Clinical Neurology and Neurosurgery 115 (2013) 2075–2081

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Aquaporin-4 in glioma and metastatic tissues harboring 5-aminolevulinic acid-induced porphyrin fluorescence

Eric Jose Suero Molina[a,*], Hilko Ardon[a,b,c], Juliane Schroeteler[a], Mark Klingenhöfer[a], Markus Holling[a], Johannes Wölfer[a], Bernhard Fischer[a], Walter Stummer[a], Christian Ewelt[a]

[a] Department of Neurosurgery, University Hospital of Münster, Münster, Germany
[b] Katholieke Universiteit Leuven, Leuven, Belgium
[c] Sint Elisabeth Ziekenhuis, Tilburg, The Netherlands

ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 7 July 2013
Accepted 9 July 2013
Available online 31 July 2013

Keywords:
Aquaporin-4
Aquaporin-1
Glioblastoma multiforme
5-Aminolaevulinic acid
Metastasis

ABSTRACT

Introduction: Aquaporin channels (AQPs) are a group of integral membrane proteins that regulate the transport of water through cell membranes. Previous studies have shown that up-regulation of AQP1 and AQP4, two of the predominant AQPs in the human brain, in high grade glial tumors contribute to cerebral edema. Others link AQPs to the regulation of human glioma cell migration and invasion. The aim of this study was to determine AQPs expression in tumor tissue harboring 5-aminolevulinic acid (ALA)-induced porphyrin fluorescence with flow cytometry and compare it to the expression in normal brain tissue.

Methods: Tissue samples were obtained from fluorescing brain tumors of 26 patients treated with ALA prior to surgery (20 mg/kg b.w.). Expression levels of aquaporin channels were measured in primary tissue cultures using a FACS CANTO I flow cytometer. A control group consisted of four non-fluorescing tissue samples, the C6 and the U87 cell line.

Results: Nineteen gliomas (14 high grade, 5 low grade) and 7 metastases were analyzed. On the 4th post-operative day, expression levels of AQP4 channels, but not of AQP1 channels, were significantly increased in samples from fluorescing tissue compared to non-fluorescing tissue. In addition we could see how ALA induces fluorescence in metastases.

Conclusion: Flow cytometry appears to be an auspicious method for the analysis of porphyrins and AQPs in primary brain cell tumor cultures. ALA fluorescing tissue showed higher AQP4 expression compared to normal brain tissue. The demonstrated expression in a context with ALA could open a targeted therapeutic spectrum, for example to selectively target AQP4.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The aquaporin channels (AQPs) are a group of integral membrane proteins that transport water through cell membranes [1]. To date, thirteen isoforms of AQPs, AQP0–AQP12, have been identified in human cells, each of them with distinct tissue distribution [2]. AQP0, 1, 2, 4, 5 and 8 are permeable for water, whereas AQP3, 7, 9 and 10, also termed aquaglyceroporins, are permeable for water, glycerol, urea and possibly other nonpolar solutes [3]. In the human brain, the predominant expression of AQP1, AQP4, and AQP9 has been described [3]. Aquaporin-4 (AQP4) is the most abundant isoform and is expressed mainly in the basolateral membrane of ependymal cells and perivascularly at the astroglial endfeet membranes [4]. In addition, AQP4 location at the blood–brain-barrier (BBB) was demonstrated by immunogold electron microscopy suggesting its key role in regulating brain water homeostasis [5]. Aquaporin-1 (AQP1) is expressed in the epithelium of the choroid plexus and participates in cerebrospinal fluid production [6,7]. Aquaporin-9 (AQP9) is expressed in astrocytes, subpopulations of neurons and endothelial cells of subpial blood vessels [8–11].

High grade gliomas are frequent and malignant cerebral neoplasms. Their diffuse and invasive growth, together with a high frequency of recurrence, are associated with a poor prognosis [12–14]. Due to the poor prognosis of these patients new therapy approaches are required. Previous studies have shown AQP1 and AQP4 to be up-regulated in high grade glial tumors compared to low grade glial tumors or normal brain tissue, possibly contributing to cerebral edema [15,16]. Other authors link AQPs to the regulation of human glioma cells migration and invasion [17].

* Corresponding author at: Department of Neurosurgery, University Hospital of Münster, Albert-Schweitzer-Campus 1, A1, D-48149 Münster, Germany.
Tel.: +49 251 8347472; fax: +49 251 83 47479.
E-mail address: eric.suero@ukmuenster.de (E.J. Suero Molina).

0303-8467/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.clineuro.2013.07.016

Five-aminolevulinic acid (ALA) induced porphyrin fluorescence is specific for high grade gliomas and other tumors [18,19]. ALA, as a non-fluorescent prodrug, leads to intracellular accumulation of fluorescent porphyrins in malignant neoplasms. Fluorescence guided neurosurgery after administration of ALA has been shown to improve resection of contrast-enhancing tumor, prolonging progression-free survival [19–22].

The objectives of the present study were to determine AQP1 and AQP4 expression in primary cultures from tumor tissue harboring 5-aminolevulinic acid (ALA)-induced porphyrin fluorescence and compare it to the expression in normal brain tissue.

2. Materials and methods

Twenty-six patients presenting intracerebral neoplasms with pre-operative imaging suggestive for malignant glial tumors were treated at the Department of Neurosurgery, University Hospital of Münster. Informed consent protocol for surgery and further laboratory investigations was approved by the Ethics Committee of the University of Münster. Twenty-six tumors (14 high grade glioma, 5 low grade gliomas and 7 metastases) were classified using the World Health Organization (WHO) grading system [23].

Control groups consisted of four normal brain samples without fluorescence, the C6 rat glioma cell line and the human glioma cell line U87. Normal brain tissue was obtained during temporal lobe resections after administration of ALA where tumor did not infiltrate the temporal pole.

Four hours before induction of anesthesia, patients were given 20 mg/kg ALA orally [20]. Tumor fluorescence was visualized using a modified operating microscope with violet-blue excitation light (375–440 nm) (Pentero surgical microscope, Zeiss, Oberkochen, Germany). Tumor samples were exclusively taken from fluorescing tissue. In order to obtain primary tumor cell cultures, specimens were immediately lysed after surgery using collagenase type 1 (Worthington, Lakewood, NJ, USA) and DNAse (Worthington, Lakewood, NJ, USA) diluted in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Darmstadt, Germany). Tumor tissue was trimmed and cells were harvested with the collagenase solution and incubated for 30 min at 37° and 5% CO_2. Subsequently, cells were centrifuged and gently washed with phosphate buffered saline (PBS) (Invitrogen, Darmstadt, Germany). All cultures were maintained as monolayer cultures in growth medium (DMEM without phenol red (Invitrogen, Darmstadt, Germany)) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM MEM-Vitamins (all Biochrom AG) and 2 mM L-glutamine (Invitrogen, Darmstadt, Germany). The cells were then cultured in T75 tissue culture flasks (Greiner BioOne, Frickenhausen, Germany) at 37 °C and 5% CO_2 and regularly passaged at 80% confluency.

To minimize effects of ALA-induced fluorescence, first experiments were carried out after four days of culture. Cultures were washed on the 4th and 14th post-operative day and harvested with trypsin (Invitrogen, Darmstadt, Germany). 10^6 cells were fixed and permeabilized saponin-mediated using BD Cytofix/Cytoperm (BD Bioscience) and afterwards, suspended in BD Perm/Wash buffer (BD Bioscience).

Cells were subsequently stained with fluorescence labeled monoclonal antibodies specific for AQP1 or AQP4 (both Santa Cruz biotechnology Inc.) and glial fibrillary acidic protein (GFAP) (BD Pharmingen) or their respective isotype controls and incubated for 20 min at 4 °C in the dark. The astrocyte marker GFAP was used to identify glial cells. After centrifugation at 500 × *g* for 5 min, cells were washed once with BD Perm/Wash buffer and centrifuged and 0.5 ml of PBS was added to the cell pellet. The expression levels of AQPs and GFAP were measured employing a FACS Canto I flow cytometer (BD Biosciences). Sample data were analyzed using BD FACS Diva V6.1.3 (BD Biosciences). Cellular events were gated based on forward and side scatter.

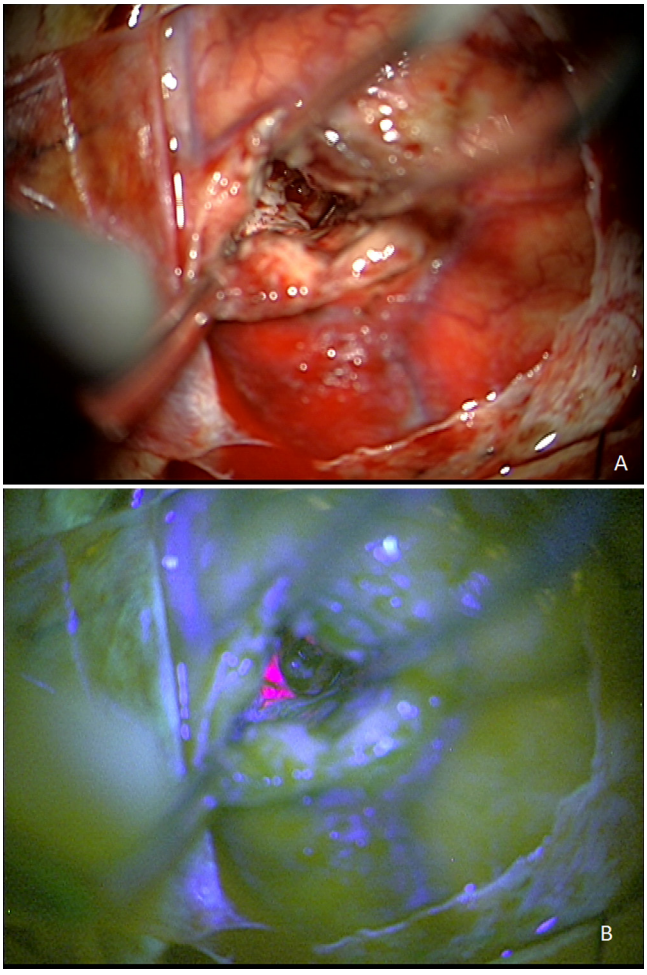

Fig. 1. ALA-mediated fluorescence guided resection. Intraoperative microscope view with white light (a) and with fluorescing light (b). All tumor samples were exclusively taken from ALA fluorescing tissue inside the tumor area. A part of specimen was analyzed histologically and immunohistochemically to define tumor entity.

All data analysis was performed using BD FACS Diva V6.1.3 (BD Biosciences), Prism (Graphpad, La Jolla, CA) and Microsoft Excel 2010 (Redmond, WA). All statistical tests were considered significant at a probability level of less than 0.05. Data are presented as mean ± standard deviation. For comparison of data, the unpaired *t*-test with Welch's correction was used. Values are given as average results and standard deviation.

3. Results

All tumor samples were exclusively taken from ALA fluorescent tissue inside the tumor area (Fig. 1). Prior to neurosurgical intervention malignant glioma was considered in all tumors as differential diagnosis. After histological and immunohistochemical investigation seven specimens where found to be metastases.

3.1. AQPs in glial tumors with ALA induced fluorescence

AQP4-expression was significantly higher in high grade gliomas ($N = 14$) than in the non-fluorescing control specimens ($N = 4$) ($^{***}P = 0.0005$). Among the positive AQP4 cells a common AQP4/GFAP cell population was found in all gliomas ($N = 19$) on

Download English Version:

https://daneshyari.com/en/article/6006568

Download Persian Version:

https://daneshyari.com/article/6006568

Daneshyari.com