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ANALYSIS

THE RADIOSTABILITY OF BETAMIPRON IN THE SOLID STATE

KAROLINA KILIŃSKA[1], JUDYTA CIELECKA-PIONTEK[1], ROBERT SKIBIŃSKI[2], ANDRZEJ MIKLASZEWSKI[3], WALDEMAR BEDNARSKI[4] and PRZEMYSŁAW ZALEWSKI[1]*

[1]Department of Pharmacognosy, Poznan University of Medical Sciences, Świecickiego 4, 60-781 Poznań, Poland
[2]Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland
[3]Institute of Material Science and Engineering, Poznan University of Technology, Jana Pawła II 24, 60-965 Poznań, Poland
[4]Institute of Molecular Physics, Polish Academy of Sciences, Smoluchowskiego 17, 60-179 Poznań, Poland

Abstract: The influence of ionizing radiation on the physicochemical properties of betamipron in solid-state was studied. No changes for betamipron irradiated with a dose of 25 kGy, required to attain sterility, was observed by spectroscopic and chromatographic methods. Solid betamipron has proven to be very stable on irradiation, and irradiation has been found to be a suitable method for its sterilization.

Keywords: betamipron, radiation sterilization, radiostability, Q-TOF, EPR

Betamipron (BP; N-benzoyl-β-alanine) is a nephroprotective agent with benzoyl and carboxyl groups in its chemical structure that is derived from an amino acid. BP is used in clinical treatment as an adjunct agent to antibacterial therapy. Its mechanism of action is based on significant inhibition of organic anion uptake by human organic anion transporter 1 (human-OAT1) and human-OAT3 in a dose-dependent manner (1, 2). Due to its ability to inhibit anionic transport in renal cortex and drug accumulation in the organism, it reduces toxicity caused, among others, by high doses of carbapenems antibiotics and inhibits deactivation of these drugs (2-5). Precisely for this reason, co-administration of betamipron with one of the first carbapenems – the panipenem was necessary during the development of commercial preparations. In clinical treatment a combination of these compounds is used in a 1 : 1 weigh ratio as a powder to prepare intravenous solution and according to the European Pharmacopoeia 9th this composition have to meet all requirements for preparations administered parenterally and containing an antibiotic component (6). One of the most advantageous methods for maintenance of sterility for this combination is irradiation, as demonstrated by radiolytic studies of other β-lactam antibiotics (7-13). Therefore, it is extremely important to verify the stability and potential changes in physicochemical properties of the component which is an adjunct agent to antibacterial therapy and during production must be subjected to the same processes.

Our investigations focused on determining the influence of ionizing radiation on betamipron in the solid-state. A standard recommended dose of irradiation (25 kGy) (14) and higher radiation doses – 50, 100, 200, and 400 kGy have been applied to understand the process of potential changes in BP after sterilization.

EXPERIMENTAL

Standards and reagents

Betamipron was obtained from TCI Chemical (Tokyo, Japan). BP is a white powder (purity > 98%), soluble in DMSO, ethanol and methanol,

* Corresponding author: e-mail: pzalewski@ump.edu.pl

sparingly soluble in water. BP is characterized by low toxicity. All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High-quality pure water was prepared using the Millipore purification system (Millipore, Molsheim, France, model Exil SA 67120).

Methods

Irradiation

BP samples were weighted into colorless glass vials in an amount of 5 mg and closed with plastic stoppers. Samples were exposed to β-irradiation in a linear electron accelerator LAE 13/9 (9.96 MeV electron beam and 6.2 μA current intensity) at 25, 50, 100, 200 and 400 kGy dose rates. Times of irradiation were 2.5, 5, 10, 20, 40 seconds respectively.

Electron paramagnetic resonance (EPR) spectroscopy

To detect free radicals creation after irradiation and determine their concentration a Bruker ELEXSYS E500 multifrequency spectrometer (Bruker, Billerica, MA, USA) was used. The powdered samples of irradiated and non-irradiated BP

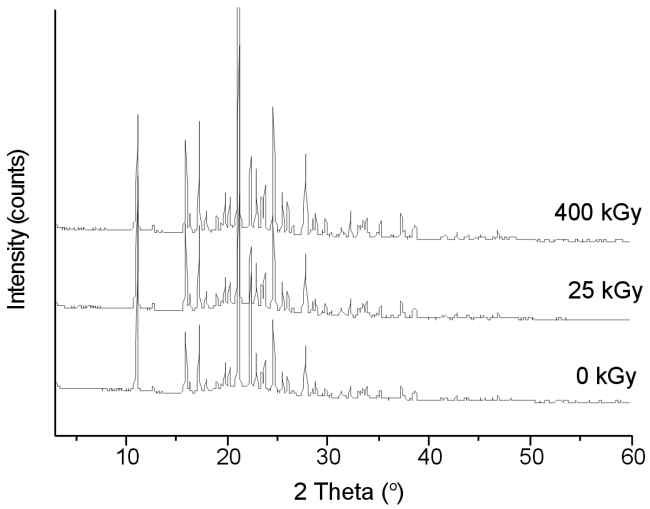

Figure 1. XRPD spectra of unirradiated and irradiated (25 and 400 kGy) betamipron

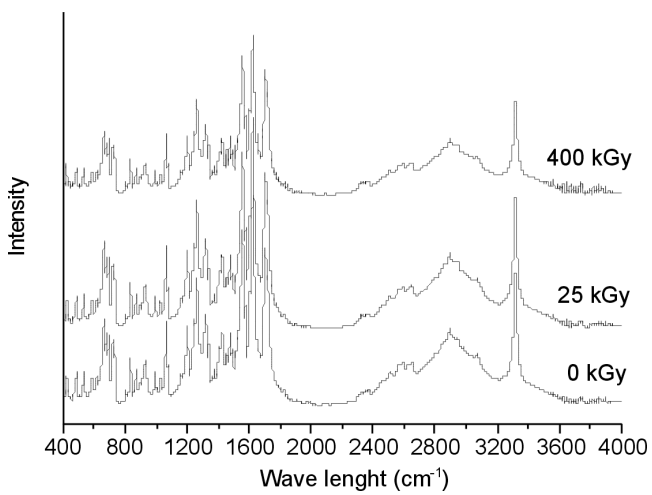

Figure 2. FT-IR spectra of unirradiated and irradiated betamipron

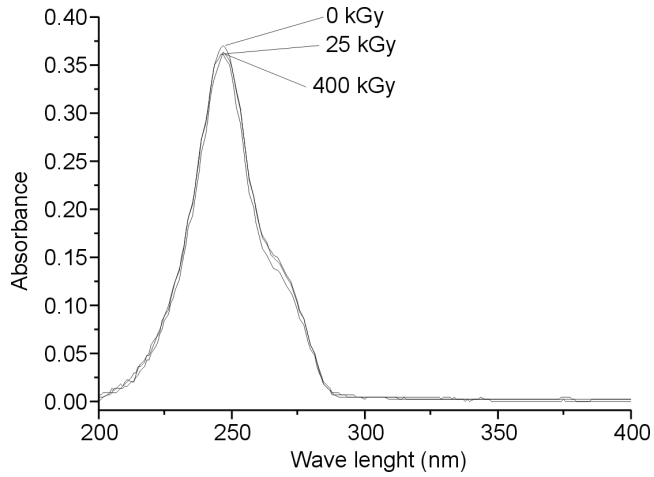

Figure 3. UV spectra of unirradiated and irradiated (25 and 400 kGy) betamipron

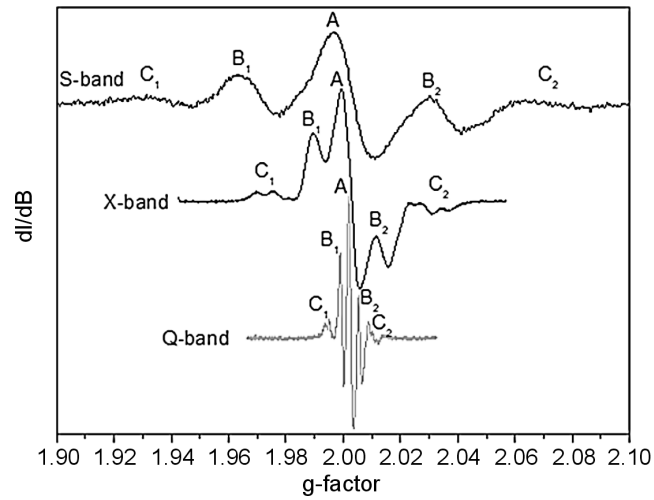

Figure 4. EPR spectra of irradiated betamipron recorded at S, X and Q – bands (radiation dose 25 kGy). Each spectrum was accumulated 10,40 times

were tested in quartz or suprasil capillaries at temperature 24°C (S-band – 3.5 GHz, X-band – 9.4 GHz, Q-band – 34 GHz). EPR spectra were recorded as a first derivative of the absorption signal. Using the method described by Mai et al. (2013) the calculation of the number of free radicals has been carried out (15).

X-ray powder diffraction (XRPD)

An X-ray powder diffraction (XRPD) was performed to assess potential changes in the crystallographic structure of BP samples. The diffractogram was obtained by using PANalitycal Empyrean system with Cu $K_\alpha 1$ radiation (1.54056 A) at a voltage of 45 kV and a current 40 mA. The irradiated and non-irradiated samples were scanned from 3° to 50° 2θ using a step size of 0.017° and the scanning rate 15 s/step with the sample spinning.

Ultraviolet-visible (UV–VIS) spectroscopy

A UV/VIS Perkin Elmer Lambda 20 spectrophotometer with the UV WinLab software

(PerkinElmer, Waltham, MA, USA) was used to verify the potential changes in stability of irradiated and non-irradiated samples. The 5.0 mg of each BP samples were dissolved in 25.0 mL of water and the 2.0 mL of the so obtained solution was diluted to 10.0 mL of water. The spectra of solutions in the final concentration 0.04 mg/mL were recorded in the wavelength range of 200–400 nm. The reference sample was the solvent – distilled water.

Fourier Transform Infrared (FT-IR) spectroscopy

For verification of potential changes in the structure of irradiated and non-irradiated samples, the FT-IR spectra were measured on Fourier Transform Infrared spectrometer, IR Affinity-1 Shimadzu. Samples were prepared by mixing BP with potassium bromide in proportions of 1 mg of sample to 300 mg KBr. Pellets were formed under pressure of 15 ton/cm^2 with a barrel of 13 mm in diameter. Absorption spectra were recorded with a resolution of 2 cm^{-1} within a wavenumber range from 4000 to 400 cm^{-1} (30 scans per spectrum).

High-performance liquid chromatography (HPLC-DAD) analysis

The kinetic study of BP samples and separation of degradation products were conducted with use of the Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and a diode array detector (Dionex, Sunnyvale, CA, USA). The stationary phase was a Lichrospher RP-18, 5 µm particle size, 150 mm × 4.6 mm (Merck, Darmstadt, Germany). The mobile phase composition was 3.12 g/L sodium dihydrogen phosphate dihydrate (pH = 7.0) – acetonitrile (9 : 1 v/v). The 5.0 mg of each BP samples were dissolved in 25.0 mL of water and the injection volume for HPLC analysis was 10 µL. Separation was performed at temperature 40°C and the wavelength of the diode array detector (DAD) was set at 225 nm. According to the International Conference on Harmonization Guidelines, the stability tests were performed (16).

HPLC-MS/MS analysis

The HPLC-MS/MS analysis was conducted with use of an Agilent Accurate-Mass Q-TOF LC/MS G6520B system with a DESI ion source and an Infinity 1290 ultra-high-pressure liquid chromatography system consisting of a G4220A binary pump, a G1330B FC/ALS thermostat, a G4226A autosampler, a G4212A DAD and a G1316C TCC module (Agilent Technologies, Santa Clara, USA). The control of the system, data acquisition and qualitative analysis were conducted with the use of the MassHunter workstation software B.04.00. Separations were performed on Hibar RP-18e, 2 µm particle size, 50 mm × 2.1 mm (Merck). The initial mobile phase composition was methanol – 0.05% acetic acid (10 : 90) during 2 min. Then gradient elution was used starting from mobile phase composition ratio (10 : 90) after 2 min to 50 : 50 within 10 min and the flow rate of the mobile phase was 0.3 mL/min. The Q-TOF detector was tuned in the positive (4 GHz) and the main parameters were optimized as follows: drying gas 10 L/min, nebulizer

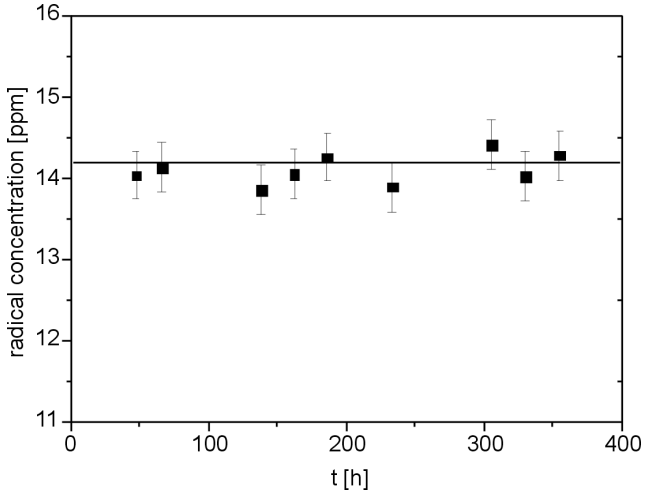

Figure 5. Concentration of free radicals vs. time after radiation sterilization (radiation dose 25 kGy)

pressure 40 psig, gas temp. 300°C, capillary voltage 3500 V, skimmer voltage 65 V, fragmentor voltage 200 V, octopole 1 radio frequency voltage 250 V. The data were acquired in the auto MS/MS mode with the mass range 50-950 *m*/*z* and the acquisition rate 1.2 spectra/s (for MS and MS/MS data). The collision energy was calculated from the formula 2V (slope)*(*m*/*z*)/100 + 6 V (offset) and maximum 2 precursors per cycle were selected with an active exclusion mode after 1 spectrum for 0.2 min. To ensure the accuracy of measurements, the reference mass correction was used and values 121.0508 and 922.0097 *m*/*z* were selected as lock masses.

RESULTS AND DISCUSSION

HPLC-UV, HPLC MS, FT-IR, UV, and XRPD analysis confirmed that after exposure to 25 and 400 kGy BP was not degraded. No differences in chromatograms, diffractograms (Fig. 1), FT-IR (Fig. 2) and UV-Vis (Fig. 3) spectra between non-irradiated and irradiated DRP samples were noticed. HPLC-MS/MS was employed to identification of BP (measured mass 194.08206, retention time t_r = 5.231 min) exposed to radiation. The molecular ion of BP was very accurately identified (4.56 ppm). HPLC-MS/MS analysis has shown no degradation product of BP after its irradiation (400 kGy).

EPR spectrum of irradiated BP (radiation dose 25 kGy) consists of a series of lines with different intensities, as presented in Figure 4. Although the spectrum is almost symmetrical at all bands, the lines belonging to groups A, B and C saturate at different microwave powers, therefore irradiation creates at least 3 types of free radicals. The most intense, central line (marked in Fig. 4 as A) is characterized by a spectroscopic coefficient (g-factor) $g = 2.0026 \pm 0.0005$, and linewidth 7,8 Gs. Because there is no hyperfine structure for the *A* signal, this radical is formed as a result of the O-H bond breaking. The doublet B_1 and B_2 can be assigned to a radical arising as a result of breaking one bond from the CH_2 group and is described by EPR hyperfine splitting 38.2 ± 0.8 Gs and $g = 2.0024 \pm 0.0005$. We also can not exclude that B_1, B_2 and part of the signal *A* form a triplet that results from breaking the N-H bond (EPR hyperfine splitting 19.1 ± 0.8 Gs and $g = 2.0024 \pm 0.0005$). The least intense lines marked as C_1 and C_2 may most probably be attributed to the radical formed as a result of breaking the C-H bond in the C_6H_5 ring. In this case, the unpaired electron should be delocalized into six carbon and partly on the remaining four hydrogens. In this case, the unpaired electron should be delocalized into six carbons and partly on the remaining four hydrogens to form an extensive hyperfine EPR structure that is partially covered by stronger lines A, B_1 and B_2. The spectrum of the non-irradiated sample does not differ from the background of the spectrometer, thus the concentration of radicals is less than 0.1 ppm. Unlike many irradiated drugs (7, 8, 10) EPR spectrum of irradiated BP sample (radiation dose 25 kGy) is very stable at room temperature, and the concentration of free radicals 14.1 ± 0.3 ppm is constant vs. time after irradiation as shown in Figure 5. Although we can not exclude that irradiation also causes other radiological defects (breaking the C-C bonds), we have shown that the radiation dose of 25 kGy does not create much radical-type damage.

The literature data confirms that the most common problem encountered after radiosterilization of solid drugs is discoloration or yellowing (12, 13, 17). In our study also a color change of samples after exposure to radiation was reported – from white to pink. In the next step, we verified the impact of packing material on irradiated betamipron. None of the tested packages (PVP and glass containers) allowed to obtain sterile BP without color changes. What is particularly important, after dissolving the samples of irradiated BP in water for injections, we get transparent and colorless solutions. Therefore, the authors tend to suggest that the color change of the BP in the solid-state after exposure to irradiation, may be associated with the presence of the radiolyzed water trapped in the crystal structure of the BP.

CONCLUSIONS

The results of conducted studies allow concluding that using radiation sterilization (E-beam) at a recommended sterilization dose 25 kGy – has no impact on the physicochemical properties of betamipron. This method can be successfully used for sterilization and decontamination of BP. Irradiation with higher doses leads to the color change from white to pink.

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