Radical decay in irradiated drugs: Flutamide, ifosfamide and aminogluthethimide

H. B. Ambroż,1*, E. M. Kornacka,1 B. Marcinić,2 G. Przybytniak1

1 Institute of Nuclear Chemistry and Technology, ul. Dorodna 16, 03-195 Warszawa, Poland
2 Department of Pharmaceutical Chemistry, K. Marcinkowski University of Medical Science, ul. Granwałdżka 6, 60-780 Poznań, Poland

(Received April 12, 2002)

Pharmaceuticals sterilized by electron beam irradiation, i.e., flutamide (FA), ifosfamide (IA) and aminogluthethimide (AA), applied in anticancer therapy, were studied in order to determine the kinetics of the radical decay and to find out the structure of some intermediates. Two species identified in flutamide were assigned to a more stable tertiary carbon-centred radical and a less stable ary1 radical or nitrogen-centred radical. Two components were found in ifosfamide, one belongs to the radical formed on the loss of chlorine atom and another reveals a broad anisotropic signal of hyperfine interaction, \( \alpha(1P) = 5.3 \text{ mT} \), together with a narrow splitting of 0.7 mT due to unassigned protons. Radiation generates in aminogluthethimide two different, highly populated radical pairs that rearrange in time into more stable radicals. Electron-beam radiolysis induced slightly more paramagnetic species in AA than in IA and about 20 times less than in FA. On a long time scale the decay rates of radicals in irradiated samples increase successively as AA<FA<IA.

Introduction

Ionising radiation can be applied as a convenient method of drug sterilization, particularly recommended for unstable substances, e.g., thermolabile or ones unsuitable for chemical sterilization.1 It allows the carrying out of radiation treatment in the final packaging stage, which ensures a long period of sterilization validity. As ionizing radiation generates, especially stable in solid state materials, paramagnetic products, it is important to control the radical concentration and their stability, i.e., the decrease in their concentration with time. Paramagnetic species may remain in a crystalline matrix even for a few years and it seems appropriate to determine post-treatment quarantine of a drug before the product is delivered to patients. This is especially important for solid drugs, although a certain type of radical, i.e., nitroxyl radicals possibly generated in certain drugs, can be detected for several hours even in aqueous solutions.

In this paper, we present EPR investigations of free radicals in electron beam irradiated drugs, chosen from the group of pharmaceutical products which were subjected to a preliminary investigation.2 The selected materials, i.e., flutamide (FA), ifosfamide (IA) and amino-gluthethimide (AA), have wide application in anticancer therapy. FA is a synthetic antiandrogen used as a therapeutic agent for prostatic tumours,3,4 whereas AA is administered to patients to block adrenal androgen secretion, e.g., in male breast cancer.5,6 Both drugs, as well as IA,3,8 play an increasing role in combination chemotherapy with other antimour agents.

This study was undertaken to determine the kinetics of the decay and, if possible, structures of the generated radical intermediates. In addition we consider probable mechanisms of formation of the radicals and their fate during storage, all on the basis of developments and transformations of their EPR spectra. Our experiments were carried out under conditions that mimic the real situation of a commercial decontamination based on economic motivation, i.e., with an electron beam generated with a pulsed electron accelerator. These are not the conditions which are most suitable for the precise interpretation of any radical structure which would require, for example, a single crystal matrix, the use of low temperatures, and gamma-irradiation at low dose rates. The results depend very much on the applied conditions and we believe that the effects we present should refer to ‘real’ conditions.

Experimental

2-Methyl-N-[4-nitro-3-(trifluoromethyl)-phenyl]propanamide (flutamide, FA), 3-(2-chloroethyl)-2-[(2-chloroethy1) amino] tetrahydro-2H-1,3,2-oxaza-phosphorine-2-oxide (ifosfamide, IA) and 3-(p-aminophenyl)-3-ethylpiperidine-2,6-dione (aminogluthethimide, AA) were obtained from the Institute of Pharmacy, Warsaw, Poland.

The samples were used in microcrystalline form without further purification. Irradiation was performed with a beam of 10 MeV electrons generated by an Elektronika linear accelerator at room temperature. The samples were irradiated at a dose of 25 kGy under aerated conditions. EPR spectra were measured with a Bruker X-band ESR-300 spectrometer at room temperature using a microwave power of 2 mW (if not indicated otherwise). After irradiation the pharmaceuticals were stored at ambient temperature.

* E-mail: ambroz@orange.ichtj.waw.pl
Double integration of the experimental spectra and the signals recorded for standard DPPH-benzene solution enabled us to estimate the concentrations of spins in the irradiated materials. APOLLO software was applied to perform analysis and deconvolution of the experimental spectra.  

Results and discussion

The experimental spectra are displayed in Figs 1 to 6 together with spectra of individual radicals separated following deconvolution and the decay curves of all paramagnetic species and some components. All EPR spectra were measured after irradiation and subsequently after days and months of storage. The recorded spectra changed their intensities and shapes over time, thus demonstrating the composite character of the processes undergone by the drug-derived free radicals trapped in the solid matrices following radiolysis. When possible, we identify the structures of the free radicals and propose mechanisms of their decay (Schemes 1 and 2).

Flutamide (FA)

Flutamide is a propanamide substituted with an aromatic ring bearing two strong electron-acceptor groups. This compound produces radicals on irradiation at room temperature, their spectra are presented in Fig. 1 (a, b). The EPR signals consist of two components of which one is partly saturated at a microwave power of 20 mW. The first one, obtained by subtraction of spectrum (b) from spectrum (a), was assigned to the major and more stable radical A (Fig. 1 (c), Scheme 1), because the unresolved singlet at $g = 2.0046$ seems to belong to a tertiary carbon-centred radical, as its stability and $g$ factor suggest. The residual part of the