lines exhibited lower CE-specific activity concerning with parent tumor, except Meningothelial base frontal G-I, atypical transitional G-II, and fibroblastic G-I (Fig3B and 3C), expressed higher CE activity in passage-1, subsequently, CE activity varies among respective passages. The reason is unknown though may be due to cell viability and studies have to be continued with more passages for the similar tumor samples.

All glioma parent tumors and most of their primary cell lines expressed significantly lower CE-specific activity as compared with a normal brain. However higher CE activity expressed in passage-1 of GBM G-IV (migration from low to high grade) and anaplastic oligodendroglioma G-III (Fig 4B) as compared to their parent tumor. While in Ewing's Sarcoma metastasis, CE specific activity increased in passage-3 but expressed lower CE activity than parent tumor (Fig 4B) which may be due to change in the viability of different cell types and growth patterns.

4.6 Significance of the CE Study in Brain Tumor and Derived Primary Cell Culture

The significant similar relation of CE activity between the meningiomas (n=50) 20.96±5.071 and gliomas (n=61) 20.77±4.4644 nmoles/min./mg respectively, exhibited significantly lower CE activity as compared to normal brain (n=106) of 52.355 ± 11.15 nmol/min./mg of protein. Primary cell cultures with respective passages expressed lower CE activities than parent tumors respectively which fluctuate and deplete as the number of passages has undergone. However, some of the Meningiomas G-I and G-II expressed higher CE activity in few passages than parent tumor but lower as compared to normal brain [33].

This suggests that one of the reasons for the failure in the metabolism activation of anticancer drugs for both benign and malignant tumor treatment.

The protein content is always higher in parent tumors and for passage-1, this refers to more number of cells (endothelial cells) that may also matter for more CE activity, and some tumor passages-2 also expressed higher CE activity as compared to parent tumor but lower than normal brain CE activity.

However, there are no reports for a low level of CE-specific activity in all types of WHO-grade brain tumors and their derived cell culture. Thus current results have shown lower CE-specific activity in the case of Benign and malignant tumors and successfully shown lower expression of CE activity in brain tumor-derived primary cell culture. The exact reason is not yet known however it may be due to cell migration, types of cells interaction, and growth.

Interestingly both Benign meningiomas and malignant gliomas exhibit similar CE-specific activity that confirms some of the main reasons for the failure of chemotherapy since the amount of CE drug-metabolizing enzymes was lower in benign and malignant brain tumors as mentioned above. These results provide an idea to increase the CE level with modulators' anticancer drug metabolism in the future/. Therefore CE has to be activated by modulators or by cloning the CE gene.

Recent findings with our current experiments of Brain tumor p53 mutant LN 229 and U251 cell lines expressed lower CE activity while expressing significantly higher CE activity for LiCl2 treated cells (not reported). This exactly confirms that Lithium is a positive modulator that enhances CE activity and has got anticancer activity. Therefore it can be conjugated with anticancer drugs for their metabolic efficiency and targeting on Brain tumor cells for better treatment.

To achieve the goal, different type's normal brain tissues should be collected from the region where the origin tumor has occurred including for eg. meningiomas transformed from meninges cells. Therefore CE activity has to be determined for meningiomas by comparing with meninges to understand the efficiency of drug metabolism. Hence Carboxyl esterase can also be made as biomarkers by developing kits for diagnosis purposes. Thus the current study promotes applications in the future by determining tumor and cell culture CE specific activity for designing anticancer drugs for efficient metabolism and Targeting.

5. CONCLUSIONS

The Native and SDS PAGE gel displayed a high intense single protein band in crude extract brain tumors as compared to normal brain. The specific activity of carboxylesterase (CE) in brain tumors for all WHO grades of meningiomas and gliomas and in their derived primary cell culture exhibited significantly lower CE activity as compared to the normal human brain.

This suggests that one of the reasons for the failure in the metabolism activation of anticancer drugs for both benign and malignant tumor treatment. Hence the current study of CE activity analysis with brain tumors confirms monitoring in the treatment and selecting the better drug for experimental designing towards efficient anticancer drug metabolism for even deciding dosage and better therapeutics.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

SUPPLEMENTARY MATERIALS

Supplementary materials is available in this link:
https://www.journalirjo.com/index.php/IRJO/libraryFiles/downloadPublic/3

CONSENT

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal." (Attached file-permission to handle the samples)

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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