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Use of hair as a diagnostic tool: X-ray fluorescence Zn monitoring in hairs of patients under parenteral nutrition

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Abstract: X-ray fluorescence excited by synchrotron radiation is a very sensitive method, allowing us to analyse trace elements along a single human hair. The results presented here demonstrate the application of the method on patients under parenteral nutrition in order to validate an adequate supplementation of Zn. We were able, by measuring different hair segments distributed along the hair, to monitor the trace element concentration during the therapy.

Special care has to be taken for the statistical interpretation of the results. Indeed, the usual techniques applied to human hair analysis yield a mean concentration, whilst the analysis of a single hair revealed important fluctuations in three levels: with time (along the hair), between hairs of one person as well as between hairs of different persons.

Key-words: Hair, Parenteral Nutrition, X-ray fluorescence, Synchrotron, Zn.

Abbreviations: PN: Parenteral nutrition

INTRODUCTION

During certain serious digestive diseases, oral nutrition can become insufficient, even impossible. The nutritional requirements must then be met by a parenteral nutrition, i.e. nutrition by intravenous way. The nutritional contribution must then be quantitatively and qualitatively perfectly adapted to the nutritional needs, especially for children for which an optimal growth must be guaranteed. Otherwise, there would be a significant risk of overload or of deficiency for certain elements. Thus, optimal supplementation of trace elements (and in particular of zinc [Anglade 1990]) is, on the clinical level, a necessity.

The nutritional contributions are carried out in the form of injectable sterile nutritive mixtures, prepared by the pharmacy of the hospital.

During prolonged parenteral nutrition, lasting possibly several years, a regular and rigorous clinical and biological follow-up is essential in order to be able, if necessary, to adapt the supplementation. This follow-up is achieved by several approaches: plasmatic and urinary zinc level evaluation are the most usual techniques but are insufficient for a good evaluation: indeed, these analyses reflect only a small proportion of the pool of trace elements - and especially of zinc - of the individual. The hair is, on the contrary, a place of accumulation of zinc [Iyengar 1988]; its composition thus reflecting at best the reserves and consequently the real status of the individual. The detailed analysis of a single hair became possible thanks to a very fine analytical technique, X-ray fluorescence.

MATERIALS AND METHODS

The aim of these analysis is to measure trace element in a very small sample (no more than one mm length of a single hair) Thus we must not only reach very low concentration (in the ppm range) but also extremely low mass detection limits (less than the ng). SXRF (Synchrotron induced X-Ray Fluorescence analysis) and PIXE (Particle Induced X-ray Emission) are, among others, two multielemental relatively easy to handle analytical methods responding to these requirements. Two reasons lead us to choose SXRF against PIXE: i) the analysis can be performed under air conditions ii) SXRF is more sensitive to heavy elements than PIXE as the fluorescence cross section scales with Z^4 for photons and roughly as Z^4 for charged particles.

Experimental set-up

The X-ray fluorescence facility is installed on beam line D15 of the LURE-DCI ring in Orsay (France). Relativistic positrons produced in an injector (Linac) are stored in a ring. During their travel in the bending magnets, positron radiate energy, resulting in an intense polychromatic (white) X-ray beam, highly collimated and polarised. In the experimental hutch the white beam first reaches the monochromator. It consists of two crystals of highly oriented pyrolytic graphite ($\text{FWHM} = 0.4^\circ$) operated in the parallel mode. The first one is a plane crystal that selects the energy with a wide band pass while the second one, due to its curved geometry, focuses the monochromatic beam on a spot of typically 1 mm diameter. The whole mechanism is under primary vacuum and all rotations and translations of the two crystals are remote controlled from a PC computer. The focal spot is in the air and at a fixed position whatever the chosen energy is in a range from 9 to 23 keV.

The energy of the incident beam is chosen depending on the element we are interested in and can be fixed so as to enhance the sensitivity on a particular element. We decided to use 14 keV photons that allow analysis of elements up to bromine on K shell electrons and up to bismuth on L shell ones. Thus sensitivity will be less for an element

like sulphur which is one of the main component of hair but this will avoid unnecessary detector saturation.

A pair of slits upstream from the monochromator allows to accept more or less of the incoming beam (maximum 4×1 cm) depending on the count rate on the sample and a collimator placed just before the focal spots can be used to reduce the spot size down to 100 microns if necessary to study small objects.

The sample is fixed to a three axis Micro-Controle remote controlled stage. Then the part of the sample under study is brought to the focal spot, which is simulated by a laser beam. The impact of the beam on the sample is visualised on a color TV screen through a microscope associated to a videocamera. An image of the screen can be obtained through a video-printer and saved as a visible image of the sample.

Finally the X-ray fluorescence spectrum is recorded with a Si(Li) detector of 150 eV energy resolution and 13 mm^2 area. An aluminum collimator is installed on the detector axis for shielding and to limit the acceptance angle. Scattering in the detector is further reduced by placing it in the plane of the synchrotron ring and perpendicular to the incoming beam. Pulses from the preamplifier are sent to a Tennelec 244 amplifier and then processed through a Nucleus Multichannel Analyzer card set in a PC computer.

Analysis of many samples can be performed automatically. The co-ordinates of the various spots that we wish to analyse on any number of samples in a 10×10 cm area are stored in a file of the program PASECH. Then, the program will automatically bring the sample to the right position, start the acquisition, give the spectrum a file name and store it. These operations are repeated until all positions have been analysed. A special sample holder was used, capable of holding up to 15 hairs of 3 to 10 cm long.

Quantitative analysis: The program WAPI [Wang, 1994] is used for deconvolution of each spectrum. It gives a list of all elements observed and the net area of the representative peaks after correction of air absorption and detectors efficiency. These final values can be used for quantitative analysis.

A particular difficulty appears concerning hair quantitative analysis by X-ray fluorescence: the fluorescence intensity depends on the volume of sample irradiated, and the different hairs are not of constant diameter. Instead of trying to apply computed corrections based on a quasi-cylindrical form and hair diameter, we decided to use the hair sulphur content as an internal standard. Indeed, keratin, the main component of hair, is a sulphur rich protein and thus sulphur hair concentration is considered as relatively constant ($\sim 4.9\%$ [Valkovic 1988]). As we are concerned with comparisons and not with absolute values, we report our results as the ratio of Zn fluorescence intensity divided by S fluorescence intensity (noted Zn/S on the diagrams). One drawback of this approach is that we can not compare our results with reference literature values, so we have to record spectra from healthy children hair as reference.

A typical X-ray fluorescence spectrum of a hair is shown in Figure 1. It is noteworthy that a wide variety of elements is detected, including minor trace elements or pollutants.

Population studied

Two series of experiments were performed including children under parenteral nutrition and control healthy children as follows:

11 healthy control children 2 to 14 years old, having a normal feeding; 9 children under parenteral nutrition (PN) 1 to 13 years old, suffering severe digestive malabsorption

(5 small bowel syndromes, 4 intractable diarrhoeas). All have been out of PN for more than 3 months, 8 out of exclusive PN and 1 out of PN as a complement.

4 PN children of: 2 years (n°10), 1 year (n°11), 18 months (n°12), 15 years (n°13) old, suffering severe digestive malabsorption (intestinal obstruction). 3 are out of exclusive PN and 1 out of PN as a complement.

All the hair samples were collected at the occipital area and subsequently cleaned following the IAEA washing procedure [Borella 1996]. Hair samples were examined under microscope before the X-ray analysis in order to eliminate damaged hairs.

RESULTS & DISCUSSION

Two different types of studies were performed:

in the first, Zn fluorescence signal for children under parenteral nutrition is compared against values for healthy children;

in the second, Zn fluorescence values along hairs of children under parenteral nutrition are recorded in order to verify the stability of Zn levels with time.

Zn fluctuations on healthy children hair

Usually, in previous studies, a mass of hairs was analysed yielding a mean value of trace element hair content. Nevertheless, important fluctuations between individuals are observed [Meng 1994, Bertazzo 1996]. The sensitivity of the X-ray fluorescence excited by synchrotron radiation allows a measure on a spot of less than 1 mm on a single hair revealing thus time variations and disparities between hairs for a single individual. A careful statistical analysis was hence necessary in order to verify the significance of an average zinc level for healthy children hair and estimate the fluctuations around this standard value.

A series of experiments was performed in order to estimate time variations along a single hair, fluctuations between hairs of the same individual, as well as disparities between individuals. Measurements were recorded for five points per hair at 5 mm intervals. The experiment was performed on different hairs of each healthy child. Analysis of variance demonstrates that all the measurements belong to the same population and can thus be averaged to compute a confidence interval around a standard value for zinc hair level on healthy children. This interval (as Zn/S fluorescence intensity) is 0.26 ± 0.17 at a 95% confidence level.

It becomes then possible to analyse hairs of children under PN (see above for detailed description of the population studied) and to verify that the Zn levels recorded belong to the confidence interval of the healthy population (Figure 2).

Zn level stability on hairs of children under parenteral nutrition

Based on a mean hair growth rate of 1 mm per 3 days, we recorded hair spectra every 5 mm (i.e. every 15 days) beginning 3 mm from the root. The experiment was performed on the four children of the second group of patients described above (patients n°10-13). We recorded data for three hairs of each child on 5 cm length, i.e. 5 months. The results obtained show clearly that the hair Zn level is stable with time, indicating a correct supplementation and assimilation of Zn (Figure 3). Relative standard deviation was less than 0.15 for all points measured (except third point of patient 12 hairs where $RSD=0.19$). A RSD value of 0.15 is acceptable since these are not mere repetitions of the same hair spot, but rather measures on three different hairs.

CONCLUSIONS

This study demonstrates the suitability of X-ray fluorescence excited by synchrotron radiation for detailed hair trace element analysis.

We were able to estimate a confidence interval for Zn level in healthy children and verify that children under parenteral nutrition were correctly supplemented.

Furthermore, a kinetic study performed by analysing spots along the hair allowed us to verify the stability of Zn level with time during the therapy.

The X-ray fluorescence detection is a multi-elementary method, allowing the same type of study to be simultaneously achieved for other trace elements.

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Fig. 1: X-ray fluorescence spectrum of hair

Fig. 2: Zn/S fluorescence intensity for patients (dark grey) compared to confidence interval (light grey) for healthy children.

Fig. 3: Zn/S fluorescence intensity along the hair for patients n° 10, 11, 12, 13 (fig. A, B, C, D respectively). Time is reversed with respect to distance (most recent point nearest the root).





