Cathepsin B and Cathepsin L Activity Levels in Different Types of Human Brain Tumors

İnsan Beyin Tümörlerinde Katepsin B ve Katepsin L Aktivite Düzeyleri

Hüray İŞLEKEL[1] Sertaç İŞLEKEL[2] Gül GÜNER[1]

Eren DEMİRTAŞ[3]

Abstract

Cathepsin B and cathepsin L are the major cysteine endopeptidases found mainly within the lysosomes of almost all mammalian cells. Besides their physiological role in the turnover of intrinsic cell proteins and extracellular proteins, these proteinases are implicated in the degradative processes of tumor invasion and metastasis. In this study, the specific activities of these enzymes were examined spectrophotometrically in normal human brain tissue (n=10) and tissues of three series of histopathologically diagnosed brain tumors; meningioma (n=16), glioblastoma multiforme (n=17) and metastatic carcinoma (n=10). Benzoyl-alanine-arginine-arginine-4-methoxy-2-naphthylamide (pH 6.2) and benzoyl-phenylalanine-arginine-4-methoxy-2-naphthylamide (pH 3.5) were used as synthetic peptide substrates for cathepsin B and cathepsin L respectively. Specific activities of cathepsins B and L were expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein (nmoles min^{-1} mg^{-1}). The specific activities of cathepsins B and L were highest in metastatic brain tumor, lower in glioblastoma and lowest in meningioma when compared to normal brain tissue. Statistically significant elevated cathepsin L activities were observed in metastatic tumor tissues (250.7±6.9) when compared to glioblastoma (99.0±14.6), meningioma (83.3±14.2) and normal brain tissue (74.3±2.2) ($p<0.05$). The cathepsin B activities were found to be significantly higher in metastatic tumor tissues (250.0±15.6) in comparison to normal brain tissue (44.9±1.6) ($p<0.05$). In conclusion, it can be stated that the activities of cathepsins B and L in human brain tumor tissues might serve as a criteria in the investigation of the invasive potential of the brain tumor.

Key Words: cathepsin B, cathepsin L, tumor, meningioma, glioma, metastasis

Özet

Katepsin B ve L hemen tüm memeli hücre lizozomlarında bulunan temel sistein endopeptidazlardandır. Hücre içi ve hücre dışı proteinlerin parçalanmalarındaki fizyolojik rollerinin yanısıra, bu proteinazlar tümör invazyon ve metastazındaki degradatif olaylardan sorumlu tutulmaktadır. Bu çalışmada, normal beyin dokusunda (n=10) ve üç farklı histopatolojik tipte beyin tümöründe; menenjiom (n=16), glioblastoma multiforme (n=17) ve metastatik karsinomda (n=10) katepsin B ve L aktiviteleri spektrofotometrik yöntemle incelendi. Sentetik substrat olarak, katepsin B için, benzoil-alanin-arjinin-arjinin-4-metoksi-2-naftilamid (pH 6.2), katepsin L için ise benzoil-fenilalanin-arjinin-4-metoksi-2-naftilamid (pH 3.5) kullanıldı. Enzim spesifik aktiviteleri mg protein başına dakikada hidroliz edilen nanomol substrat miktarı olarak ifade edilmektedir. Her iki proteinazın aktiviteleri normal beyin dokusuna kıyasla, sırasıyla metastatik tümörlerde, glioblastoma multiforme ve menenjiomda daha yüksek

1 Dokuz Eylül University Faculty of Medicine Department of Biochemistry Narlıdere İzmir, 35340
2 Ege University Faculty of Medicine Department of Neurosurgery Bornova İzmir, 35100
3 Ege University Faculty of Medicine Department of Pathology Bornova İzmir, 35100

Biyokimya Dergisi
Temmuz-Ağustos-Eylül/ 1999
Sayı : 3, Cilt : 24

olarak bulundu. Katepsin L aktiviteleri, glioblastom (99.0±14.6), meninjiom (83.3±14.2) ve normal beyin dokusu (74.3±2.2) ile karşılaştırıldığında, metastatik beyin tümörlerinde (250.7±6.9) istatistiksel olarak daha yüksek bulundu ($p<0.05$). Katepsin B aktivitelerinde ise sadece metastatik beyin tümörleri (250.0±15.6) ile normal beyin dokusu (44.9±1.6) arasında istatistiksel olarak anlamlı fark tespit edildi. Sonuç olarak; beyin tümör dokularında katepsin B ve katepsin L aktivitelerinin belirlenmesi tümörün invaziv karekterinin incelenmesinde potansiyel bir kriter olarak kabul edilebilir.

Anahtar Sözcükler: katepsin B, katepsin L, tümör, menenjiom, gliom, metastaz

INTRODUCTION

Cathepsins are lysosomal proteolytic enzymes participate in various physiological processes such as turnover of intrinsic cell proteins and extracellular proteins, hormone action and inactivation, growth and ageing, fertilization, memory, modelling, tissue resorption, degradation of endocytosed material, and creation of immunologically recognizable molecules. In addition, cathepsins play an important role in a number of pathological situations such as muscular dystrophy, cachexia, multiple sclerosis, diabetes, cancer, and ischemia-reperfusion injury (1-3). In the last decade, there are several investigations related to the activities of these enzymes in different pathologies of the brain tissue (4-7)

Invasive and metastatic properties and recurrence at the site of initial lesion are the major problems in the study of the tumorigenic process. To invade the surrounding tissue, tumor cell needs passage through the biological barriers mainly the extracellular matrix (ECM) of the host tissue. It has been proposed that tumor cell migration through the extracellular matrix may be facilitated by the secretion of proteolytic enzymes, which may partially degrade elements of ECM (8). Members of the proteinase super-family, particularly the lysosomal cysteine proteinases cathepsin B (CB) and cathepsin L (CL) are reported to be the major proteolytic enzymes involved in the invasion and metastatic processes (8,9). If these lysosomal proteinases, which are normally safely sequestered behind the lysosomal membranes, become released either into the cytoplasm or extracellular matrix, they can cause major damage as seen with invasion and metastasis of malignant tumors (10). Studies on human tumor tissue specimens as well as tumor cell lines revealed elevated levels of activity or overexpression of cathepsin B and/or cathepsin L (11-14). Positive associations between increased cathepsin activities as well as their expression and tumor invasion have been noted in human glioma and meningioma tissues (5,8,15-18). At present, there is no previous study comparing cathepsin activities with benign, malignant and metastatic brain tumors. In the current investigation, we aimed to determine the activities of the two important cysteine proteinases CB and CL in human brain metastatic carcinoma, glioblastoma multiforme, meningioma and normal brain tissues, with the objective of comparing these parameters in the most commonly encountered human brain tumors. The enzyme determinations were performed on tissue extracts following homogenization and cold extraction to release these enzymes out of the lysosomes.

MATERIALS AND METHODS

Patients and Tissue Samples

Tumoral tissue samples were obtained from the Department of Neurosurgery, Ege University Medical School within one hour after surgical intervention between January 1997 and September 1997. All samples were examined and diagnosed histopathologically. The study group consisted of meningioma (n=16), glioblastoma multiforme (n=17) and metastatic brain tumors from primary lung carcinoma (n=10). Normal brain tissues (n=10) were obtained at autopsy within a maximum of five hours following death.

This study met the criteria of the local ethical committee.

All samples were freed of blood and visible blood vessels, thoroughly washed in 0.9% NaCl and immediately stored at -20°C before analysis.

Preparation of Tissue Extracts

The frozen tissues were minced and thawed on

ice, and macroscopically homogeneous pieces of tissues (0.1g) were cut out of the specimens. Ten percent tissue homogenates (w/v) prepared in ice- cold distilled water using a glass Teflon homogenizer (B.Braun Welsungen, W Germany), were frozen and thawed three times for cold extraction. The tissue extracts thus obtained were centrifuged at 17,000 x g for 50 min at +4°C using a Centrikon T 1180 (Cedex France).

Biochemical Analysis of Cathepsin B and L

Cathepsin B and L activities were determined by the spectrophotometric method of Shuja (19). The principle of the method depends on the hydrolysis of the synthetic peptide substrates by CB and CL in the sample at 37°C and the formation of color with fast blue B. Benzoyl-alanine-arginine-arginine - 4 - methoxy-2-naphthylamide (Z-Ala-Arg-Arg-MNA) (pH 6.2) and benzoyl-phenylalanine-arginine-4-methoxy-2-naphthylamide (Z-Phe-Arg-MNA) (pH 3.5) (Enzymes Systems Products, Dublin, CA) were used as synthetic peptide substrates for CB and CL, respectively. The pink color developed by the reaction of amino acid residues with fast blue B was measured at 520 nm. This color is proportional to the enzyme activity. The enzyme activity is determined as nanomoles substrate hydrolyzed per minute. 4-methoxy-2-naphthylamine (Sigma) was used as standard. The total protein concentration was measured by the method of Lowry et al (20) using bovine serum albumin (BSA) as standard. The specific activities of cathepsins B and L were expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein (nmoles min^{-1} mg^{-1}).

Statistical Analysis

All values are expressed as the mean plus or minus standard error of mean (SEM). Cathepsins B and L activities were analyzed for statistical differences between malignant and normal groups using analysis of one way ANOVA followed by the DUNCAN test.

RESULTS

The specific activities of CB and CL expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein (nmoles min^{-1} mg^{-1}) are given in Table I. Each value represents the mean ±SEM of the enzyme assay results. CB and CL activities were highest in metastatic brain tumor, lower in glioblastoma and lowest in meningioma when compared to normal brain tissue. Statistically significant elevated CL activities were observed in metastatic tumor tissues (250.7±6.9) when compared to glioblastoma (99.0±14.6), meningioma (83.3±14.2), and normal brain tissue (74.3±2.2) ($p<0.05$). CB activities were found significantly higher in the metastatic tumor tissues (250.0±15.6) in comparison to normal brain tissue (44.9±1.6) ($p<0.05$). No significant difference was observed for CB, between the metastatic tissue and other tumoral tissues.

DISCUSSION

The present study revealed that CB and CL activities are increased in human brain metastatic carcinoma, glioblastoma multiforme, and meningioma

Table I. Specific activities of CB and CL in normal brain and tumor tissues (Mean±SEM)

	Normal Brain (n=10)	Meningioma (n=16)	Glioblastoma Multiformu (n=17)	Metastasis (n=10)
Cathepsin B[1]	44.9±1.6	118.1±19.5	159.6±27.6	250.0±15.6*
Cathepsin L[1]	74.3±2.2	88.3±14.2	99.01±14.6	250.7±6.9**

[1]Expressed in (nmoles min^{-1} mg^{-1})

* Statistically significant difference as compared to normal brain tissue group ($p<0.05$)

** Statistically significant difference as compared to normal brain, meningioma and glioblastoma multiforme tissue groups ($p<0.05$)

tissues compared to normal human brain tissue. For CL activities, statistically significant increment was observed in metastatic tumor tissue extracts when compared to glioblastoma multiforme, meningioma, and normal brain tissue, whereas significantly higher CB activities were found in the metastatic tumor tissue as compared to normal brain tissue. There was no significant difference between the metastatic tumor tissue and other tumoral tissues regarding the CB activities.

Sivaparvathi et al (21) have found highest levels of CL activities in glioblastoma, followed by anaplastic astrocytoma, low-grade glioma and normal brain tissues. They also showed that the expression of CL is upregulated in malignant glioma and this correlated with the malignant progression of human glioma in vivo. In a similar study, the CB activity and the protein content were observed highest in glioblastoma, lower in anaplastic astrocytoma and lowest in low grade glioma and normal brain tissue [18). The results of different immunohistochemical studies performed on various degrees of malignancy of human glioma revealed that CB plays a role in human glioma progression and invasion (4,5). These studies correlate well with our results showing increased CB and CL activities in glioblastoma multiforme. Our literature survey reveals that there are no previous reports comparing CB and CL activities with metastatic brain tumor, glioblastoma multiforme and meningioma in the same study.

The present investigation has demonstrated that both of the cysteine endopeptidases are significantly high in metastatic brain tumor tissues originating from distant organ carcinomas. The metastasizing tumor cell must penetrate the stromal tissue and the biological barriers, such as basement membranes, in order to enter and leave the circulation. Passage of a cell through such a matrix is considered to involve a three-step process: initial adherence, enzymatic degradation of the matrix components and locomotion across the barrier (22). The degradation of matrix component occurs through proteolytic enzymes produced and released by tumor cells. Cathepsin B (CB) and cathepsin L (CL) are the major lysosomal cysteine proteinases that have been implicated in local basement membrane degradation with proteolytic processes, a prerequisite to tumor invasion and metastasis (23). In our study, the significantly higher enzyme activities observed in metastatic brain tumor compared with other studied types of tumors and the normal brain tissue for CL and the significantly higher CB activity found in metastatic brain tumors compared to the normal brain tissue support the involvement of these proteolytic enzymes in invasive and metastatic processes.

On the basis of our results, it seems that CL is more specific than CB for the discrimination of metastatic brain tumor from other tumor types. This observation is in aggrement with Kamboj et al who declared that cathepsin L has a more potent endopeptidase action compared to cathepsin B or cathepsin H in brain tissue [24].

In conclusion, it can be stated that the activities of CB and CL in human brain tumor tissues might serve as a criteria in the investigation of the invasive potential of the tumor. These results may lead further to the investigation of protease inhibitors for their possible potential in the limitation of tumor growth at a new site through blood-borne micrometastasis.

ACKNOWLEDGEMENTS:

This project was financially supported by Dokuz Eylül University Research Projects Administration (No. 0923. 94. 01. 03)

REFERENCES

1. Kos J. (1996) Cathepsins and their inhibitors-new factors for prognosis in cancer. E Clin Lab, June, 16
2. Agarwal SK (1990) Proteases cathepsins-a view. Biochem Edu, 18 (2), 67-72
3. Kohda Y, Yamashima T, Skuda K, Yamashita J, Ueno T, Kominami E, Yoshioka T. (1996) Dynamic changes

of cathepsins B and L expression in monkey hippocampus after transient ischemia. Biochem Biophys Res Commun, 228, 616-662

4. Mikkelsen T, Yan PS, Ho KL, Sameni M, Sloane BF, Roselinum ML. (1995) Immunolocalization of cathepsin B in human glioma: implications for tumor invasion and angiogenesis. J Neurosurg, 83, 285-290

5. Rempel SA, Rosenblum ML, Mikkelsen T, Yan PS, Ellis KD, Glombieski WA, Sameni M, Rozhin J, Ziegler G, Sloane BF. (1994) Cathepsin B expression and localization in glioma progression and invasion. Cancer Res, 54, 6027-6031

6. İşlekel H, İşlekel S, Güner G, Özdamar N. (1999) Evaluation of lipid peroxidation, cathepsin L and acid phosphatase activities in experimental brain ischemia-reperfusion. Brain Res, 843, 18-24

7. Nitatori T, Sato N, Kominami E, Uchiyama Y, (1996) Participation of cathepsins B, H and L in perikarial condensation of CA1 pyramidal neurons undergoing apoptosis after brief ischemia. Adv Exp Med Biol 398, 177-85

8. McCormick-D. (1993) Secretion of cathepsin B by human gliomas in vitro. Neuropathol Appl Neurobiol, 19, 146-151

9. Luthgens K, Ebert W, Trefz G, Gabriejelcic D, Turk V, Lah T. (1993) Cathepsin B and cysteine proteinase inhibitors in bronchoalveolar lavage fluid of lung cancer patients. Cancer Detect Prev, 17 (3), 387-397

10. Higashiyama M, Doi O, Kodama K, Yokouchi H, Tateishi R. (1993) Cathepsin B expression in tumor cells and laminin distribution in pulmonary adenocarcinoma. J Clin Pathol, 46, 18-22.

11. Heidtmann HH, Salge U, Abrahamson M, Bencina M, Kastelic L, Kopitar-Jerala N, Turk V, Lah TT. (1997) Cathepsin B and cysteine proteinase inhibitors in human lung cancer cell lines. Clin Exp Metastasis 15, 368-381.

12. Khan A, Krishna M, Baker SP, Banner BF. (1998) Cathepsin B and tumor-associated laminin expression in the progression of colorectal adenoma to carcinoma. Mod Pathol, 11, 704-708.

13. Krepela E, Prochazka J, Karova B, Cermak J, Roubkova H. (1997) Cathepsin B, thiols and cysteine protease inhibitors in squamous-cell lung cancer. Neoplasma, 44, 219-239.

14. Nakae T, Horai T, Imamura F, Akedo H, Higashino K. (1998) Expression of cathepsin B in small cell lung carcinoma cells in relation to in vitro invasiveness. Tumour Biol, 19, 118-125.

15. Rooprai HK, McCormick D. (1997) Proteases and their inhibitors in human brain tumours: a review. Anticancer Res, 17, 4151-4162

16. Sivaparvathi M, McCutcheon I, Sawaya R, Nicolson GL, Rao JS. (1996) Expression of cysteine protease inhibitors in human gliomas and meningiomas. Clin Exp Metastasis,14, 344-350.

17. Sivaparvathi M, Sawaya R, Gokaslan ZL, Chintala SK, Rao JS. (1996) Expression and the role of cathepsin H in human glioma progression and invasion. Cancer Lett, 104, 121-126

18. Sivaparvathi M, Sawaya R, Wang SW, Rayford A, Yamamoto M, Liotta LA, Nicolson GL, Rao JS. (1995) Overexpression and localization of cathepsin B during the progression of human gliomas. Clin Exp Metastasis, 1349-1356

19. Shuja S, Sheahan K, Murnane MJ. (1991) Cysteine endopeptidase activity levels in normal human tissues, colorectal adenomas and carcinomas. Int.J.Cancer, 49, 341-346.

20. Lowry OH, Rosebrough NJ, Farr AL., Randall RJ. (1951) Protein measurement with the folin phenol reagent. J Biol Chem, 193, 265-275.

21. Sivaparvathi M, Yamamoto M, Nicolson GL, Gokaslan ZL, Fuller GN, Liotta LA, Sawaya R, Rao JS. (1996) Expression and immunohistochemical localization of cathepsin L during the progression of human gliomas.

Clin Exp Metastasis,14, 27-34.

22. Boike G, Lah T, Sloane BF, Rozhin J, Honn K, Guirguis R, Stracke ML, Liotta LA,Schiffmann E A. (1992) Possible role for cysteine proteinase and its inhibitors in motility of malignant melanoma and other tumor cells. Melanoma Research, 1, 337-340

23. Budihna M, Strojan P, Smid L, Skrk J, Vrhovec I, Zupevc A, Rudolf Z, Zargi M, Krasovec M, Svetic B, Kopitar-Jerala N, Kos J. (1996) Prognostic value of cathepsins B, H, L, D and their endogenous inhibitors stefins A and B in head and neck carcinoma. Biol Chem Hoppe Seyler, 377, 385-390

24. Kamboj, R.C., Pal, S., Raghav, N. and Singh, H. (1993) A selective colorimetric assay for cathepsin L using Z-Phe-Arg-4-methoxy-b-naphthylamide. Biochemie, 75, 873-878.