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A comparative in vivo and in vitro L-band EPR study of irradiated rat incisors

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Abstract

L-band (∼ 1 GHz) EPR has the potential to measure the absorbed radiation dose in human teeth inside the mouth (in vivo analyses). One crucial point in the development of the method is to know if dosimetry evaluation carried out in vivo after accidental exposures can be reliably based on calibration curves built in vitro. The aim of the present work is to specifically address this point. First, we compared L-band in vitro and in vivo analyses in irradiated rat teeth and estimated the possible loss in in vivo experiments due to rat movements and mouth proximity. Second, the lower pair of rat incisors were analysed by L-band EPR before and after irradiation (50 Gy), first on the living rat, then on the same dead rat, finally after extraction of the teeth. X-band powder spectra were also taken after crushing of the two teeth. Irradiations of dead rats and extracted teeth were also carried out. Comparing L-band spectra obtained with living rats and removed heads does not show any significant difference due to possible small rat movements or breathing. Relative standard deviations of the amplitudes of the dosimetric signal are quite high (27–54%). Nevertheless, it seems to be a tendency to have higher signals in irradiated extracted teeth than in irradiated animals.

Keywords: EPR dosimetry; L-band; Rat incisors

1. Introduction

Very similar X-band EPR spectra of irradiated teeth are observed whatever the origin: human teeth (Wieser et al., 2000) or teeth of animals, for example elephant (Debuyst et al., 2000), reindeer (Klevezal et al., 1999), walrus (Hayes et al., 1998), cow (Toyoda et al., 2003), rat (Brik et al., 2000; Miyake et al., 2000), mouse (Toyoda et al., 2003; Khan et al., 2003). The EPR spectrum principally consists of two signals, one due to the stable CO_2^- radicals ("dosimetric signal") and the other to the so-called "native" radicals.

Although EPR dosimetry with human teeth is usually performed at X-band (∼ 9.7 GHz) and with enamel powder (Wieser et al., 2000), it was recently suggested to use L-band (∼ 1 GHz) EPR spectroscopy to measure the absorbed radiation dose in human whole teeth in situ, without removing the tooth from the mouth (Miyake et al., 2000). The L-band spectrum of a whole tooth is reduced to a single composite line, sum of the dosimetric and native signals. In using a surface coil resonator placed on top of the tooth, enamel was found to be responsible of 88% of the total EPR signal (Zdravkova et al., 2002a). This type of resonator was therefore identified as the best choice for in vivo L-band measurements. Different in vitro L-band studies with photon

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and neutron irradiated extracted human molars were then undertaken in the framework of an European project "EPR Dose Reconstruction with Teeth" in order to check on the feasibility and the reliability of the method with the available spectrometers and resonators (Zdravkova et al., 2002a, b, 2003a, b).

A further step in the development of measurement procedures for living objects involves in vivo L-band measurements of irradiated teeth. To our knowledge, only the L-band study of Miyake et al. (2000) refers to this subject describing measurements carried out in alive rats. These authors presented a linear dose-response in the dose range 0–40 Gy obtained with X-ray irradiated anaesthetized rats whose upper pair of incisors were surrounded by the twin spiral loops of their L-band resonator. They obtained similar dose responses for rat teeth irradiated in vivo and in vitro.

On the other hand, following the X-band results obtained by Brik et al. (2000) with enamel powder from X-ray irradiated rat teeth, the efficiency of producing CO_2^- radicals by irradiation is different for teeth in living and dead rats. These authors compared EPR spectra from rats sacrificed after irradiation, rats suffocated just before irradiation and teeth extracted from dead rats and subsequently irradiated (70 Gy). While the intensities from sacrificed and suffocated rat teeth enamel produced by the same radiation dose were approximately equal, the signal from extracted teeth was roughly twice as intense.

The crucial point from these controversial observations is to know if dosimetry evaluation carried out in vivo after accidental exposures can be reliably based on calibration curves built in vitro. The aim of the present work is to specifically address this point. First, we compared L-band in vitro and in vivo analyses and estimated the possible loss in in vivo experiments due to rat movements and mouth proximity. Second, we verified whether the factor of 2 between in vitro and in vivo irradiations observed by Brik et al. (2000), but not by Miyake et al. (2000), could be reproduced in L-band and X-band. Therefore, the lower pair of rat incisors were analysed by L-band EPR before and after irradiation, first on the living rat, then on the same dead rat, finally after extraction of the teeth. X-band powder spectra were also taken after crushing of the two teeth. Irradiations of dead rats and extracted teeth were also carried out.

2. Materials and methods

2.1. Animals

About 40 male Wistar rats with body weight 300–400 g were purchased from the UCL animal house facilities unit of the faculty of medicine. Rats were anaesthetized with chloral hydrate (400 mg/kg i.m.) for irradiation and subsequent EPR measurements lasting around 3 h.

2.2. Radiation

The rats and extracted incisors were X-ray irradiated (Philips 250 RT, 250 kV, 15 mA, 1 mm Cu filter; field size 1.0 cm diameter for the rat mouth) with a dose rate of 1.2 Gy/ min. A single dose of 50 Gy (dose in water) was delivered to all teeth using fullface irradiations.

2.3. EPR measurements

Whole teeth EPR measurements were carried out using a L-band Magnettech EPR spectrometer (Berlin, Germany) equipped with a low frequency microwave bridge operating at 1.2 GHz and an extended loop resonator (surface coil of 3.5 mm inner diameter and 2 mm thickness, and twin spiral loops which fit closely around the lower pair of the rat incisors) specially designed and built by Dr T. Walczak (Dartmouth Medical School, Hanover, NH, USA) for use in living rat as in Miyake et al. (2000) (see figure 3 of the last reference). A frequency counter (CUB RF minicounter, Optoelectronics, USA) enabled the measurement of the microwave frequency. The spectrometer settings of EPR parameters for measurement of rat teeth were: 95 mW input microwave power, 3 mT sweep field, 1024 data points, 1 min scan time, 0.06 s time constant, 0.34 mT modulation amplitude, 40 scans.

Powder EPR spectra were taken at X-band with a Bruker EMX-8/2.7 spectrometer (100 kHz modulation frequency, 5 mW microwave power, 0.34 mT modulation amplitude, 5 mT field range, 1024 data points, 20.48 ms conversion time, 10.24 ms time constant, 20 scans). The magnetic field was measured by a Bruker ER 036 TM NMR teslameter and the microwave frequency by a Bruker EMX 040-1161.8A frequencymeter. The Bruker weak pitch signal was used for signal intensity normalization.

2.4. EPR spectra analysis

The spectra deconvolution was done with the DOSIMETRY software package developed by the GSF-National Research Centre for Environment and Health of Munich and the Institute of Metal Physics of Ekaterinburg (Koshta et al., 2000; Zdravkova et al., 2003a). EPR spectra were deconvoluted into one or two isotropic native signals and an anisotropic CO_2^- species. The latter signal was approximated either by a linear combination of two Gaussian functions whose line positions, widths and relative contributions can be adjusted, or by a simulated powder spectrum.

2.5. Procedures

Following Brik et al. (2000), three different procedures were used:

Procedure 1: anaesthesia of the rat; in vivo L-band spectrum of the unirradiated lower incisors; 50 Gy irradiation; in vivo L-band spectrum; killing of the rat by use of overdose