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Monoamine oxidase B activity is increased in human gliomas

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Abstract

Glial tumours are the most common type of brain neoplasm in humans. Tumour classification and grading represent key factors for patient management. However, current grading schemes are still limited by subjective histological criteria. In this context, gliosis has been linked to increases in monoamine oxidase B (MAO-B) activity. Thus, in the present study, MAO-B activity in membranes of glial tumours ($n = 20$), meningiomas ($n = 12$) and non-pathological human brains ($n = 15$) was quantified by [^{14}C]PEA oxidation. MAO-B activity was significantly greater in glioblastoma multiformes than in postmortem control brains ($p < 0.01$) or meningiomas ($p < 0.001$). There were no significant differences in MAO-B activity between glioblastoma multiformes ($n = 11$) and low-grade astrocytomas ($n = 3$) or anaplastic astrocytomas ($n = 6$). In conclusion, the present results demonstrate a significant and selective increase in MAO-B activity in human gliomas when compared with meningiomas or non-tumoural tissue. These results suggest that the quantification of MAO-B activity may be a useful diagnostic tool for differentiating glial tumours from other types of brain tumours or surrounding normal brain tissue.

Keywords: MAO-B; Gliomas; Human brain

Glial brain tumours span a wide range of neoplasms with distinct clinical, histopathological, and genetic features (Kleihues et al., 2002). Furthermore, gliomas constitute the most frequent group of malignant primary brain neoplasm in humans and one of the most aggressive forms of cancer. Thus, accurate histopathological diagnosis is a first crucial prerequisite for patient treatment (Andratschke et al., 2001). However, the current morphological classification of glial brain tumours remains unsatisfactory (Behin et al., 2003). It is therefore essential to identify new molecular and biological markers for the diagnosis and grading of gliomas (Rasheed et al., 2002).

Monoamine oxidase (MAO, EC 1.4.3.4) exists in two forms: MAO-A and MAO-B. These isoenzymes differ in their sensitivities to the inhibitors clorgyline and deprenyl, and by their substrate specificities (Youdim et al., 2006). The MAO-B is the predominant subtype in the human brain (Stenström et al., 1987), where it is mainly localized in glial cells (Westlund et al., 1988). MAO-B activity in human brain has been described to increase with age both in postmortem tissue (Saura et al., 1997) as well as in living subjects (Fowler et al., 1997). Similarly, increased MAO-B activity has also been reported in the brains of patients with neurodegenerative disorders such as Alzheimer's and Huntington's diseases (Oreland and Gottfries, 1986; Mann et al., 1986; Sherif et al., 1992; Saura et al., 1994). Furthermore, this increase in MAO-B activity has been linked to gliosis involving reactive astrocytes (Oreland and Gottfries, 1986; Nakamura et al., 1990).

The aim of the present work was to evaluate the activity of the MAO-B in human glial tumours.

1. Experimental procedures

1.1. Brain samples

Small pieces of brain tissue containing tumour were collected at the time of craniotomy for tumour resection at the Neurosurgery Service of Cruces Hospital (Bizkaia, Spain) and stored at −70 °C until binding assays were performed. A second sample from each patient was also taken for diagnosis performed by

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neuropathologists and in accordance with the International Classification of CNS tumours drafted under the auspices of the World Health Organization (WHO). The tumours were diagnosed as low-grade astrocytoma (WHO grade I–II; $n = 3$), anaplastic astrocytoma (WHO grade III; $n = 6$), glioblastoma multiforme (WHO grade IV; $n = 11$) or meningioma ($n = 12$).

Human brain samples used as controls were obtained at autopsy in the Instituto Vasco de Medicina Legal (Bilbao, Spain) from 15 subjects without a history of neuropathological or psychiatric disorders and who had died suddenly, mainly in car accidents. Toxicological screening was negative for all these subjects and brain samples were histologically determined as normal. The frontal cortex of each subject was dissected at the time of autopsy, stored at −70 °C until assay and encoded in order to protect the identity of the subject. The time interval between death and autopsy (postmortem delay at 4 °C) was 35.6 ± 4.8 h. Sample collection was performed in accordance with approved protocols of the Instituto Vasco de Medicina Legal (Bilbao, Spain) for postmortem human studies.

All tissue samples were collected following protocols approved by the Human Studies Committee of each of the institutions involved. Informed consent was obtained from each surgical subject.

There were no significant differences in either male/female ratio or in age between the different experimental groups (Table 1). The age range for the different groups was as follows: 34–69 years for control brains, 39–68 years for glioblastomas, 36–70 years for meningiomas, 31–62 years for low-grade astrocytomas and 27–74 years for anaplastic astrocytomas.

1.2. Membrane preparation and MAO-B assays

Tumour containing samples were carefully dissected in order to isolate the abnormal tissue. Tissue samples of each subject (∼200 mg) were thawed and homogenized in 5 ml of ice-cold Tris–sucrose buffer (5 mM Tris–HCl, 250 mM sucrose, EDTA 5 mM, pH 7.4). The crude homogenate was centrifuged at 1100 × *g* (4 °C) for 10 min and the supernatant was recentrifuged at 40,000 × *g* (4 °C) for 10 min. The resultant pellet was washed twice in 2 ml of incubation buffer (50 mM Tris–HCl, pH 7.5) and recentrifuged in similar conditions. The final pellet was resuspended in an appropriate volume of incubation buffer for MAO-B assays. Final protein content was 1.42 ± 0.11 mg/ml for control brain ($n = 15$), 0.84 ± 0.15 mg/ml for glioblastomas ($n = 11$), 0.76 ± 0.12 mg/ml for meningiomas ($n = 12$), 1.18 ± 0.39 mg/ml for low-grade astrocytomas ($n = 3$) and 1.26 ± 0.20 mg/ml for anaplastic astrocytomas ($n = 6$). Protein content was determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Munich, Germany) using bovine albumin as standard.

MAO-B activity was assessed with a radioenzymatic method (Fowler and Tipton, 1981; Soto et al., 1999) in which radiolabelled β-[ethyl-1-^{14}C]phenylethylamine HCl ([^{14}C]PEA) was used as the substrate. Briefly, 50 μl of the membrane suspension were preincubated with 150 μl potassium phosphate buffer (50 mM; pH 7.2) at 37 °C for 5 min. Then the enzymatic reaction was started by adding 25 μl of the substrate solution (180 μM). Following a 4 min incubation period at 37 °C, the reaction was stopped with 100 μl citric acid (2 M) and the oxidation products were extracted into 2 ml ethyl acetate at −20 °C. Finally, 1 ml from the ethyl acetate phase from each tissue sample was transferred on to a vial containing 10 ml of Optiphase Hisafe II cocktail (Packard) and its radioactivity level was quantified by liquid scintillation spectrometry (Packard model 2200CA). Non-specific activity of MAO-B was considered as that obtained in the presence of deprenyl (3 μM). The non-specific activity was determined in every sample and consequently subtracted from the total activity in order to obtain the specific MAO-B activity. The results are expressed as pmol PEA min^{-1} mg^{-1} protein.

1.3. Statistical analyses

Values are expressed as means ± standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) with post hoc application of the Tukey's multiple comparison test as well as *t*-test were used for statistical evaluation. Level of significance was established at $p < 0.05$.

1.4. Materials

[^{14}C]PEA (specific activity 41.8 mCi/mmol) was purchased from Du Pont NEN (Belgium). Deprenyl HCl was from R.B.I. (USA). Clorgyline HCl and PEA were obtained from Sigma (USA). All other chemical reagents were of analytical quality and were purchased from Merck (Darmstadt, Germany) or Sigma (USA).

2. Results

In order to determine the degree of selectivity of the radioenzymatic assay with [^{14}C]PEA for the A and B forms of monoamine oxidase, inhibition experiments were performed in control ($n = 3$) and glioblastoma samples ($n = 3$) with the selective irreversible inhibitors clorgyline and deprenyl (10^{-12} to 10^{-3} M). MAO activity, defined as [^{14}C]PEA oxidation, was sensitive to inhibition by nanomolar concentrations of the MAO-B selective inhibitor deprenyl (Fig. 1). This effect was similar in control brain membranes ($IC_{50} = 5.56 \pm 1.14$ nM, $n = 3$) and glioblastomas ($IC_{50} = 5.20 \pm 1.21$ nM, $n = 3$). Conversely, the MAO-A selective inhibitor clorgyline only inhibited MAO activity at high micromolar concentrations ($IC_{50} = 10.72 \pm 1.19$ μM, $n = 3$; and 9.88 ± 1.20 μM, $n = 3$, for control brain and glioblastoma membranes, respectively) (Fig. 1). These results confirmed the selectivity of the assay for determining MAO-B activity.

Table 1
Demographic characteristics and MAO-B activity for the different human brain tumours and normal cerebral tissues

	Age (years)	Gender	MAO-B activity (pmol min^{-1} mg^{-1} protein)	*n*
Postmortem control	54 ± 3	9M/6F	692 ± 93	15
Low-grade astrocytoma	39 ± 8	2M/1F	2506 ± 739	3
Anaplastic astrocytoma	49 ± 7	4M/2F	2676 ± 744	6
Glioblastoma multiforme	54 ± 3	6M/5F	2834 ± 930$^{**,\#\#\#}$	11
Meningioma	55 ± 3	6M/6F	205 ± 72	12

Values are expressed as mean ± S.E.M. $^{**}p < 0.01$ vs. postmortem control; $^{\#\#\#}p < 0.001$ vs. meningioma (ANOVA followed by the post hoc Tukey's multiple comparison test).

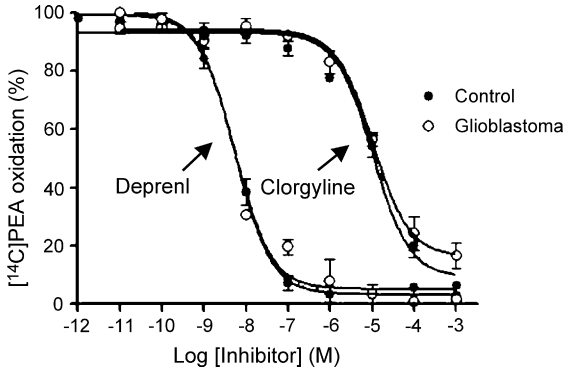

Fig. 1. Inhibition of specific MAO activity by indicated concentrations of deprenyl or clorgyline. Tissue homogenates were preincubated with different concentrations of deprenyl or clorgyline. MAO activity was assayed with 1 μM [^{14}C]PEA as substrate. Each point is the mean ± S.E.M. of three determinations.