Radical Mechanisms in the Radiosterilization of Metoprolol Tartrate Solutions

Catherine Slegers,[1,3] Gérard Baldacchino,[2] Dominique Le Parc,[2] Bernard Hickel,[2] and Bernard Tilquin[1]

Received April 28, 2003; accepted August 11, 2003

Purpose. Study of the radical mechanisms in the radiosterilization of metoprolol tartrate aqueous solutions in order to determine the parameters governing its radiostability.

Methods. Pulse radiolysis with pseudo-first-order kinetics to measure the reaction rate constants of hydrated electrons and hydroxyl radicals with metoprolol tartrate. Chemsimul® was used to solve the decay kinetics of transients and to simulate the radiolysis of metoprolol tartrate solutions.

Results. Hydrated electrons react with metoprolol and the tartrate ion with rate constants of 6.8×10^7 M^{-1} s^{-1} and 1.7×10^7 M^{-1} s^{-1}, respectively. Hydroxyl radicals react with metoprolol and the tartrate ion with rate constants of 5.2×10^9 M^{-1} s^{-1} and 5.5×10^8 M^{-1} s^{-1}, respectively. The hydroxyl-metoprolol transients are found to scavenge the superoxide anion (5.5×10^{10} M^{-1} s^{-1}), react with oxygen (1.0×10^8 M^{-1} s^{-1}), and follow a biradical decay (2.0×10^8 M^{-1} s^{-1}). A simplified radical mechanism is used to simulate the loss of potency of metoprolol tartrate aqueous solutions during radiosterilization.

Conclusions. To decrease the loss of potency of metoprolol tartrate, the sterilization dose must be lowered and very high dose rates used.

KEY WORDS: radiosterilization; drug aqueous solution; rate constant measurements; computer simulation; metoprolol tartrate.

INTRODUCTION

β-Blockers are among the first choice drugs to treat hypertension and heart failure (1). Many of them are injectable drugs that are sterilized by aseptic filtration because of their heat sensitivity. Aseptic processing is costly and does not provide a recommended 10^{-6} sterility assurance level; furthermore, pharmaceutical guidelines are evolving toward terminal sterilization methods (2–4).

Sterilization by ionizing radiation could be a good alternative because it can be applied on the final packaged product and gives no rise in temperature (5,6). There is a consensus that radiosterilization should not be applied to drugs in aqueous solution because of the greater degradation, or loss of potency, of the drug compared to the solid state (7–10). Decision trees for sterilization methods do not even propose radiosterilization for drugs in aqueous media (4). However, no research has been done on the fundamental mechanisms of the radiolysis of drugs in aqueous solutions.

β-Blockers are found to be quite stable to radiosterilization in the solid state (11), but no research has been done on their radiostability in aqueous solution. The present study investigates the radical mechanisms in the radiolysis of metoprolol tartrate aqueous solutions. The structure of metoprolol tartrate is shown in Fig. 1A. Metoprolol tartrate is a highly soluble salt and dissolves completely in water to yield two molecules of metoprolol per tartrate ion. In the chemical equations metoprolol is denoted as MET, and the tartrate ion as TART:

$$(\text{metoprolol})_2\text{tartrate} \rightarrow 2\ \text{MET} + \text{TART} \qquad (1)$$

The transformation of a drug in aqueous solution is brought about essentially by the attack of free radicals generated by the water radiolysis (12,13):

$$H_2O \rightsquigarrow e^-_{(aq)},\ {}^\bullet OH,\ {}^\bullet H,\ H_2,\ H_2O_2,\ H_3O^+ \qquad (2)$$

Pulse radiolysis (14,15) is used to study the radical mechanisms in the radiolysis of metoprolol tartrate solutions and to measure the associated reaction rate constants. The reaction rate constants of the hydrated electron and the hydroxyl radical with metoprolol tartrate are measured with pseudo-first-order kinetics. The biradical decay of the transients and their reaction with oxygen and the superoxide anion are modeled with a computer simulation program.

The reaction rate constants are essential for the computer simulation of the radiolysis of the drug in aqueous solution, thus allowing predictions on the drug stability toward ionizing radiation. The loss of potency of the drug can be simulated to test the different parameters influencing its radiostability. These include the conditions of irradiation such as the choice of ionizing radiation, the dose rate, and the absorbed dose (10). The parameters of the drug formulation such as its concentration, the presence of radioprotecting excipients, and its gas atmosphere may also be simulated. The computer simulation will play a vital role in determining the feasibility and/or optimizing the radiation processing of drugs in aqueous solution.

MATERIALS AND METHODS

Pulse Radiolysis Setup

The irradiations are performed on a Febetron 707. This electron accelerator produces a single pulse of 1.8 MeV electrons during 15 ns. The drug solutions are placed in a quartz Suprasil® irradiation cell of 2.5 cm optical path. The solutions are changed after each electron pulse, using a system of valves. The formation and decay of free radicals are monitored by time-resolved absorption spectrometry. The experimental setup is described in earlier publications (14,16–17).

The dose is determined by measuring the total charge per pulse with a charge integrating current. Calibrations are made with the thiocyanate dosimeter (12,15).

UV/VIS Absorption Spectra

A Uvikon 933 double-beam UV/VIS Spectrophotometer with quartz Suprasil® precision cells of 1 cm optical path are

[1] Unité d'Analyse Chimique et Physico-chimique des Médicaments, Université Catholique de Louvain, Brussels, Belgium.
[2] Laboratoire de Radiolyse, CEA/Saclay, DSM/DRECAM/SCM/URA331CNRS, Gif-sur-Yvette, Cedex, France.
[3] To whom correspondence should be addressed. (email: catherine.slegers@cham.ucl.ac.be)

ABBREVIATIONS: MET, metoprolol; TART, tartrate; $M^\bullet$, hydroxylmetoprolol transients; $T^\bullet$, hydroxyltartrate transients; HPLC, high-performance liquid chromatography; UV, ultraviolet light; VIS, visible light.

0724-8741/03/1200-1977/0 © 2003 Plenum Publishing Corporation

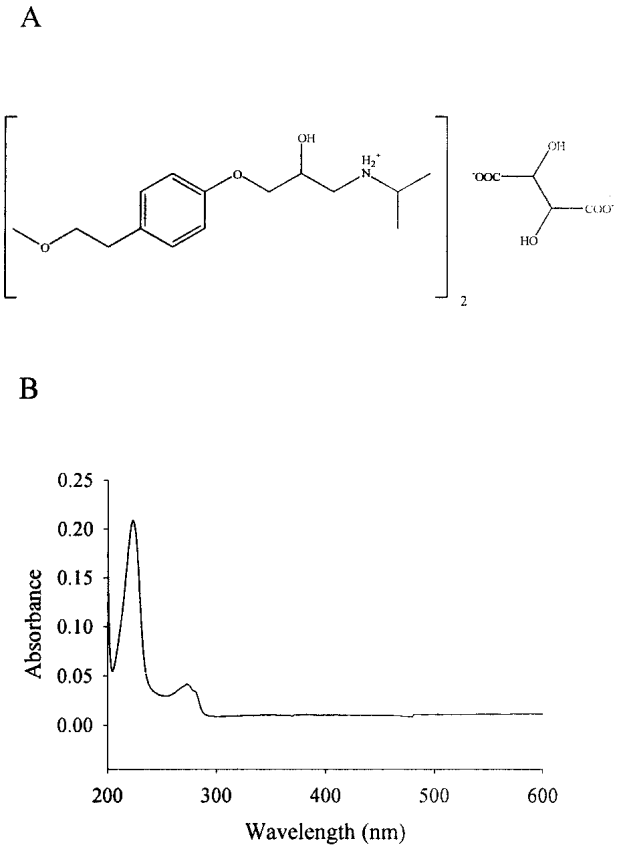

Fig. 1. (A) The structure of metoprolol tartrate. (B) The absorption spectrum of a 10^{-5} M solution of metoprolol tartrate.

used to measure the absorption spectrum of metoprolol tartrate. The absorption spectrum of a 10^{-5} M metoprolol tartrate solution is shown in Fig. 1B.

Chemicals

Metoprolol tartrate with minimum 99% purity and sodium tartrate with minimum 99.6% purity are purchased from Sigma®. Argon, nitrous oxide/oxygen (90/10) and pure oxygen, all of high purity, are purchased from Air-Liquide®.

Ultrapure water is obtained from a Millipore Purification Pak Alpha-Q system. The water resistivity is maximum 18.2 MΩ cm and the water carbon content, ranging from 6 to 14 ppb, is measured with a Millipore A10 Anatel TOC Monitor.

Glassware

All the glassware to prepare the solutions and the irradiation cell are detergent washed, rinsed with ultrapure water, and then dried and passed in the oven at 400°C for 4 h in order to remove all traces of organic contaminants.

Reaction of Hydrated Electrons with Metoprolol Tartrate

The solutions are degassed using argon to remove any traces of oxygen and carbon dioxide, as these readily react with the hydrated electron (18).

The hydrated electron can be observed directly by time-resolved absorption spectrometry as it has an intense absorption spectrum, with a maximum at 720 nm (ε = 20, 000 M^{-1} cm^{-1}) (13). The pseudo-first order decay kinetics of the hydrated electron are measured at 650 nm for 10^{-4}-10^{-1} M concentrations in metoprolol tartrate and doses of 25-55 Gy.

To distinguish between metoprolol and its tartrate salt, similar measurements are made on sodium tartrate solutions.

Reaction of Hydroxyl Radicals with Metoprolol Tartrate

To scavenge hydrated electrons and quantitatively convert them into hydroxyl radicals the solutions are saturated with nitrous oxide (18):

$$e^-_{(aq)} + N_2O + H_2O \rightarrow {}^\bullet OH + OH^- + N_2;\ k = 9.1 \times 10^9\ M^{-1}\ s^{-1} \quad (3)$$

The concentration of nitrous oxide in saturated solutions is 2×10^{-2} M and the radiation chemical yield in hydroxyl radicals at such a solute strength, is 6.0×10^{-7} mol J^{-1} (13).

The hydroxyl radical absorbs weakly in the UV region of the spectrum and therefore its decay cannot be directly observed without interference from the absorption of the drug solute. The absorption spectrum of the transients formed by the reaction of the hydroxyl radical with metoprolol tartrate is scanned from 300–500 nm, so as not to interfere with the absorption of metoprolol tartrate, shown in Fig. 1B. The pseudo-first-order formation kinetics are measured at 300 and 330 nm for 10^{-4}–10^{-3} M concentrations in metoprolol tartrate and doses of 35–75 Gy.

To distinguish between hydroxylmetoprolol transients and hydroxyltartrate transients, the experiments are repeated on sodium tartrate solutions.

Decay of Transients

The decay of the transients formed by the reaction of the hydroxyl radical with metoprolol tartrate is studied in nitrous oxide saturated solutions. Under these conditions the transients can decay by radical-radical or by radical-molecule reactions. The transients are followed at 300 and 330 nm with doses of 40–65 Gy. Solutions of 10^{-2} M metoprolol tartrate are used in order to completely scavenge the hydroxyl radicals to form the transients.

Decay of Transients with Oxygen

A mixture of 90% nitrous oxide and 10% oxygen is used to saturate 10^{-2} M solutions of metoprolol tartrate to study the influence of oxygen on the decay of the transients. The reaction of the hydrated electron with oxygen (4) cannot compete with that of nitrous oxide (3) at such low oxygen concentrations. The oxygen concentration in solution is 1.25×10^{-4} M and that of nitrous oxide 1.8×10^{-2} M (13).

$$e^-_{(aq)} + O_2 \rightarrow O_2^{\bullet -};\ k = 1.9 \times 10^{10}\ M^{-1}\ s^{-1} \quad (4)$$

Under these conditions 1.4% of hydrated electrons react with the oxygen in solution to form the superoxide anion and the rest are transformed into hydroxyl radicals. The decay kinetics of the transients are measured at 300 and 330 with doses of 250-300 Gy in 10^{-2} M metoprolol tartrate solutions. These are compared to the decay without oxygen in the medium.

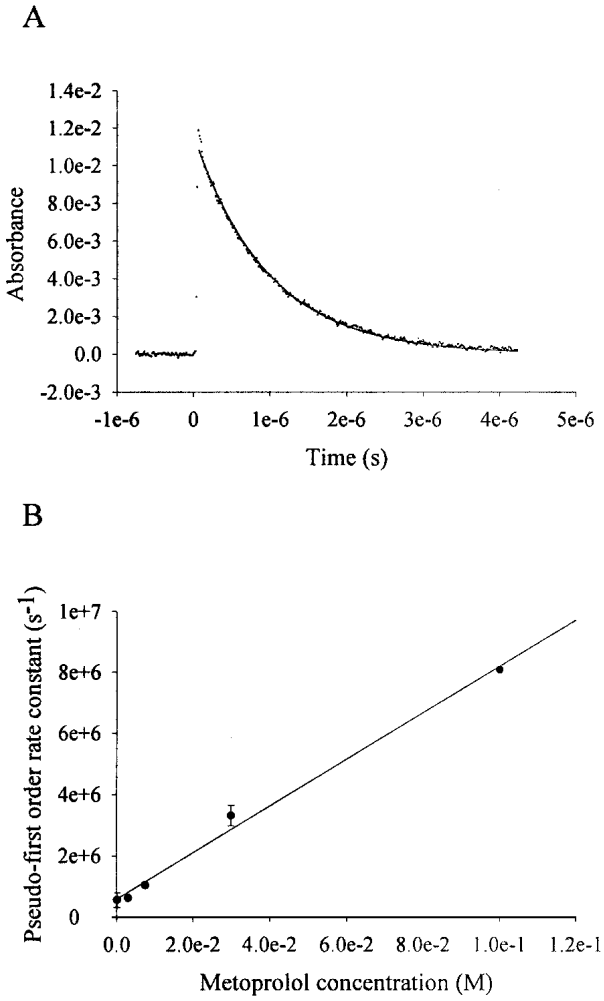

Fig. 2. (A) Absorbance of the hydrated electron at 650 nm as a function of time (·). Exponential decay regression (–) with equation $D_t = D_0\ e^{-\ kobs\ t}$ and $D_0 = 1.16 \times 10^{-2}$, $k_{obs} = 1.03 \times 10^6\ s^{-1}$. Argon-saturated solution of 3.738×10^{-3} M metoprolol tartrate, dose of 1 Gy. (B) Pseudo-first-order rate constant (s^{-1}), k_{obs}, for the hydrated electron decay vs. metoprolol concentration (M) (●). Linear regression (–) with equation $k_{obs} = k_{global}$ [MET] + b, and $k_{global} = 7.6 \times 10^7\ M^{-1}\ s^{-1}$.

Decay of Transients with the Superoxide Anion

Oxygen saturated solutions are used to study the decay kinetics of the transients when the superoxide anion is present in solution. The oxygen concentration in saturated solutions is 1.25×10^{-3} M and it effectively scavenges the hydrated electron to form the superoxide anion according to reaction (4). The decay kinetics of the transients are measured at 300 and 330 nm with doses of 25-60 Gy in 10^{-2} M solutions of metoprolol tartrate. The decay kinetics are compared to that in other media.

Computer Simulation Program

Chemsimul® is used to solve the complex decay kinetics of the transients in different media and to simulate the radiolysis of metoprolol tartrate solutions. Chemsimul® solves the non-linear differential equations of the reaction rates of all the species in the irradiated solution. The simulation program is adapted to the radiolysis of aqueous solutions as it allows the input of the radiation chemical yields, the absorbed dose and the dose rate of the ionizing radiation. The 30 or more reactions of the water radiolysis used in the computer simulation program are well documented in other publications (19,20).

The simulation results are outputted as data tables of the molar concentrations of all the species in the irradiated medium as a function of time. To reproduce the experimental results, Beer's Law is included to graphically output the chosen species' absorbance as a function of time.

RESULTS AND DISCUSSION

Reaction of Hydrated Electrons with Metoprolol Tartrate

The absorbance of the hydrated electron at 650 nm as a function of time in a solution of metoprolol tartrate is repre-

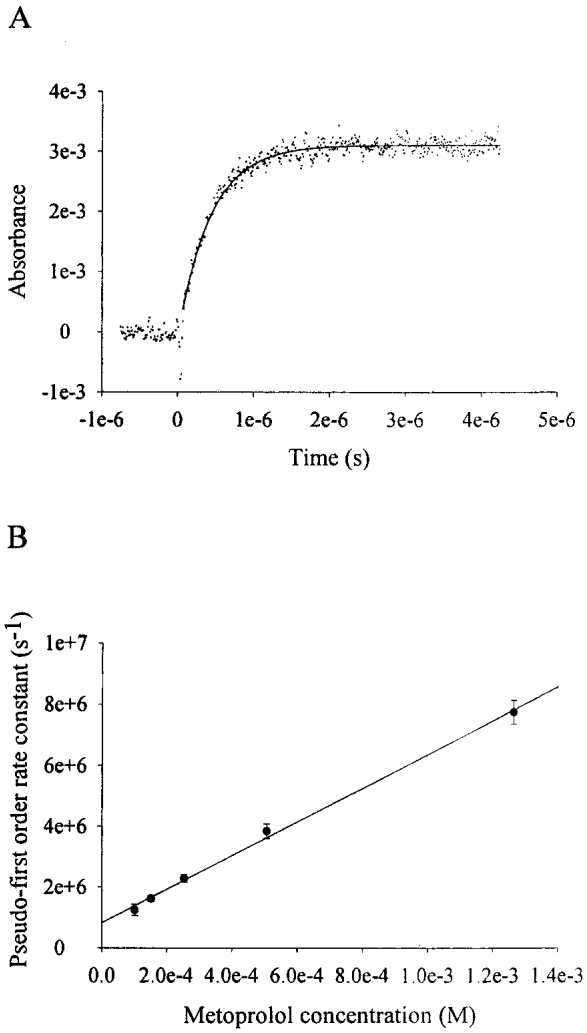

Fig. 3. (A) Absorbance of the transients formed by the reaction of the hydroxyl radical with metoprolol tartrate at 300 nm as a function of time (·). Exponential rise to maximum regression (–) with equation $D_t = D_{inf}\ (1 - e^{-kobs\ t})$ and $D_{inf} = 3.11 \times 10^{-3}$, $k_{obs} = 2.30 \times 10^6\ s^{-1}$. Nitrous oxide saturated solution of 1.265×10^{-4} M metoprolol tartrate, dose of 1 Gy. (B) Pseudo-first-order rate constant (s^{-1}), k_{obs}, vs. metoprolol concentration (M) for the formation of the transient species (●). Linear regression (–) with equation $k_{obs} = k_{global}$ [MET] + b, and $k_{global} = 5.5 \times 10^9\ M^{-1}\ s^{-1}$.

Table I. Summary of the Computer Simulation Parameters and Results for the Formation and Decay of Transients in Different Media

Transients	Wavelength (nm)	Molar absorptivity coefficient (M^{-1} cm^{-1})	Rate constant of formation (M^{-1} s^{-1})	Equation
$M^{\bullet}$	300	2955	5.2×10^9	(12)
	330	2246		
$T^{\bullet}$	300	1808	5.5×10^8	(13)
	330	833		
M_2	300	1000	2.0×10^8	(16)
	330	250		
$MO_2^{\bullet}$	330	100	1.0×10^8	(17)
MO_2^-	300	1000	5.5×10^{10}	(18)
	330	500		

sented by dots in Fig. 2A. The exponential decay half-life is constant from the beginning to the end of the curve, which is consistent with a first-order process. A regression with equation $D_t = D_0\ e^{-\ kobs\ t}$, is fitted through the experimental results and superimposed on the graph. D_0 is the initial absorbance, D_t is the absorbance at time t and k_{obs} is the observed rate of decay of the hydrated electron, in the pseudo-first order conditions.

The slope of the graph of the pseudo-first order rate constant vs. metoprolol concentration, shown in Fig. 2B, represents the global rate constant, k_{global}, and equals 7.6×10^7 M^{-1} s^{-1}. The global rate constant is the sum of the reaction rate constants of the hydrated electron with metoprolol and its tartrate ion.

Similar results are obtained for the reaction of the hydrated electron with sodium tartrate; only the highest sodium tartrate concentration is used because lower concentrations show competing reactions for the hydrated electron from the water radiolysis. The rate constant for the reaction of the hydrated electron with the tartrate ion, $k_{e\text{-}(aq)TART}$ is found to be 1.7×10^7 M^{-1} s^{-1}.

The rate constant of metoprolol with the hydrated electron, $k_{e\text{-}(aq)MET}$ is calculated with equation (5) and equals 6.8×10^7 M^{-1} s^{-1}.

$$k_{global} = k_{e\text{-}(aq)MET} + \tfrac{1}{2}\, k_{e\text{-}(aq)TART} \qquad (5)$$

These reaction rates are slow and high concentrations of metoprolol tartrate are needed in order to compete for hydrated electrons in the reactions of the water radiolysis. Metoprolol tartrate has no functional groups that are susceptible to react with the hydrated electron, as this one reduces carbonyl groups or acts as a nucleophilic reagent toward aromatic rings/double bonds with adjacent electron-withdrawing substituents (12).

The following mechanism is incorporated into the simulation program for the reaction of hydrated electrons with metoprolol and the tartrate ion:

$$MET + e^-_{(aq)} \rightarrow M^- ;\ k_{e-(aq)MET} = 6.8 \times 10^7\ M^{-1}\ s^{-1} \qquad (6)$$

$$TART + e^-_{(aq)} \rightarrow T^- ;\ k_{e-(aq)TART} = 1.7 \times 10^7\ M^{-1}\ s^{-1} \qquad (7)$$

Reaction of Hydroxyl Radicals with Metoprolol Tartrate

The absorbance at 300 nm of the transients formed from the reaction of the hydroxyl radical with metoprolol tartrate is shown as dots in Fig. 3A. The absorbance of the transients reaches plateau, which means that all the hydroxyl radicals have reacted and the reaction is complete. A regression with equation $D_t = D_{inf}\ (1 - e^{-\ kobs\ t})$ is fitted through the experimental results and superimposed on the graph. D_t is the absorbance at time t, D_{inf} is the absorbance of the plateau, k_{obs} is the pseudo-first order rate constant of formation.

The slope of the graph of the pseudo-first order rate constant vs. metoprolol concentration, shown in Fig. 3B, is the global rate constant, k_{global}, and equals 5.5×10^9 M^{-1} s^{-1}. It is the sum of the hydroxyl rate constants with metoprolol and the tartrate ion. The rate constant of metoprolol with the hydroxyl radical, $k_{\bullet OHMET}$, can be calculated knowing the tartrate-hydroxyl rate constant, $k_{\bullet OHTART}$, and using eq. (8).

$$k_{global} = k_{\bullet OHMET} + \tfrac{1}{2}\, k_{\bullet OHTART} \qquad (8)$$

The same experimental conditions are used to determine the rate constant of the hydroxyl radical with sodium tartrate. The hydroxyl rate constant with the tartrate ion is found to be 5.5×10^8 M^{-1} s^{-1}, which is in accordance to other publications (18). The hydroxyl rate constant with metoprolol is calculated to be 5.2×10^9 M^{-1} s^{-1}.

The reaction of metoprolol tartrate with hydroxyl radicals is fast enough to compete with those of the water radiolysis. Metoprolol is found to be an effective hydroxyl radical scavenger, which is not unusual since the hydroxyl radical reacts with most organic compounds (13,18). The hydroxyl radical can react by hydrogen abstraction or by electrophilic addition on double bonds/aromatic rings (12). There are many different possible hydroxyl radical attack sites on the metoprolol molecule, such as the aromatic ring, the mobile hydrogen of the alcohol/amine functional groups, or the hydrogen in α to the ether groups. It is proposed that different transients are formed and for simplification, the hydroxylmetoprolol transients are represented as $M^{\bullet}$ and the hydroxyltartrate transients as $T^{\bullet}$:

$$MET + {}^{\bullet}OH \rightarrow M^{\bullet} ;\ k_{\bullet OHMET} = 5.2 \times 10^9\ M^{-1}\ s^{-1} \qquad (9)$$

$$TART + {}^{\bullet}OH \rightarrow T^{\bullet} ;\ k_{\bullet OHTART} = 5.5 \times 10^8\ M^{-1}\ s^{-1} \qquad (10)$$

Similarly, the rate constant $k_{\bullet OHMET}$ is the sum of all the hydroxyl radical reactions on the metoprolol molecule, and $k_{\bullet OHTART}$ that of the tartrate ion. Metoprolol and its tartrate ion compete with each other for the capture of hydroxyl radicals and the transients $M^{\bullet}$ and $T^{\bullet}$ are formed in a 19:1 proportion.

It is not possible to distinguish between the different hydroxyl radical-transients, especially in UV spectrometry because it is not a selective detection method. Furthermore,

radiolysis products generally have the same chromophore as the parent compound (21–22). The simplified radiolysis mechanism is enough for the computer simulation of the hydroxyl radical reactions on metoprolol tartrate.

DECAY OF TRANSIENTS

The decay of the hydroxyl-tartrate transients is not studied because they contribute little to the total absorbance, which is the sum of the absorption spectra of $M^{\bullet}$ and $T^{\bullet}$. This is because $M^{\bullet}$ and $T^{\bullet}$ are formed in a 19:1 proportion and the molar absorptivity of $T^{\bullet}$ is smaller compared to that of $M^{\bullet}$, as can be seen in Table I. A simplification is made to consider only the hydroxyl-metoprolol transients.

The decay of the transients at 300 nm in nitrous oxide saturated solutions of metoprolol tartrate is shown as dots in Fig. 4A. The absorbance (Dt) does not go back to the baseline (D_{inf}) and persists even after 10^{-2} s because the product(s) formed absorb(s) at the same wavelength. The decay kinetics are complicated because the measured absorbance represents the sum of the decay of transients and the formation of products.

Chemsimul® is used to calculate the rate of transient decay and product formation. Several hypotheses are proposed for the radical mechanism but only one simulation reproduced the experimental results: the bi-radical decay:

$$M^{\bullet} + M^{\bullet} \rightarrow M_2 \; ; \; k_{simM2} \qquad (11)$$

The molar absorptivity coefficient of M_2 and the rate constant for the bi-radical decay, k_{simM2}, are varied to fit the experimental curve as shown in Fig. 4A. The simulation parameters and results are in Table I. The bi-radical decay rate of the transients is calculated to be $2.0 \pm 0.3 \times 10^8$ M^{-1} s^{-1}.

Decay of Transients with Oxygen

The absorbance of transients at 300 nm in the presence of oxygen is shown as dots in Fig. 4B. The rate of decay increased compared to the nitrous oxide medium, suggesting a reaction with the oxygen in solution. A reaction of the transients with oxygen is included in the simulation program:

$$M^{\bullet} + O_2 \rightarrow MO_2^{\bullet} \; ; \; k_{simMO2\bullet} \qquad (12)$$

The values of $k_{simMO2\bullet}$ and the molar absorptivity coefficient of $MO_2^{\bullet}$ are varied to reproduce the experimental curve, as is shown in Fig. 4B. The simulation parameters and results are in Table I. The reaction rate constant of the transients with the dissolved oxygen is calculated to be $1.0 \pm 0.5 \times 10^8$ M^{-1} s^{-1}.

Decay of Transients with the Superoxide Anion

The transients decayed much faster in the presence of the superoxide anion as can be seen in Fig. 4C. The decay of transients with the superoxide anion is in the 10^{-6} s time scale, whereas the bi-radical decay and the decay with oxygen in the

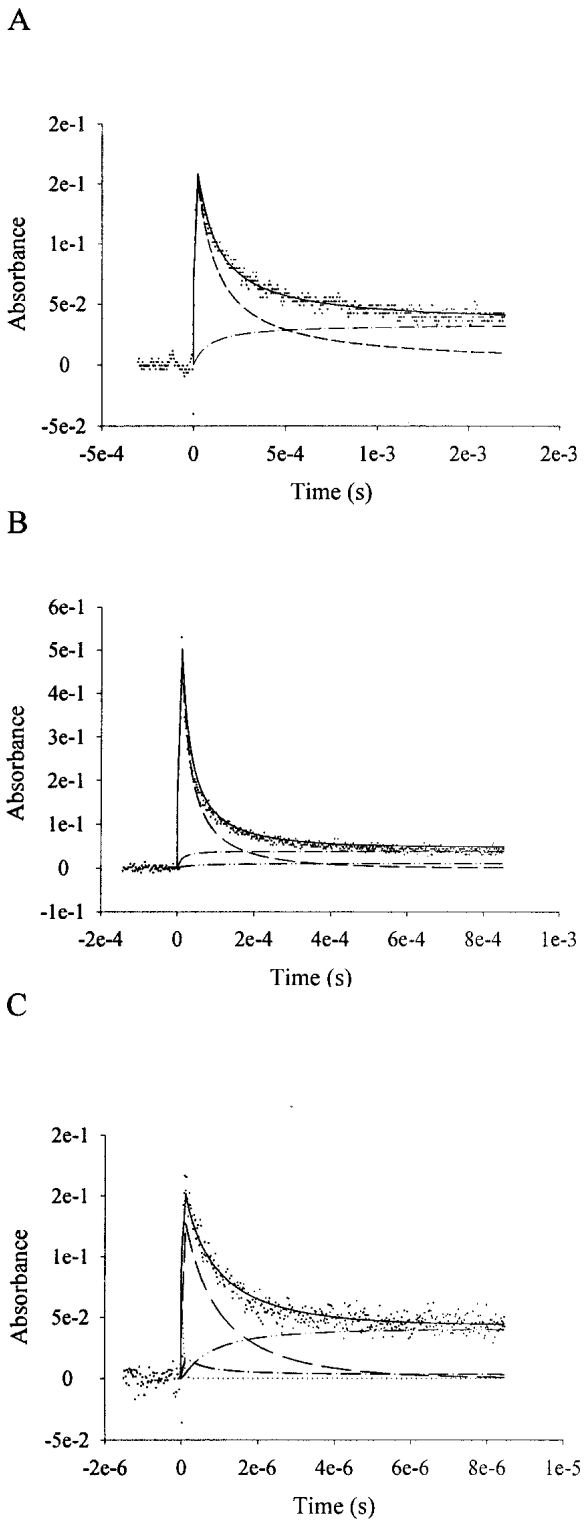

Fig. 4. (A) Decay of the hydroxylmetoprolol transients at 300 nm in nitrous oxide saturated solutions of 1.503×10^{-2} M metoprolol tartrate, dose of 43 Gy (·). Computer simulation of the decay of the hydroxylmetoprolol transients: total absorbance (—), absorbance of $M^{\bullet}$ (– –) and M_2 (– · –); $k_{simM2} = 2.0 \times 10^8$ M^{-1} s^{-1}. (B) Decay of the hydroxyl-metoprolol transients at 330 nm in N_2O/O_2 (90/10) saturated solutions of 5.000×10^{-3} M metoprolol tartrate, dose of 300 Gy (·). Computer simulation of the decay of the hydroxylmetoprolol transients: total absorbance (—), absorbance of $M^{\bullet}$ (– –), M_2 (– · –), and $MO_2^{\bullet}$ (– ·· –); $k_{simMO2\bullet} = 1.0 \times 10^8$ M^{-1} s^{-1}. (C) Decay of the hydroxyl-metoprolol transients at 300 nm in O_2 saturated solutions of 5.025×10^{-3} M metoprolol tartrate, dose of 60.76 Gy (·). Computer simulation of the decay of the hydroxyl-metoprolol transients: total absorbance (—), absorbance of $M^{\bullet}$ (– –), $e^-_{(aq)}$ (····), $O_2^{\bullet -}$ (– · –) and MO_2^- (– ·· –); $k_{simMO2-} = 5.5 \times 10^{10}$ M^{-1} s^{-1}.

10^{-4} s time scale. The reaction of the superoxide anion with the transients is added in the simulation program.

$$M^{\bullet} + O_2^{\bullet -} \rightarrow MO_2^{-} ; k_{simMO2-} \quad (13)$$

The values of $k_{simMO2-}$ and the molar absorptivity coefficient of MO_2^- are varied to reproduce the experimental curve as is shown in Fig. 4C. The simulation results and parameters are in Table I. The reaction rate constant of the transients with the superoxide anion, $k_{simMO2-}$, is calculated to be $5.5 \pm 1.2 \times 10^{10}$ M^{-1} s^{-1}.

There is a competition reaction for the transients between the dissolved oxygen and the superoxide anion. The transients $MO_2^{\bullet}$ and MO_2^- are formed in a 1:6 proportion for an average dose of 50 Gy. This proportion is dependant on the absorbed dose because if the latter is increased, the yield in superoxide anions increases.

Nature of the Transients and Future Perspectives

The structure identification of the transients and the final products is important in order to elucidate the missing steps in the proposed radical mechanism for the radiolysis of metoprolol tartrate solutions. The radical mechanism is initiated by the attack of free radicals on metoprolol tartrate, propagated by the decay of transients and terminated by reactions that may lead to stable radiolysis products.

A direct method to detect and analyze transient radicals is the spin trapping technique, which stabilizes short-lived radicals as adducts and then analyzes them by electron paramagnetic resonance to elucidate their structure (23).

An alternative method is the qualitative analysis of the final radiolysis products by HPLC-mass spectrometry. The final products of radiolysis are unique because they derive from radical mechanisms (22,24,25). Therefore, the structure identification of the final radiolysis products can provide clues as to the radicals they originate from.

The structure identification of the main final radiolysis products of metoprolol tartrate solutions will be done in future work and will hopefully elucidate the nature of the transients $M^{\bullet}$ and $T^{\bullet}$ as well as the reaction pathways that predominate.

Computer Simulation of the Radiolysis of Metoprolol Tartrate Solutions

A simplified radical mechanism is proposed for the degradation of metoprolol tartrate in order to run simulations on the loss of potency of the beta-blocker during radiosterilization of aqueous solutions. The parameters of drug concentration, absorbed dose and dose rate are purposely varied to study their effect on the relative loss of potency of metoprolol. In the simulation two different dose rates are used, a low dose rate of 1kGy/h to represent gamma irradiation, and a very high one of 1kGy/ns to represent the upper limit of electron beam irradiation. The metoprolol concentrations used in the simulation are varied around that of commercialized injectables (1mg/ml). The simulation results are shown in Fig. 5.

The relative loss in potency of metoprolol is proportional to the absorbed dose because the greater the absorbed dose, the higher the yield in reactive radicals. In recent radiosterilization quality assurance guidelines, an absorbed dose of 25 kGy is no longer recommended (2–4) and lower doses can be validated with appropriate sterility tests (26–27).

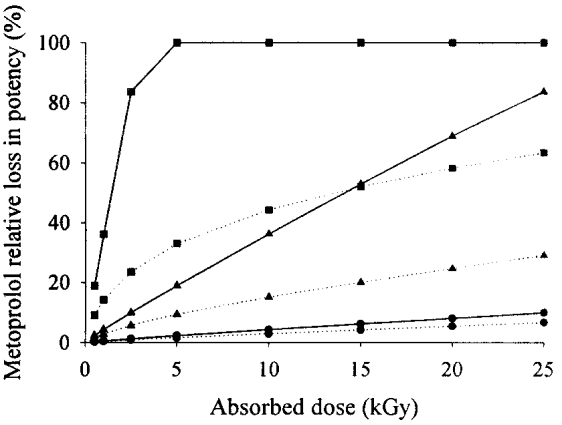

Fig. 5. Computer simulation of the relative percentage loss in potency of metoprolol as a function of the absorbed dose with a dose rate of 1 kGy/h (–) and 1 kGy/ns (····) for metoprolol concentrations of 10^{-1} M (●), 10^{-2} M (▲), and 10^{-3} M (■).

The lower the concentration of metoprolol, the greater the influence of the dose rate on the relative loss in potency. A 10^{-3} M metoprolol solution has a 50% relative loss in potency after an absorbed dose of 1.4 kGy for a dose rate of 1 kGy/h and after 14 kGy for a dose rate of 1 kGy/ns; whereas 10^{-1} M solutions of metoprolol have a relative loss of potency < 10% for both dose rates. The higher the dose rate, the greater the radical-radical recombination reactions and the lesser the radical-drug solute reactions. An increase in the drug concentration to reduce its relative degradation is not applicable for injectable drugs, but could be done for sterile topical drug solutions. Very high dose rates to lower the drug degradation can be achieved because radiation technologies are evolving toward powerful e-beam accelerators.

The effect of radioprotecting excipients can also be simulated if their rate constants are known with the products of the radiolysis of water. Antioxidants or co-solvents frequently used in injectable drug formulations could be investigated in regards to their free radical scavenging capacity. Some reactions of cosolvents are already described (18).

In order to validate the calculations, the computer simulation results will be compared to experimental results from chromatographic analysis. Future work will be done to quantitatively measure the influence of the parameters of irradiation and drug formulation on the radiostability of metoprolol tartrate aqueous solutions.

CONCLUSION

The degradation of metoprolol tartrate during radiosterilization of aqueous solutions is essentially due to the attack of hydroxyl radicals. To decrease the loss of potency of metoprolol tartrate, the sterilization dose must be lowered and very high dose rates used.

ACKNOWLEDGMENTS

The pulse radiolysis experiments were rendered possible by grant support from the Ministère de la Communauté française, Concours des Bourses de Voyage 2002.

REFERENCES

1. B. G. Katzung. *Basic and Clinical Pharmacology, 7th ed.* Appleton & Lange, McGraw-Hill, NewYork, 1998.
2. *European Pharmacopoeia 4th ed.* Council of Europe, Strasbourg, 2001.
3. *The United States Pharmacopoeia National Formulary 18*, United States Pharmacopeial Convention, Rockville, MD, 1995.
4. EMEA Decision trees for the selection of sterilisation methods (CPMP/QWP/054/98 Corr), 2000.
5. F. M. Nordhauser and W. P. Olson. (eds.). *Sterilization of Drugs and Devices: Technologies for the 2000,* Interpharm Press, Illinois, 1998.
6. B. D. Reid. Gamma processing technology: an alternative technology for terminal sterilization of parenterals. *PDA J. Pharm. Sci. Technol.* **49**:83–89 (1995).
7. N. G. S. Gopal. *Radiation Sterilization and Treatment of Medical Products: Current Practices, Regulations and Standards.* Consultant's Meeting "Training Guidelines for Industrial Radiation Sterilization," IAEA, Jerusalem, 27–30 November 1995.
8. C. Boess and K. W. Bögl. Influence of radiation treatment on pharmaceuticals—A review: alkaloids, morphine derivatives and antibiotiques. *Drug Dev. Ind. Pharm.* **22**:495–529 (1996).
9. B. Tilquin. Radio-stérilisation des médicaments. *J. Pharm. Belg.* **46**:396–398 (1991).
10. B. Tilquin and B. Rollmann. Recherches à conseiller pour l'application de la stérilisation ionisante des médicaments. *J Chim Phys* **93**:224–230 (1996).
11. A. Engalytcheff, V. Deridder, R. Debuyst, and B. Tilquin. Determination of radical yields in solid-state drugs as one technique to identify drugs that will withstand radiosterilization: radioresistance of beta-blockers. *Radiat. Res.* **160**:103–109.
12. J. W. T. Spinks and R. J. Woods. *An Introduction to Radiation Chemistry, 3rd ed.* Wiley-Interscience, John Wiley & Sons, New York, 1990.
13. C. Ferradini and J. Pucheault. *Biologie de l'action des rayonnements ionisants*, Masson, Paris, 1983.
14. B. Hickel. Pulse radiolysis in chemistry: instruments and prospects. *J. Chim. Phys.* **85**:9–12 (1988).
15. J. H. Baxendale and F. Busi. *The Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*, D. Reidel Publishing Company, Dordrecht, 1982.
16. A.-S. Crucq, B. L. Tilquin, and B. Hickel. Radical mechanisms of cephalosporins: A pulse radiolysis study. *Free Radic. Biol. Med.* **18**:841–847 (1995).
17. A.-S. Crucq and B. L. Tilquin. Attack of cefotaxime by different radicals: comparison of the effects. *Free Radic. Biol. Med.* **21**:827–832 (1996).
18. G. V. Buxton, C. L. Greenstock, P. W. Helman, and A. B. Ross. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($^{\bullet}OH/^{\bullet}O^{-}$) in aqueous solution. *J. Phys. Chem. Ref. Data* **17**:513–886 (1988).
19. E. Bjergbakke, K. Sehested, L. O. Rasmussen, and H. Christensen. *Riso-M-2430: Input files for Computer Simulation of Water Radiolysis*, Riso National Laboratory, Roskilde, 1984.
20. E. Bjergbakke, Z. D. Draganic, K. Sehested, and I. G. Draganic. Radiolytic products in waters part I: Computer simulation of some radiolytic processes in the laboratory. *Radiochimica Acta.* **48**:65–71 (1989).
21. M. Gibella and B. Tilquin. Detection of the radiolysis of solid ampicillin by UV-spectroscopy. *Analusis.* **27**:657–662 (1999).
22. N. Barbarin and B. Tilquin. Study of nonvolatile degradation compounds produced by radiosterilization of cefotaxime. *Radiat. Phys. Chem.* **60**:359–367 (2001).
23. S. I. Dikalov and R. P. Mason. Spin trapping of polyunsaturated fatty acid-derived peroxyl radicals: reassignment to alkoxyl radical adducts. *Free Radic. Biol. Med.* **30**:187–197 (2001).
24. F. Zeegers, M. Gibella, and B. Tilquin. Analysis of some products from the irradiation of solid chloramphenicol. *Radiat. Phys. Chem.* **50**:149–153 (1997).
25. N. Barbarin, B. Tilquin, and E. de Hoffmann. Radiosterilization of cefotaxime: investigation of potential degradation compounds by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A* **929**:51–61 (2001).
26. *EN 552 Sterilisation of medical devices: Validation and routine control of sterilisation by irradiation,* European Standardisation Organisation, Brussels, 1994.
27. *ISO 11137 Sterilization of health care products: Requirements for validation and routine control: Radiation sterilization,* International Organization for Standardization, Geneva, 1995.