SHORT REPORT

Imidazoline I₂ receptor density increases with the malignancy of human gliomas

L F Callado, J I Martín-Gómez, J Ruiz, J M Garibi, J J Meana

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Background: Current glioma grading schemes are limited by subjective histological criteria. Imidazoline I₂ receptors are principally expressed on glial cells.

Objective: To investigate the feasibility of using the measurement of imidazoline I₂ receptor expression to differentiate glial tumours from other types of brain tumours and for grading the different gliomas.

Methods: The specific binding of [³H]idazoxan to imidazoline I₂ receptors was measured in homogenates from human gliomas of different grades.

Results: The density of imidazoline I₂ receptors was significantly greater in the three types of malignant glial tumours than in postmortem control brain or non-glial tumours. The increase in density correlated with the malignancy grade of the gliomas. No significant differences in affinity values were observed.

Conclusion: These results suggest that the density of imidazoline I₂ receptors may be a useful radioligand parameter for the differentiation of glial tumours from other types of brain tumours and for grading the different gliomas.

Gliomas constitute the most important group of brain tumours in humans,¹ and accurate histopathological diagnosis is a crucial prerequisite for patient treatment.² However, it has been suggested that diagnostic accuracy and reproducibility may be compromised by interobserver variability and subjective histological criteria used to classify and grade gliomas.^{3–4} Therefore, there is now increased interest in finding new molecular and biological markers to establish the diagnosis and grade of gliomas.⁵

The imidazoline I₂ receptors (I₂-IRs) are widely distributed in the brain and seem to be found principally on glial cells.⁶ It has been suggested that glial I₂-IRs have a direct role in the regulation of glial fibrillary acidic protein (GFAP) concentrations.⁷

We have previously demonstrated an increased density of imidazoline receptors of the pharmacological I₂ receptor subtype in human glioblastomas.⁸ Thus, the aim of our present study was to assess whether this increase in density was specific to glial tumours and correlated with the grade of malignancy.

MATERIALS AND METHODS

Small pieces of brain tumour were collected at craniotomy for resection. Samples were also taken for diagnosis by neuropathologists according to the World Health Organisation international classification of central nervous system tumours. Surgical control samples were obtained from patients who underwent surgery for other pathologies and which were found to be histologically normal. Postmortem brain controls were obtained at necropsy from patients with

no history of neuropathological or psychiatric disorders. Toxicological screening was negative for all subjects. Frontal cortex and white matter were dissected and stored at –70°C until assay.

All tissue samples were collected under an approved protocol from each institution's human studies committee. Informed consent was obtained from each patient undergoing surgery.

Animal experiments were conducted in accordance with the European Union animal welfare guidelines in male Sprague-Dawley rats. Rats were dosed with dexamethasone (0.2 mg/kg, intraperitoneally (i.p.), every 24 hours) and phenytoine (20 mg/kg, i.p., the first day, and 5 mg/kg, i.p. every 24 hours for the rest of the treatment period), or saline for 14 days. Twenty four hours after the last dose the rats were sacrificed, the brain cortex was excised and stored at –70°C until assay.

Membrane P2 fractions were prepared as described previously.⁹ Protein concentrations (0.5–1.4 mg/ml) were not different between the groups.

[³H]idazoxan saturation experiments (1.25–40 nM; eight concentrations) were performed at 25°C for 30 minutes in 550 µl aliquots of membranes. To prevent the binding of [³H]idazoxan to α₂ adrenoreceptors, (–)adrenaline (5 × 10^{–6} M) was added to the final incubation medium. Non-specific binding was estimated in the presence of 10^{–4} M naphazoline.

Data were analysed by non-linear least square curve fitting with the program LIGAND.¹⁰ Values were expressed as means (SEM). The affinity values (K_d) were normalised as log K_d value to be compared.¹¹ ANOVA and covariance (ANCOVA) with post hoc application of Tukey's multiple comparison test were used for statistical evaluation at p < 0.05.

[³H]idazoxan ((1,4-[6,7-³H]benzodioxan-2-yl)-2-imidazoline HCl; 43–44 Ci/mmol) was purchased from Amersham (Little Chalfont, Buckinghamshire, UK). (–)Adrenaline bitartrate, dexamethasone, and naphazoline HCl were from Sigma (St Louis, Missouri, USA). Phenytoine (Epanutin®) was from Parke Davis SI (Detroit, USA).

RESULTS

The specific binding of [³H]idazoxan (1.25–40 nM) in the presence of (–)adrenaline (5 × 10^{–6} M) to membranes from human control brain tissue or tumours was a saturable process of high affinity. In all cases, non-linear analyses of individual saturation isotherms revealed the existence of a single population of sites.

White matter obtained from normal brains, ependimomas, schwannomas, and metastases from other primary tumours showed similar [³H]idazoxan binding properties to postmortem frontal cortex, which was used as control (table 1). Similarly, there were no differences in K_d or B_{max} between

Abbreviations: GFAP, glial fibrillary acidic protein; I₂-IR, I₂ imidazoline receptor; i.p., intraperitoneally; PET, positron emission tomography

Table 1 Demographic characteristics and [³H]idazoxan binding parameters for the different human brain tumours and normal cerebral tissues

	Age	Sex	B _{max} (fmol/mg protein)	K _d (nM)	N
Postmortem control	49 (4)	13M/12F	71 (6)	13.3 (1.2)	25
Surgical control	44 (3)	2M/1F	76 (21)	15.4 (3.7)	3
White matter	52 (7)	4M/3F	91 (14)	12.9 (2.2)	7
Low grade astrocytoma	45 (5)	4M/4F	123 (17)*	14.4 (1.9)	8
Anaplastic astrocytoma	44 (6)	3M/2F	195 (31)**	14.4 (4.4)	5
Glioblastoma	55 (4)	7M/7F	268 (33)**	16.9 (2.3)	14
Meningioma	59 (3)	5M/7F	14 (6)*	14.2 (3.4)	12
Ependimoma	33 (7)	2M/4F	86 (17)	10.8 (2.7)	6
Schwannoma	34 (12)	5M	44 (14)	14.2 (4.4)	5
Metastasis	60 (4)	8M/2F	106 (40)	23.6 (3.8)	10

Values are expressed as mean (SEM). B_{max} represents the density of imidazoline I₂ receptors. K_d represents the equilibrium dissociation constant for [³H]idazoxan. *p<0.005, **p<0.001 v control (ANOVA with post hoc application of the Tukey's multiple comparison test).

control, postmortem brain samples, and normal surgical brain tissue (table 1). Meningiomas had a significantly reduced density compared with controls, with no changes in the affinity (table 1).

The mean density of I₂-IRs obtained from individual B_{max} values was significantly greater in the three types of malignant glial tumours than in postmortem control brain (table 1; fig 1). This significance did not change when controlled for age (ANCOVA: F[3,42] = 18.782; p < 0.0001). The B_{max} for [³H]idazoxan binding was also significantly greater in anaplastic astrocytomas and glioblastomas when compared with low grade astrocytomas (p < 0.05 and p < 0.001, respectively). Similar analyses showed no differences in affinity values between glial tumours and control tissue or between the different types of glial tumours (table 1; fig 1).

To avoid a possible bias induced by pharmacological treatment of patients before surgery, binding experiments were performed in brain cortical membranes of rats treated with dexamethasone plus phenytoine and corresponding saline controls.

In these assays [³H]idazoxan (1.25–40nM), in the presence of (–)adrenaline (5 × 10^{–6}M), bound with high affinity to a single population. The parameters were similar in treated (K_d = 14.7 (1; B_{max} = 85 (4; n = 8)) and control rats (K_d = 15.1 (1.2; B_{max} = 94 (5; n = 8)).

DISCUSSION

We found a significant increase in I₂-IR density in human gliomas when compared with normal brain tissue and other intracranial non-glial tumours. Moreover, this increase seemed to correlate with the degree of malignancy in human gliomas. Thus, in glioblastomas, the density of I₂-IRs was 1.4 times higher than in anaplastic astrocytomas and 2.2 higher than in low grade astrocytomas. Accordingly, the density of these receptors in anaplastic astrocytomas was 1.6 times higher than in low grade astrocytomas.

An increase in the density of I₂-IRs has been reported in the human brain in association with ageing,¹² probably related to the enhanced gliosis that appears with ageing. However, the influence of this factor in our results can be discarded because differences were also significant when the age variable was controlled. Another possible bias could be related to the treatment given to the patients before the tumour was excised. In our health service, dexamethasone and, occasionally, phenytoine are given to patients with brain tumours before neurosurgery. However, the experiments performed in animals demonstrated that this treatment did not significantly change the [³H]idazoxan binding parameters.

Figure 1 (A) Individual B_{max} and (B) log K_d values of [³H]idazoxan binding in postmortem brain controls, low grade astrocytomas, anaplastic astrocytomas, and glioblastomas. The lines represent the mean value of each population.

As compared with normal glial cells, gliomas are characterised by specific genomic alterations that may induce overexpression of receptors. In agreement with our results, the increased expression of other receptors, such as peripheral benzodiazepine receptors, has been reported in gliomas.¹³ Moreover, in some cases receptor expression correlated with the malignancy of the tumour.¹⁴ This increase in receptor density may be related to signal transduction or cell cycle alterations that contribute to abnormal proliferation in tumours. The simultaneous increase in receptor density and tumour malignancy shown in our results could be explained

in this context. Accordingly, blockade of overexpressed receptors has been reported to reduce the growth of gliomas.^{15–16} In this context, despite the functional role of I₂-IRs still being unclear, they could regulate the expression of GFAP.⁷

The classification and grading of human gliomas rely on histopathological and immunohistochemical criteria. However, there are many problems with reproducibility and interobserver variability in establishing the classification and grading of primary gliomas.⁴ An incorrect diagnosis can affect immediate patient care decisions, resulting in inadequate treatment of the tumour.^{3–4} In this context, several studies have reported molecular alterations in gliomas that could provide new and more accurate approaches for the evaluation and classification of these tumours.^{17–19} For example, recent technological advances allow us to study tumours in living patients using in vivo imaging. The application of positron emission tomography (PET) has proved useful for grading and prognosis.²⁰ Thus, energy metabolism measurements with PET are widely performed in gliomas in vivo.²⁰ The fact that we found increased I₂-IR density to be specific for glial tumours and to correlate with the degree of malignancy may indicate that these receptors could be used in the diagnosis of gliomas. The binding technique used in our study confirmed that the recognition of these receptors in gliomas is not only specific but of high affinity. Thus, radiolabelled ligands for I₂-IRs could be developed as suitable tools for in vivo studies using PET.

In conclusion, our results demonstrate not only a significant and selective increase in the density of I₂-IRs in human gliomas, but a correlation between this parameter and the degree of malignancy of the tumours. Thus, the density of I₂-IRs might be a useful parameter permitting differentiation of gliomas from other types of brain tumours and even supporting their classification.

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Authors' affiliations

I F Callado, J I Martín-Gómez, J Ruiz, J J Meana, Department of Pharmacology, University of the Basque Country, E-48940 Leioa, Spain
J M Garibi, Neurosurgery Service, Cruces Hospital, Barakaldo, Bizkaia, Spain

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Correspondence to: Dr I F Callado, Department of Pharmacology, University of the Basque Country, E-48940 Leioa, Bizkaia, Spain; kfbcahel@lg.ehu.es

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