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ELECTRON PARAMAGNETIC RESONANCE STUDIES OF GAMMA IRRADIATED AZITHROMYCIN

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Gamma irradiated azithromycin – antibiotic important inter alia in dermatology was examined by the use of an X-band (9.3 GHz) electron paramagnetic resonance spectroscopy. The following doses of irradiation [kGy]: 5, 10, 15, 20, and 25, were used. EPR spectra with complex shape were recorded for all the irradiated samples. Continuous microwave saturation of EPR spectra indicates slow spin-lattice relaxation processes in irradiated azithromycin. The strongest paramagnetism characterizes azithromycin irradiated with a dose 25 kGy. Amplitudes of EPR lines of irradiated azithromycin decrease with increasing of storage time for all doses. The performed EPR studies point out that gamma irradiation should be rejected as sterilization method of azithromycin.

INTRODUCTION

According to Polish Pharmacopoeia sterilization is process leading to elimination microbes both vegetative and spore forms [Polish Farmacopoeia]. It is possible to sterilize solids, liquids and gases. Depending on type, characteristics and way of production of pharmaceutic different methods of sterilizations are apply. Polish Pharmacopoeia accepts following sterilizations methods: heat and gas exposure, filtration and ionizing radiation. Radiation sterilization uses two sources of radiation: electron accelerators and isotopes sources Co^{60} and Cs^{137}. The most often putting into use of gamma radiation source is Co^{60} [Polish Farmacopoeia].

Radiation sterilization technology is more actively used now that any time because of its many advantages. Gamma radiation has high penetrating power, relatively low chemical reactivity and causes small temperature rise [Basly, Longy & Bernard, 1997; [a)]Basly, Basly & Bernard, 1998]. Besides it is possible to sterilize drugs in their final containers what is very advantageous from economic point of view [[b)]Basly, Basly & Bernard, 1998; Gibella, Crucq, Tilquin, Stocker, Lesgards & Raffi, 2000; Fauconnet, Basly & Berdard, 1998; [c)]Basly, Basly & Bernard, 1998]. But on the other hand radiosterilization can lead to radiolytic products appearing. Pharmaceutical solids have been observed to undergo radiative degradation of about 1–5 % at the normal sterilization dose of 25 kGy [Basly, Longy & Bernard, 1997]. Among radiodegradation products we can observe free radicals what can by particularly dangerous during radiosterilized drugs therapy.

Electron paramagnetic resonance (EPR) spectroscopy is one of the leading method for identification of paramagnetic centers in gamma irradiated pharmaceuticals [Basly, Longy & Bernard, 1997; [a)]Basly, Basly & Bernard, 1998; [b)]Basly, Basly & Bernard, 1998; Gibella, Crucq, Tilquin, Stocker, Lesgards & Raffi, 2000; Varshney & Dodke, 2004; Fauconnet, Basly & Berdard, 1998; [c)]Basly, Basly & Bernard, 1998; Onori, Pantaloni, Fattibene, Ciranni – Signoretti, Valvo & Santucci, 1996; Basly, Doroux & Bernard, 1996; Yurus, Ozbey & Korkmaz, 2004]. Paramagnetic centers are not expected in properly sterilized drugs.

The aim of this work was to study formation of paramagnetic centers in gamma irradiated antibiotic: azithromycin. Properties of paramagnetic centers in irradiated drug were examinated. Effect of irradiation dose versus paramagnetic centers amounts in the gamma irradiated azithromycin was also discussed.

MATERIALS AND METHODS

Samples

Antibiotic azithromycin in form of tablets commercially available for clinical use were analyzed. Each tablet contains 500 mg of azithromycin and also contains the following inactive ingredients: pregelatinized starch, sodium croscarmellose, dibasic calcium phosphate, magnesium stearate, sodium lauryl sulfate and an aqueous film coat consisting of hypromellose, titanium dioxide, macrogol, talc, simethicone, polisorbat. The mass of the whole tablet was about 865 mg.

Azithromycin, is an azalide, a subclass of macrolide antibiotics. It has molecular structure given in Fig. 1 [Zejc & Gorczyca, 2002]. Azithromycin is derived from erythromycin. Following oral administration, azithromycin is rapidly absorbed and widely distributed throughout the body. Rapid distribution of azithromycin into tissues and high concentration within cells result in significantly higher azithromycin concentrations in tissues than in plasma or serum. Azithromycin is active against broad spectrum both gram positive and negative bacteria and also against anaerobic microorganisms. It works by binding to a site on bacterial ribosome what blocks peptides chains translocation causing peptides synthesis inhibition [Janiec, 2001; Dzierżanowska, 2000; Kostowski & Herman, 2003; Janiec & Krupińska, 2002].

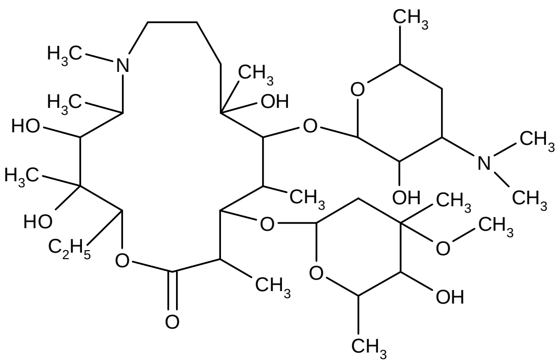

Fig. 1. Chemical structure of azithromycin [Zejc & Gorczyca, 2002].

Iradiation

Samples were irradiated in form of tablets in a cobalt-60 gamma chamber Theratron 780E. The dose rate was about 12,5 kGy/h. Gamma irradiation was made at room temperature. Investigations were performed for samples irradiated at five different doses (5, 10, 15, 20 and 25 kGy). An unirradiated sample was kept as reference.

Measurments

First derivative EPR spectra were obtained by an X-band (9.3 GHz) electron paramagnetic resonance spectrometer with modulation of magnetic field 100 kHz produced by RADIOPAN (Poznań). Microwave frequency was measured with MCM 101 recorder produced by EPRAD (Poznań).

All spectra were recorded at low microwave power 0.7 mW to avoid microwave saturation of EPR signals. Amplitudes (A) of central line in the spectra were determined. Influence of irradiation dose and storage time on this amplitude were tested. Distances ΔB between exemplary chosen peaks in the complex EPR spectra were compared for samples irradiated with different doses. The comparisons were done up to 20 days after antibiotic irradiation. Influence of microwave power in the range 0.7-70 mW on the mentioned A and ΔB values was examined.

RESULTS AND DISSCUSION

Unirradiated azithromycin sample presents no EPR signal. Free radicals do not exist in the original no sterilized drug. Gamma irradiation lead to formation of paramagnetic centers in azithromycin. Strong EPR lines were observed for the gamma irradiated antibiotic. All irradiated samples revealed complex EPR spectra consisting of many resonance peaks. Spectra of azithromycin irradiated at five different doses are given in Figure 2.

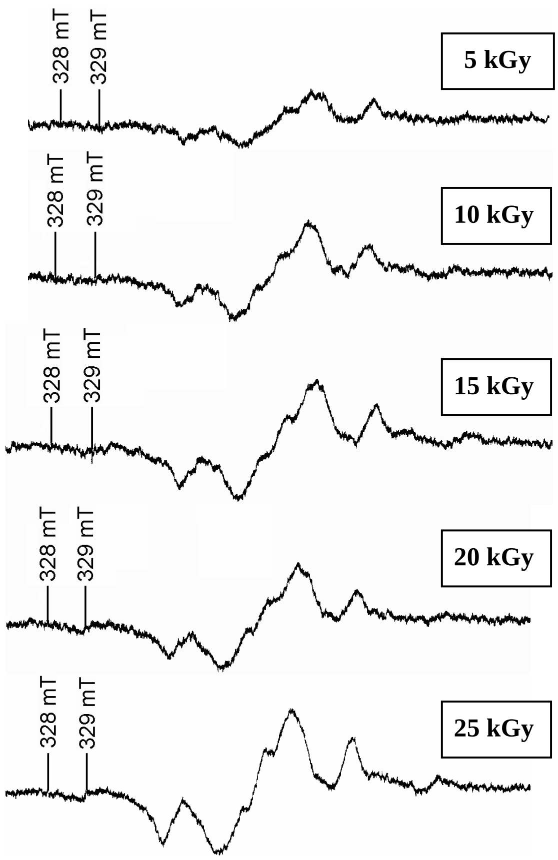

Fig. 2. EPR spectra of gamma irradiated azothromycin with five different doses [kGy]: 5, 10, 15, 20, and 25. Spectra recorded with microwave power 0.7 mW one day after irradiation are presented. Examined amplitudes are shown.

Complex spectra point out that a few types of paramagnetic centers exist in gamma irradiated samples.

The main peak indicated by arrows in Figure 2 origin from azithromycin, because irradiated tablet mainly contains the studied antibiotic. The tested tablets contained additionally inactive ingredients. It is expected that paramagnetic centers appear also in these irradiated substances and give signals in EPR spectra. It is possible that unresolved hyperfine structure for some paramagnetic centers may be visible in the spectra. It is difficult to simulate lineshape of such multi-component resonance curves, but electron paramagnetic resonance method pointed out directly strong paramagnetism of azithromycin after irradiation. We suggest practical application of our EPR results in medicine and pharmacy. Gamma irradiation as sterilization method for azithromycin should be rejected.

Paramagnetism characterized azithromycin irradiated with a different doses from 5 to 25 kGy and for a long time after irradiation (Fig. 3).

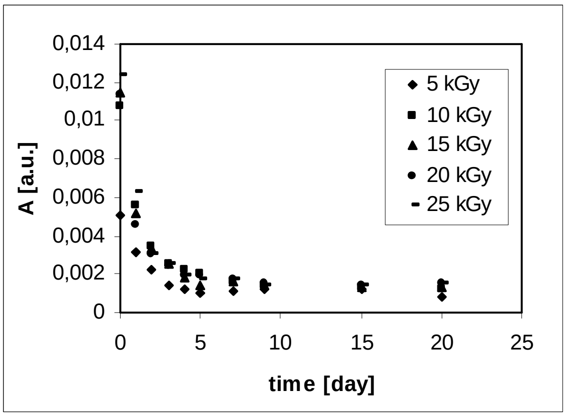

Fig. 3. Decrease of amplitudes of EPR signals which were signed in figure 1 with increasing of storage time. Data for gamma irradiated azythromycin with doses 5-25 kGy are presented.

The results of our studies showed that heights of resonance peaks depend on dose of gamma irradiation. The lowest amplitudes were obtained for dose 5 kGy. Relationship dose versus amplitudes for antibiotic irradiated with doses 10, 15, and 20 kGy were not visible. The highest amplitudes correspond with the highest irradiation dose of 25 kGy.

The evolution of EPR spectra was observed over a period of 20 days by recording spectra in regular time interval (24 hours). Changes of amplitudes A with increasing time after irradiation of drug are presented in Figure 3. Results of this study clearly shows that amount of paramagnetic centers in irradiated azithromycin which is proportional to EPR amplitude depend on storage time. Decrease of amplitudes and amount of paramagnetic centers in irradiated azithromycin indicates two-steps kinetics (Fig. 3). EPR amplitudes fast decrease up to 5 days from irradiation and after that time, they only slowly decrease. It was checked that only after 3 month after irradiation the sample storage at room temperature in air environment is still remains paramagnetic.

Influence of storage time on EPR parameter ΔB is shown in Figure 4.

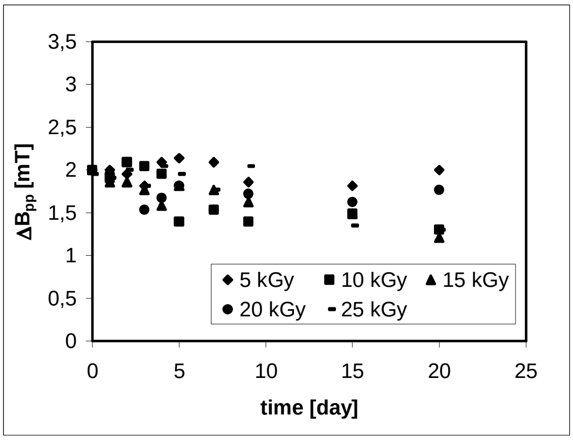

Fig. 4. Changes of distances between peaks signed in figure 1 with increasing of storage time. Data for gamma irradiated azythromycin with doses 5-25 kGy are presented.

As one can see ΔB values for azithromycin's lines for all doses (5, 10, 15, 20, and 25 kGy) do not considerably change with time after irradiation. It can be concluded that magnetic interactions in the analysed gamma irradiated antibiotic responsible for linewidths only slightly change with time.

It was observed that lineshape of EPR spectra of gamma irradiated azithromycin strongly depends on microwave power. Microwave power was modulated by the use of different attenuations (15-0.5 dB). Influence of microwave power on exemplary azithromycin's EPR spectrum is shown in Figure 5. Different changes of the individual components of this EPR spectrum with microwave power causes the visible changes in lineshape.

Presented in this work results bring to light that electron paramagnetic resonance spectroscopy is a useful technique to examine method and conditions of drug sterilization. Paramagnetic properties of sample is not expected for drug sterilized at optimal conditions. EPR measurements may be useful to chose radiation or thermal sterilization for drug. Examined in this work gamma sterilization is not good for azithromicin. Paramagnetic centers formed during irradiation of this antibiotic will probably react with biological molecules in cells and tissues. Biochemical interactions of paramagnetic centers may stimulate large amount of negative processes in living organism.

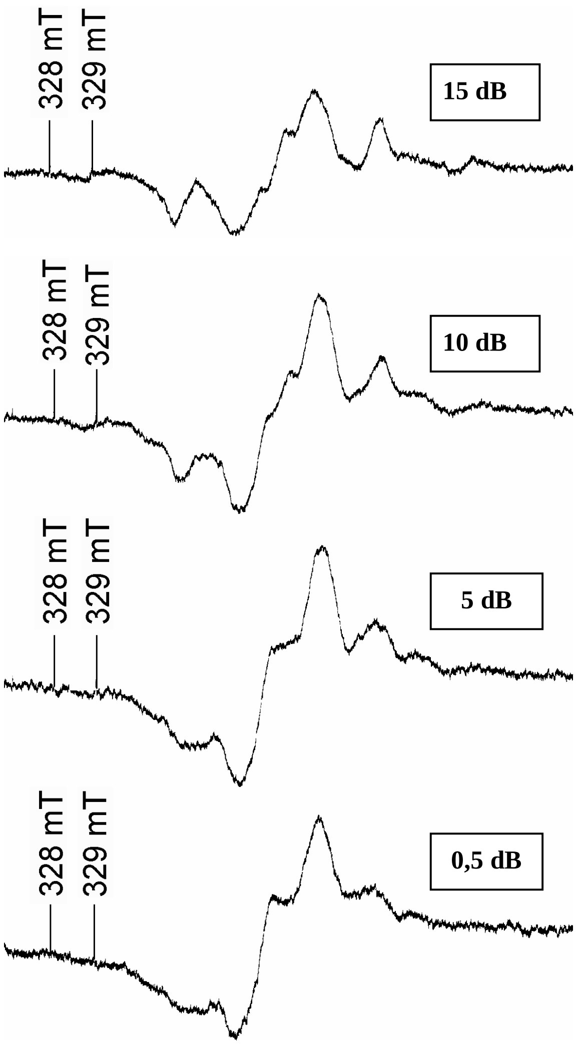

Fig. 5. EPR spectra of gamma irradiated azythromycin recorded with different microwave power attenuations (15-0.5 dB). Data for sample irradiated with 15 kGy one day after irradiation.

CONCLUSIONS

On basis of EPR examination of gamma irradiated azithromycin the following conclusions may be drawn:

1) Gamma irradiation of azithromycin with doses 5-25 kGy causes paramagnetic centers formation in this antibiotic.

2) Amplitudes of azithromycin's paramagnetic centers increase with increasing of irradiation dose.

3) For all doses amplitudes of EPR lines of irradiated azithromycin decrease during storage of this drug.

4) Azithromycin remains paramagnetic only after 3 months after gamma irradiation.

5) It is possible that gamma sterilized azithromycin may cause toxic free radicals effects in living organisms during farmacotherapy.

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