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Free Radicals in Irradiated Drugs: An EPR Study

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Exposure of dry powder forms of the drugs nitrendipine, nifedipine, felodipine, and nimodipine to γ-radiation results in the formation of free radicals detected by electron paramagnetic resonance (EPR) spectroscopy. The four structurally related drugs show qualitatively identical EPR spectral features in terms of *g*-values, the qualitative descriptive parameter. These radicals are very stable, surviving long periods of time in excess of 9 months and possibly beyond conventional shelf-life of the drugs. The residual radical population is high enough to be detectable after long storage. Administration of such radiation-treated drugs may present patients with quantities of free radicals and possibilities of secondary cell damage.

Keywords: γ-Irradiation, free radicals, nitrendipine, nifedipine, felodipine, nimodipine

INTRODUCTION

Sterilisation of medical equipment by the use of ionising radiation is rapidly becoming common practice in preference to autoclaving and liquid chemical disinfectants. Irradiation is also being successfully applied to food and drug preservation in many countries. A large number of foods and pharmaceuticals are currently sterilised using γ-radiation.

The break-through in bringing radiation-sterilised drugs on the market came partly due to the ecological problems associated with ethylene oxide sterilisation and the reduction in the maximum daily personnel exposure dose of ethylene oxide to 1 ppm by the US Occupational Safety and Health Authorities. Another contributing factor was the acceptance of average dose of up to 10 kGy for irradiated food by the World Health Organisation. γ-Radiation has been tested on a number of drugs; dry solid,[1,2] ointments,[3] aqueous solutions of antibiotics,[3] steroids and hormones,[3,4] alkaloids,[3] vitamins and enzymes,[5] excipients and raw materials.[6] The general finding is that γ-radiation can be used as an alternative method of sterilisation, however, caution must be exercised in its use on certain pharmaceuticals, particular restrictions being put on water-based preparations due to radiolytic

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products of water and the high potentials of secondary damage effects.[7] The chemical structure of the drug may also limit the use of irradiation. The presence of aromatic side chains as in penicillin, double bonds in β-carotene, amide bonds in hormones, high sulphur content as in mercaptides, all decrease the stability of the drug with respect to γ-radiation.

An additional effect and possibly very serious factor to be considered in many cases is the long stability of radiation-generated radicals. Produced though in small quantities, these radicals may be stable for long enough time, up to point of sale and even beyond shelf-life.[8] They would therefore constitute undesirable constituents of drug preparations. This study looks at the formation and longevity of radicals in a select group of drugs, calcium channel blockers, after treatment with low dose γ-radiation.

MATERIALS AND METHODS

The compounds nitrendipine, nifedipine, felodipine, and nimodipine, Figure 1, (all calcium channel blockers interacting with cardiac membrane structures), were synthesised at the Pharmaceutical Institute in Poznan, Poland. Their purity is in accordance with USPXXI. Powder samples of the compounds were exposed to γ-radiation from a ^{60}Co γ-ray source at a dose rate of 1.02 Gy/h for a total of 4 h at room temperature. Previously irradiated samples had been exposed to a total dose of 20 kGy before storage at 278–283 K for about 9 months. First derivative EPR spectra were recorded at room temperature (~293 K) on an X-band EPR spectrometer (Bruker 6/1 EMX model) operating in the 9 GHz range at a modulation frequency of 100 MHz. Instrumental parameters used for measurement of spectra were as follows; centre field 3382 G, sweep width 400 G, microwave power 10 mW, modulation amplitude 4 G, sweep time 167.7 s, microwave frequency 9.499 GHz.

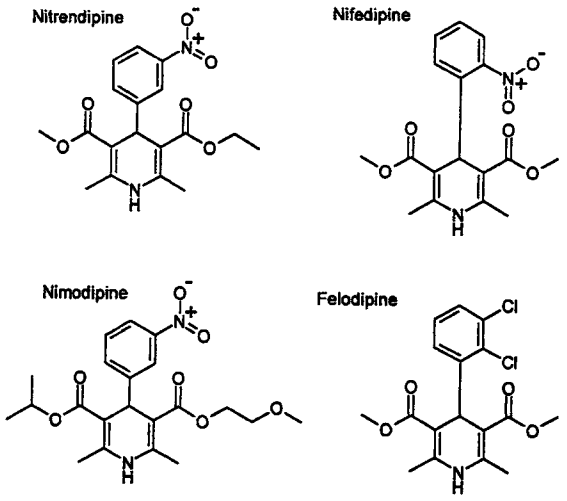

FIGURE 1 Structures of the select group of calcium channel blockers.

RESULTS AND DISCUSSION

Formation of free radicals as a result of exposure to ionising radiation is a well-known phenomenon.[7] In all cases free radicals are generated as the primary products of radiolytic and photolytic processes (γ-rays, X-rays, or hν). However they are most often short-lived especially at non-cryogenic states, either as a result of radical–radical recombination or secondary processes leading to formation of new products with concomitant loss of free radical characteristics. In cases where the free radicals are stable at room temperature, it is as a result of trapped electrons in the crystal lattice or changes in chemical structure. In all cases however, detection of EPR signals is classical confirmation for formation of free radicals. Samples of the compounds nitrendipine, nifedipine, felodipine, and nimodipine exposed to γ-irradiation of 4.08 kGy at room temperature (298 K) give well defined EPR spectra, Figure 2.

The spectra were essentially the same for all the compounds but for differences in intensities despite identical dose input. Characteristics of the spectrum are mainly an isotropic singlet perpendicular feature with an incompletely

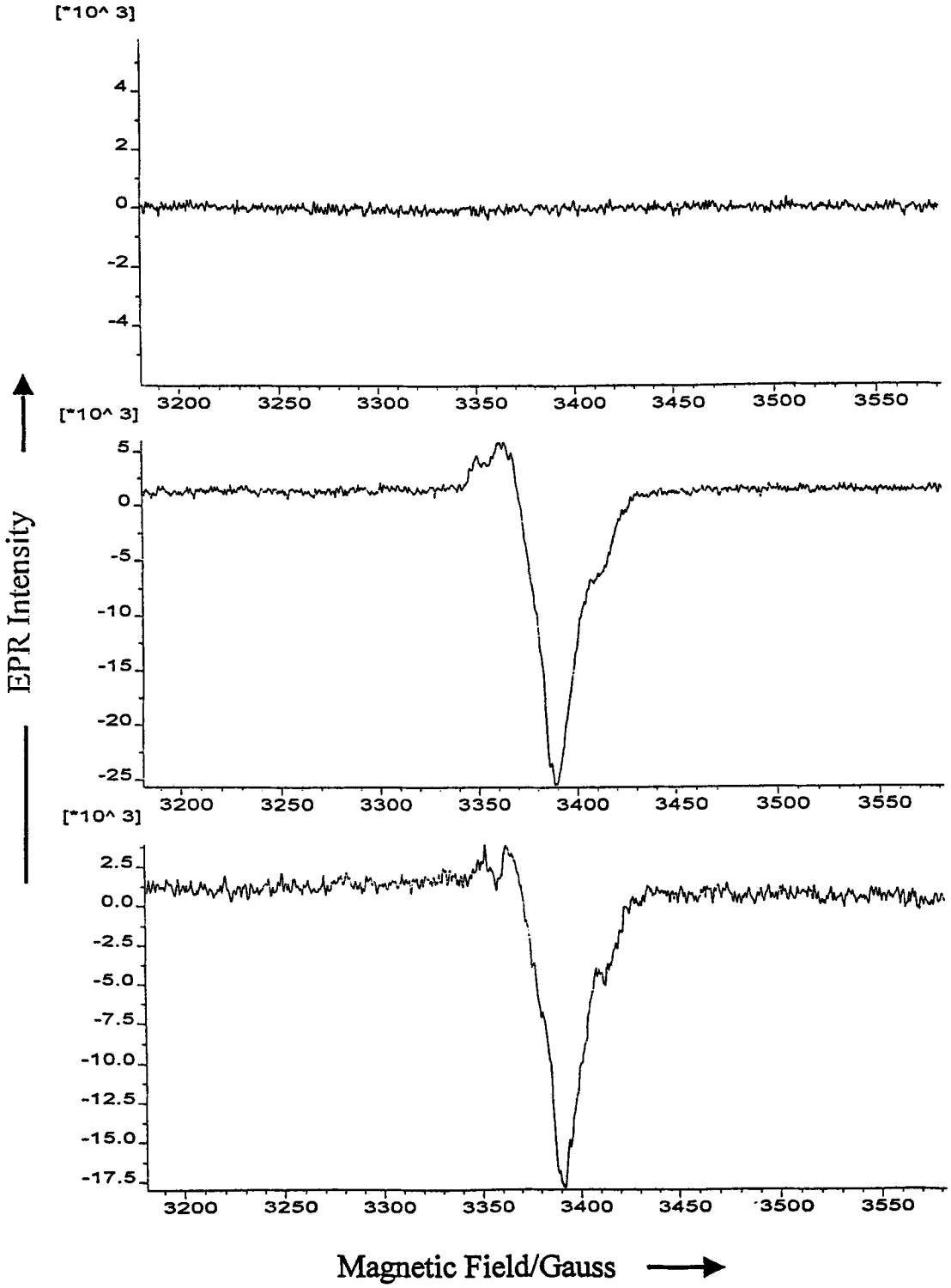

FIGURE 2 X-Band EPR spectra of nifedipine: (top) before γ-radiation, (middle) after recent irradiation, using 4.08 kGy, (bottom) 9 months after first trial irradiation using 20 kGy.

resolved doublet at the free spin region. Minor features are at *g*-values 2.025 and 1.99, the signal at 2.025 with the isotropic singlet conforms with the characteristics of the oxygenated organic radical $RO_2^{\bullet}$.[8] These results provide strong evidence for the generation of free radicals in these compounds, and such radicals are very stable at 298 K. Comparison with recent spectra of same samples previously irradiated (9 months!) shows that the spectra are the same. This clearly indicates that the radicals must be the same as those freshly generated, being primary products of radiolysis. They must be quite stable to be detectable after such long storage. The spectra of previously irradiated samples were not recorded at the time hence we are unable to calculate kinetic parameters of the decay characteristics from our present data. Further work on this aspect is now in progress.

Previous studies on the irradiation of formoterol fumarate[9] showed the formation of a stable radical. The recorded EPR spectrum was a rather symmetric broad singlet centred at about free spin with line width *ca.* 1.5 G, interpretable as an $R^{\bullet}$ radical. On close observation of the spectrum this in fact may be an incompletely resolved doublet for reasons of high modulation amplitude. Our results as for all four compounds are consistently of rhombic symmetry like the $RO_2^{\bullet}$ type radical. This is understandable because the irradiation was performed in an ambience of atmospheric oxygen. Any first-formed $R^{\bullet}$ radicals would immediately undergo oxygen addition to give the $RO_2^{\bullet}$ species.

In this study we have used a dose of 4.08 kGy, a value much less than 25 kGy which is prescribed for sterilisation against strains *C. botulinum* and *B. subtlilis*,[10] and yet there is formation of measurable magnitudes of stable free radicals. By implication, beneficial doses for effective sterilisation would precipitate very high amounts of these radicals. On the general use of irradiation for preservation of drugs and pharmaceuticals, the stability of these radicals suggests a definite possibility of ingestion of radicals in the course of chemotherapy. A similar stability of radicals had been reported in radio-preservation of fish 8 months after the treatment.[11] Oral and topical administration of such products may be followed by formation of water- or lipid-derived secondary radicals of the type $^{\bullet}OH$, $R^{\bullet}$, and $RO_2^{\bullet}$, which are known to attack cells through lipid peroxidation.[12–14] Lesions in the mouth, the oesophagus through to the stomach may be real possibilities.

CONCLUSION

In this study we present evidence for the generation and stability of free radicals produced in a select group of drugs by ionising radiation. Implications are drawn for possible toxicological effects after administration during chemotherapy.

Acknowledgements

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