Particle Induced X-Ray Emission Examination in Post-Mortem Brains

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ABSTRACT

Background: Particle induced X-ray emission (PIXE) allows to demonstrate the distribution of different minor and trace elements in post-mortem brains.

Objective: This article reviews the available data on PIXE in normal and diseased human post-mortem brains.

Methodology: Fresh non-formalin brain samples have to be used. The brain dissection has to be performed in a sterile room and in order to avoid the risk of metal contamination it is performed with molybdenum instruments. The brain samples are weighed, freeze-dried and weighed again to determine the wet-to-dry weight ratio. Afterwards the samples are submitted to acid digestion. Ten µl of the obtained solution are piped onto a target film and submitted to proton beam irradiation. The PIXE spectrum shows the levels of potassium, calcium, manganese, iron, cupper, zinc, selenium and rubidium.

Results: The distribution of the minor and trace elements in normal brains vary according to the regions and in structures, involved in the same physiological function or morphologically similar. They predominate in the gray matter structures. In the rare diseased brains examined with PIXE the alteration of the minor and trace elements differ according to the underlying disease.

Discussion: It can be concluded that post-mortem PIXE analysis can be an additional tool to compare the pathology of normal to diseased brains.

Keywords: Particle induced X-ray emission, Post-mortem brains, Wet-to-dry weight ratio, Potassium, Calcium, Manganese, Cupper, Zinc, Selenium, Rubidium.

INTRODUCTION

Particle induced X-ray emission (PIXE) is an X-ray spectrographic technique, which can be used for the non-destructive, simultaneous elemental analysis of solid, liquid or aerosol filter samples. Energetic protons initiate the X-ray spectrum by exciting the inner shell electrons in the target atoms [1]. PIXE allows a multi-analysis of biological material [2]. In the human brain it demonstrates a spectrum of eight elements. The dry weight concentrations of potassium (K), calcium (Ca), manganese (Mn), iron (Fe), cupper (Cu), zinc (Zn), selenium (Se) and rubidium (Rb) can be determined with this technique [3]. The accuracy of the method has been previously shown by comparison of animal brain matter examined with PIXE analysis and with instrumental neutron activation analysis [4]. There are topographic differences in concentrations of these elements depending on the degree of neuronal density of the examined structure [5].

Brain metal homeostasis is altered in neurodegenerative and cerebrovascular diseases [6]. Using other methods such as magnetic resonance imaging and quantitative susceptibility mapping many studies have mainly focussed on the quantification of the Fe content in normal and diseased brains [7-9].

In rat brains the Fe concentration is the highest in the cytoplasm of neocortical oligodendrocytes, followed by microglia and astrocytes and less of all in neurons [10].

METHODOLOGY

Formalin fixation of the brain has to be avoided mainly because of the lost of extra-cellular and intra-cellular fluid of the tissues. It shows only a part of the complete biochemical “picture” of the tissue samples [11].

For the dissection of the non-formalin fixed brain special adequate surgical instruments and tools as well as an adequate sampling procedure are needed [12]. The brain dissection has to be...
performed in a sterile room and in order to avoid the risk of metal contamination it is performed with molybdenum instruments [13].

In our laboratory fresh brains are removed at autopsy, according to standard procedures, within 72 hours after death and sealed in a polyethylene bag. They are then placed in a deep-freezer at -30°C for a period of 14 days. At about 12 hours before the dissection the frozen brains are removed from the freezer, and placed in a refrigerator at -2°C until the actual dissection is performed. The tissue samples are taken from representative parts of the cerebral cortex, cerebral white matter, basal ganglia, thalamus, brainstem and cerebellar cortex. In our laboratory 50 samples of each brain are removed. They are weighed, freeze-dried and weighed again, allowing the determination of the wet-to-dry weight ratio. Afterwards the samples are submitted to acid digestion. Ten µl of the obtained solution are piped onto a target film and submitted to proton beam irradiation. All concentration data obtained are expressed on a dry weight base. The PIXE spectrum of the individual samples is obtained by proton beam irradiation, measured with a Silicon detector. This procedure does not produce alterations in the trace element levels of the individual brain structures [14].

RESULTS

PIXE Spectrum in Normal Brains

The wet-to-dry weight ratio is the highest in the cerebral and cerebellar cortex (6-7 times), followed by the basal ganglia and thalamus (5-6 times) and the lowest in the cerebral white matter (3-4 times).

The PIXE values of K, Ca and the 6 trace elements are expressed in dry weight ratios [14]. The K content is between 15,000 and 20,000 µg/g in the cerebral and cerebellar cortex, and in the basal ganglia and the thalamus. In the cerebral white matter it is between 5,000 and 10,000 µg/g dry.

The Ca content is between 500 and 700 µg/g in the cerebral and cerebellar cortex. In the basal ganglia, thalamus and cerebral white matter it is between 250 and 350 µg/g.

The Mn content is between 2.2 and 3.2 µg/g in the basal ganglia and the thalamus and between 1.1 and 1.5 µg/g in the cerebral and cerebellar cortex, and in the cerebral white matter.

The Fe content is more than 500 µg/g in the basal ganglia and between 200 and 280 µg/g in the cerebral and cerebellar cortex, and in the thalamus. In the cerebral white matter the level is between 140-200 µg/g.

The Cu content is more than 36 µg/g in the basal ganglia and cerebellar cortex. In the cerebral cortex and thalamus it is between 18 and 26 µg/g, while in the cerebral white matter it is as low as 9 up to 13 µg/g.

The Zn content is between 60 and 84 µg/g in the cerebral cortex, basal ganglia, thalamus and cerebellar cortex. It is between 30 and 42 µg/g in the cerebral white matter.

The Rb content is between 14 and 16 µg/g in the cerebral cortex, thalamus and cerebellar cortex. In the basal ganglia and the cerebral white matter it is between 10 and 12 µg/g.

During normal aging the concentrations of K and Rb tend to decrease while there is an augmentation of Ca, Fe, Zn and Se [15].

According to some studies the major and trace element concentrations differ between the right and left hemispheres [16-17], depending of the individuals are right- or left handed [18].

PIXE Spectrum in Brain Diseases

Only a few neurodegenerative diseases have been examined with PIXE.

In Alzheimer’s disease the senile plaques contain significantly more Fe, Cu and in particular of Zn, compared to normal brains [19,20].

With PIXE analysis the increase of Fe in the substantia nigra is confirmed in Parkinson’s disease [21].

In status epilepticus a Ca increase linked to a decrease of K is observed in the pars reticularis of the substantia nigra and the globus pallidus [22].

In acute non-haemorrhagic cerebral infarcts the wet-to-dry weight ratio and the Ca content are significantly increased mostly in the basal ganglia and cerebral white matter (Fig.1-2). The Fe level is mainly augmented in the cerebral cortex and white matter. On the other hand, there is a severe loss of K, Cu and Rb, while the levels of Zn and Mn remain unchanged (Fig. 3-4) [23]. In haemorrhagic cerebral infarcts all these changes are more pronounced compared to non-haemorrhagic ones [24]. In post-mortem brains of patients with an acute cerebral infarct,
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treated with anti-oedema medication, the wet-to-dry ratio is significantly decreased in the treated group, compared to the non-treated one. The Ca and Fe concentrations are lowered in the treated group while the K, Rb and Cu levels are higher. No differences are observed for Zn and Se [25].

**PIXE Analysis of Middle Cerebral Artery Infarct**

*Figure1.* In the normal brain (on the left) the wet-to-dry weight ratio is the highest in the cerebral and cerebellar cortex, followed by the basal ganglia and thalamus and low in the cerebral white matter. In the acute middle artery cerebral infarct (on the right) it is increased in the cerebral white matter and basal ganglia.

**PIXE Analysis of Middle Cerebral Artery Infarct**

*Figure2:* In the normal brain (on the left) the K level is high in the cerebral and cerebellar cortex, basal ganglia and thalamus while low in the cerebral white matter. In the cerebral infarct and its penumbra (on the right) it is globally decreased.
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**Figure 3:** In the normal brain (on the left) the Ca level is moderately high in the cerebral and cerebellar cortex, while low in the basal ganglia, thalamus and the cerebral white matter. In the cerebral infarct and its penumbra (on the right) it is globally increased in the cerebral infarct and in the cerebral cortex of the penumbra.

**Figure 4:** In the normal brain (on the left) the Mn level is moderately high in the basal ganglia and thalamus, while low in the cerebral and cerebellar cortex, and the cerebral white matter. In the cerebral infarct (on the right) the Mn levels are unchanged compared to the normal control.

**DISCUSSION**

This study demonstrates the heterogeneous distribution of minor and trace elements in the different structures of the normal brain. There is a clustering of all gray matter structures and a clustering of all white matter components. Also, structures, involved in the same physiological function or morphologically similar regions often conglomerate in one substructure [14]. K, Mn and Cu levels can increase in specific regions of the brain during physiological processes such as memory consolidation [26].

In Alzheimer’s disease it is known that increased levels of Cu, Fe and particularly Zn can accelerate aggregation of amyloid beta peptide [19].
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Previous studies have already demonstrated Fe accumulation in the substantia nigra of Parkinson brains, mainly in the severe forms of the disease [27]. In the early stages of the disease it is found to be restricted to the pars compacta of the substantia nigra [28]. In advanced stages Fe deposition extends to the pars reticularis of the substantia nigra, the red nucleus and the globus pallidus [29].

The accumulation of Ca and the decrease of K in the deep brain structures in status epilepticus is a secondary phenomenon due to the intensity of the ischaemic damage [30].

Brain oedema, reflected by the increased wet-to-dry ratio, is due to disruption of the blood-brain barrier in acute ischaemic stroke, leading to accumulation of Ca and Fe in the infarcted tissue. Also a further increase of Fe is observed in older infarcts compared to recent ones, while the Ca level remains unchanged. This must be explained by a still ongoing leakage at the level of the blood-brain barrier. Also Mn, Zn and Se levels are enhanced, whereas K and Rb levels show a tendency to return to normal [31].

It can be concluded that neuropathological PIXE analysis can be an interesting additional tool to examine post-mortem normal and diseased brains.

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