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X-ray fluorescence study of the concentration of selected trace and minor elements in human brain tumours

Aleksandra Wandzilak [a,*], Mateusz Czyzycki [a,b], Edyta Radwanska [c], Dariusz Adamek [c], Kalotina Geraki [d], Marek Lankosz [a]

[a] AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, al. A. Mickiewicza 30, 30-059 Krakow, Poland
[b] International Atomic Energy Agency, Department of Nuclear Sciences and Applications, Division of Physical and Chemical Sciences, Physics Section, Wagramer Str. 5, A-1400 Vienna, Austria
[c] Jagiellonian University, Faculty of Medicine, Chair of Pathomorphology, Department of Neuropathology, Grzegorzecka 16, 31-531 Krakow, Poland
[d] Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxon OX11 0DE, UK

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ABSTRACT

Neoplastic and healthy brain tissues were analysed to discern the changes in the spatial distribution and overall concentration of elements using micro X-ray fluorescence spectroscopy. High-resolution distribution maps of minor and trace elements such as P, S, Cl, K, Ca, Fe, Cu and Zn made it possible to distinguish between homogeneous cancerous tissue and areas where some structures could be identified, such as blood vessels and calcifications. Concentrations of the elements in the selected homogeneous areas of brain tissue were compared between tumours with various malignancy grades and with the controls. The study showed a decrease in the average concentration of Fe, P, S and Ca in tissues with high grades of malignancy as compared to the control group, whereas the concentration of Zn in these tissues was increased. The changes in the concentration were found to be correlated with the tumour malignancy grade. The efficacy of micro X-ray fluorescence spectroscopy to distinguish between various types of cancer based on the concentrations of studied elements was confirmed by multivariate discriminant analysis. Our analysis showed that the most important elements for tissue classification are Cu, K, Fe, Ca, and Zn. This method made it possible to correctly classify histopathological types in 99.93% of the cases used to build the model and in as much as 99.16% of new cases.

1. Introduction

Neoplastic processes involve changes in cell metabolism. The activities of many enzymes and hormones change. This can alter the concentration and distribution of trace elements taking part in metabolic processes in the tissues. Trace elements play an important role in metabolic processes [1–5]. Iron participates in the formation of reactive oxygen species and consequently contributes to the oxidative stress. Zinc and copper as components of superoxide dismutase (CuZnSOD) are involved in the reduction of the superoxide anion radical. Both these elements can therefore affect the level of reactive oxygen species — the main causes of lipid peroxidation and damage to DNA and other parts of the cell. In addition, copper plays an important role in angiogenesis in a growing tumour [6].

In recent years there has been an interest in determining how trace elements concentration and distribution changes as a corollary of neoplastic processes. Such knowledge may prove helpful in diagnosing cancer and shedding light on the mechanism of processes associated with carcinogenesis. A number of studies have been published on both tumours in animals and various types of human cancer. Feldstein et al. [7] performed experiments on mice inoculated with various types of cancer. The experiment showed changes in the concentration of trace elements as a function of time elapsed from inoculation. They noted a considerable increase in the concentration of some elements (ten-fold in the case of rubidium) and a decrease in the case of Fe with time elapsed from inoculation. Changes in the concentration of some trace elements in cases of breast cancer were studied by Silva et al. [8]. They found that the concentrations of Ca, Fe, Cu and Zn are greater in cancerous tissues compared to the control, although interestingly, the level of Fe was greater in benign tumours than in malignant ones. Geraki et al. [9] also studied breast cancer and noted greater concentration of K, Cu, Zn and Fe in cancerous tissues. Studies on human brain tumours are more difficult due to the limited availability of samples, which are taken intraoperatively and frequently are not accompanied by healthy tissue for comparison. An attempt to use the information on the trace elements distribution in human brain tissues for tumour type classification was undertaken in study by Szczerbowska-Boruchowska et al.

* Corresponding author. Tel.: +48 12 617 39 15.
E-mail address: aleksandra.wandzilak@fis.agh.edu.pl (A. Wandzilak).

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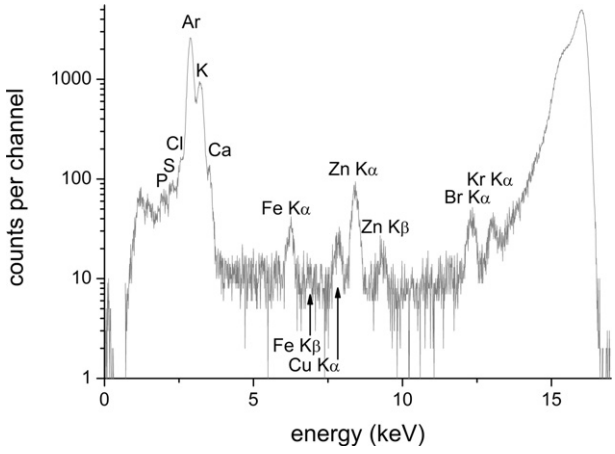

Fig. 1. XRF spectrum of cancerous brain tissue obtained at an energy of 16.5 keV.

Table 1
Limits of detection and limits of quantitation expressed in μg/cm2.

	P	S	Cl	K	Ca	Fe	Cu	Zn
LOD	0.48	0.11	0.11	0.02	0.023	0.0082	0.0021	0.0023
LOQ	1.61	0.36	0.37	0.07	0.077	0.0274	0.0069	0.0077

using the method of multivariate discriminant analysis (MDA) [10]. Other researchers focussed on investigating not only the overall changes in the concentration of trace elements caused by the neoplastic process, but also the accompanying variations in the oxidation state of trace elements [11–13]. In our recent publication [14] we demonstrated a correlation between the oxidation state of iron present in human brain tumour samples and their malignancy grade as defined by WHO [15]. We observed that the ratio of Fe2 + to Fe3 + increases with the tumour malignancy grade. The information on changes in the quantity of iron and other biologically significant elements in the brain samples we have been studying compliments the data on their oxidation states.

In this paper an attempt is made to show how the neoplastic process affects the concentration of trace elements such as Fe, Cu and Zn and minor ones such as P, S, Cl, K and Ca in brain tissues. For this purpose X-ray fluorescence microprobe (μ-XRF) was employed, as this method is particularly well suited for measuring concentrations of elements at the ppm level. Additionally, this method is non-destructive, which is essential in the case of hard-to-obtain human brain samples. Also, to obtain information on whether these elements are useful for the identification of histopathological types of brain tumours, MDA was used. This research is a step forward in determining the most promising areas for the further studies on the potential role of trace and minor elements in the neoplastic process.

2. Materials and methods

2.1. Sample preparation

The samples were obtained from the material collected during resection of brain tumours conducted in the Clinic of Neurosurgery and Neurotraumatology, Collegium Medicum, Jagiellonian University in Krakow, Poland. The tissues were cryo-sectioned and specimens were prepared for both histological examination and elemental analysis. One section of each sample was stained with haematoxylin–eosin to determine the type and grade of malignancy of the tumour and the adjacent piece of tissue was cut to a thickness of 20 μm, and then placed on 4 μm thick X-ray-transparent Ultralene foil stretched on a polymer disc. Next the sample was dried at a temperature of −80 °C. The analysed tissues represented various types of brain tumours whose malignancy grades were determined in accordance with the World Health Organisation (WHO) classification [15]. These included multiform glioblastoma (WHO IV), anaplastic astrocytoma (WHO III), anaplastic oligodendroglioma (WHO III), diffuse astrocytoma (WHO II), and meningothelial meningioma (WHO I). Benign tumour, containing numerous calcifications, was also analysed. Samples taken from patients who died of causes other than cancer (ruptured aneurysm) were used as controls. The histopathological examination was conducted at the Department of Neuropathology, Collegium Medicum UJ.

The study was approved by the Jagiellonian University Bioethical Committee (KBET/101/B/2010).

2.2. Measurement conditions

Experiments were carried out at beamline I18 of Diamond Light Source in Didcot, UK. The synchrotron radiation was monochromatized by a double crystal Si (111) monochromator. The beam was focused to a size of 2 μm x 4 μm using a pair of Kirkpatrick–Baez mirrors. The energy of the exciting radiation was set to 16.5 keV. The fluorescence measurements were performed with the sample surface positioned at 45° with respect to the beam direction and 45° to the detector. Both the samples and the reference materials were measured in the same geometry. The characteristic fluorescence radiation was detected by a 4-element SDD detector. Normalisation to the beam current was achieved by monitoring with an ionisation chamber the beam impinging on the sample. The measurements were conducted in air. All X-ray scans were conducted at room temperature. As reference materials, certified NIST SRM 1832 and SRM 1833 standards with certified amounts of elements expressed in μg/cm2 were used [16]. They were measured in the same geometry as the samples.

3. Results and discussion

XRF spectroscopy was used to measure emission spectra on twenty six areas on eleven brain samples. The size of each area was between 50 μm × 100 μm and 100 μm × 300 μm. An example XRF spectrum

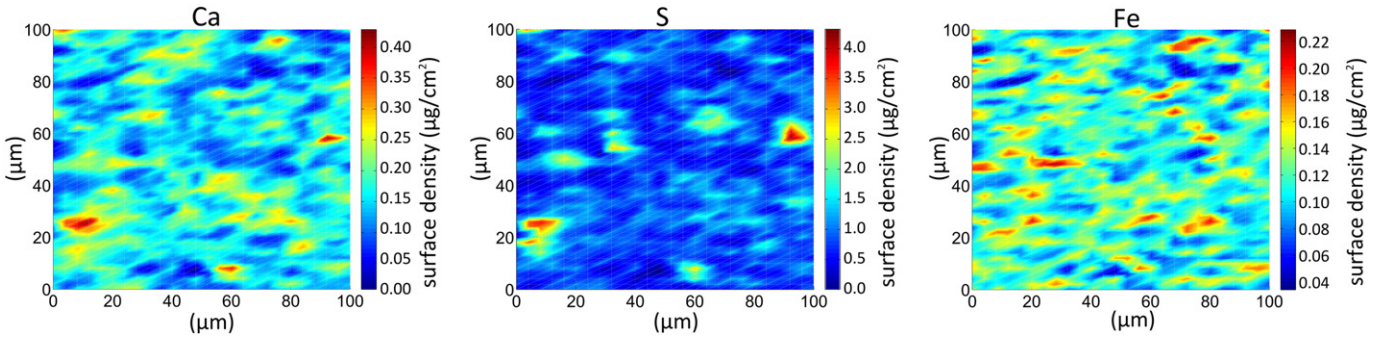

Fig. 2. Distribution of Ca, S and Fe in a section of diffuse astrocytoma.