Pergamon

Free Radical Biology & Medicine, Vol. 18, No. 5, pp. 841–847, 1995

0891-5849/95 $9.50 + .00

0891-5849(94)00204-5

Original Contribution

RADICAL MECHANISMS OF CEPHALOSPORINS: A PULSE RADIOLYSIS STUDY

ANNE-SOPHIE CRUCQ,* BERNARD L. TILQUIN,* and BERNARD HICKEL†

* Unité d'Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, Bruxelles, Belgique; and †C.E.A., CEN Saclay, DRECAM-SCM, CNRS-URA, Gif-sur-Yvette, Cedex, France

(*Received* 11 *April* 1994; *Revised* 15 *September* 1994; *Accepted* 22 *September* 1994)

Abstract—Radiosterilization induces radicals, and it is very important to describe radical mechanisms before the possible use of cephalosporins gamma sterilization. Moreover, physiological or radiotherapeutically induced free radicals also initiate radical mechanisms. For this study, pulse radiolysis was used. This method permits to avoid in vivo direct study difficulties of bioradical processes and gives quantitative data. Reactions of solvated electron (e^-_{aq}), hydroxyl radical ($^{\bullet}OH$), azide radical ($N^{\bullet}_3$), dibromine radical anions ($Br_2^{\bullet -}$), oxygen, and superoxide radical ($O_2^{\bullet -}$) with three cephalosporins have been studied . Absorption spectra and rate constants have been determined. It has been found that both e^-_{aq} and $^{\bullet}OH$ quickly react ($k \simeq 10^{10}$ mol^{-1} L s^{-1}) with the molecules to give radicals with similar absorption spectra. $N^{\bullet}_3$ gives an absorption spectra that has been attributed to an electron transfer, whereas a part of $^{\bullet}OH$ and $Br_2^{\bullet -}$ could add themselves to an unsaturated bond.

Keywords—Pulse radiolysis, Cephalosporins, Radical mechanisms, Rate constants, Free radicals

INTRODUCTION

Cephalosporins are β-lactam antibiotics that are the most frequently prescribed antibacterial drugs.[1] Commercially available as powder for injections, a sterilization is required. Because of their degradation, especially at high temperature, which eliminates conventional sterilization methods such as autoclaving, a costly sterilizing filtration in aseptic conditions is used. Therefore, the gamma radiosterilization could be a better process: the packed drug is sterilized and undergoes very little degradation.[2] Moreover, the sterility control can be easily made by measuring the absorbed dose (chemical dosimetry). However, the ionizing treatment leads to new substances produced by radical mechanisms.[3] Thus, it seems essential to study this radical mechanism by methods like electron spin resonance (ESR) in solid state[3] or pulse radiolysis in liquid state (this study). ESR measurements give qualitative features only in favorable studies like the radiolysis of solid ceftazidim, where the ESR spectra have been recorded during the warming up of the irradiated samples.[4] ESR study of the two other cephalosporins is in progress.

Moreover, the use of quantitative data obtained in vitro by pulse radiolysis could be of fundamental interest in vivo and, perhaps, could give new views on drugs metabolisms: free radicals as hydroxyl radical, superoxide radical, and so forth, are physiological products and can react to administered cephalosporins.[5]

Pulse radiolysis is a method commonly used to study the radicals formed by irradiation of dissolved molecules or ions. The electron pulse produces a high initial concentration of reactive intermediates with lifetimes of microseconds or longer. The change in concentration of these species can be followed by the changes in optical density of the irradiated solutions. Spectroscopic and kinetic data about the transient species formed by the attack of different free radicals (e^-_{aq}, $^{\bullet}OH$, $N^{\bullet}_3$, $Br_2^{\bullet -}$) on three cephalosporins—sodium cefotaxim, sodium cefuroxim, and pentahydrated ceftazidim (Fig. 1)—are described.

There are very few publications on the radiolysis of cephalosporins. Most of them deal with the loss of antibacterial activity under gamma irradiation, but no mechanism and no rate constant are given.[6–8] The only one that uses pulse radiolysis gives the rate constant of

Address correspondence to: Anne-Sophie Crucq, Unité d'Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, 72, av. E. Mounier, 1200 Bruxelles, Belgique.

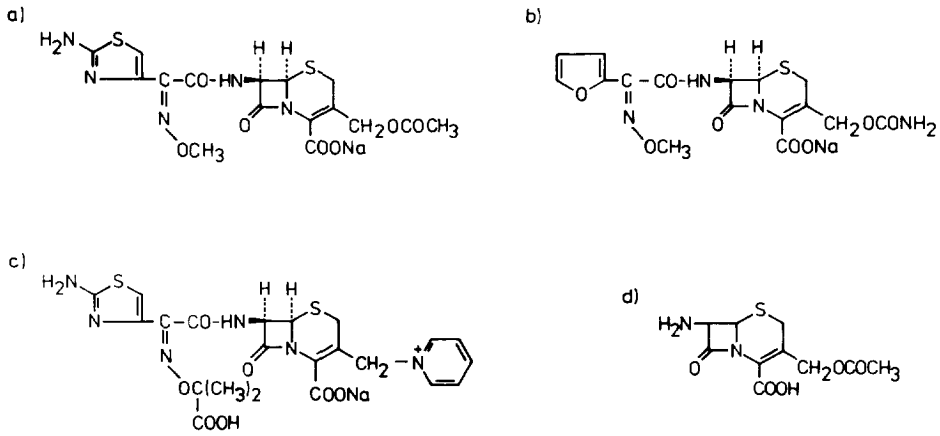

Fig. 1. Chemical stuctures of (a) sodium cefotaxim, (b) sodium cefuroxim, (c) ceftazidim, and (d) 7-aminocephalosporanic acid.

hydrated electron with cephalosporin C, cephaloridine, and cephalothin to study the interaction of these compounds with serum albumine.[9]

MATERIALS AND METHODS

Sodium cefotaxim was obtained from Hoechst, sodium cefuroxim and hydrated ceftazidim were obtained from Glaxo. Ceftazidim contains Na_2CO_3, CO_2, and N_2. Terbutanol, sodium azide, and potassium bromide pro analysis were used without further purification. All solutions were prepared with tri-distilled water.

Pulse radiolysis experiments were performed using a modified Febetron 707, which delivers electron pulse of 1,8 MeV during 15 ns. The irradiation cell in quartz had an optical path length of 2.5 cm. The solution inside the cell was changed after each pulse. The variation of dose from pulse to pulse was monitored by measuring the total pulse charge with a charge integrating current. The doses were calibrated with Fe $(CN)_6^{4-}$/ Fe $(CN)_6^{3-}$, $\epsilon_{420} = 1040\ M^{-1}cm^{-1}$ at room temperature, and $G = 5{,}8.10^{-7}$ mol J^{-1}. The fast spectrophotometric detection system has been described in earlier publications.[10,11]

RESULTS

It is known that water radiolysis leads to radical (e_{aq}^-, $^{\bullet}OH$, $H^{\bullet}$) and molecular (H_2O_2, H_2) products. To simplify the radical mechanisms, some of these radicals were selected by using different working conditions.[12]

1. Reducing conditions (e_{aq}^-)—Aqueous solutions were degazed with argon, and terbutanol was added to convert hydroxyl radicals into nonreactive terbutanol radicals.

$$^{\bullet}OH + (CH_3)_3COH \rightarrow H_2O + (CH_3)_2{}^{\bullet}CH_2 - COH$$

In such solutions, the total radiolytic yield (G) of e_{aq}^- is $2{,}9.10^{-7}$ mol J^{-1}.

2. Oxidizing conditions ($^{\bullet}OH$)—Solutions were saturated with nitrous oxide (N_2O) to convert e_{aq}^- into hydroxyl radicals.

$$N_2O + e_{aq}^- \rightarrow N_2 + OH^- + {}^{\bullet}OH$$

$$G(^{\bullet}OH) = 5{,}8.10^{-7}\ \text{mol}\ J^{-1}$$

3. Azide radical ($N_3^{\bullet}$) or dibromine radical anion ($Br_2^{\bullet -}$) selection—In N_2O saturated solutions, high concentrations of NaN_3 or KBr were added to transform quantitatively $^{\bullet}OH$ radicals into $N_3^{\bullet}$ or $Br_2^{\bullet -}$, respectively.

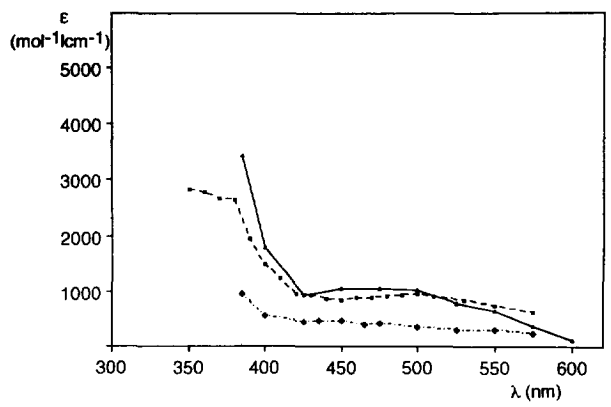

Fig. 2. Differential absorption spectra of 4×10^{-3} M cefotaxim (—), cefuroxim (· – ·), and ceftazidim (- - -) in terbutanol 1% aqueous solutions, argon-saturated.

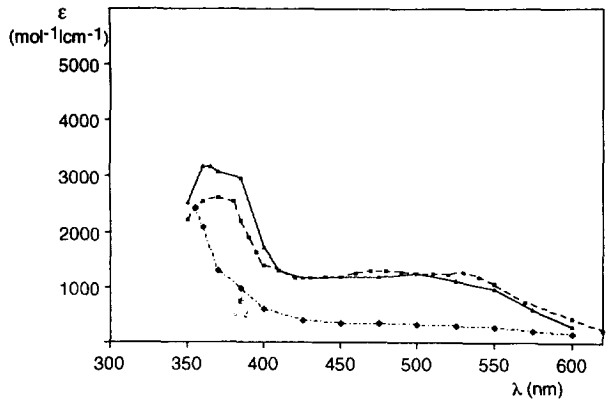

Fig. 3. Differential absorption spectra of 1×10^{-3} M cefotaxim (—), cefuroxim (· – ·), and ceftazidim (- - -) in aqueous solutions, N_2O-saturated.

$$^{\bullet}OH + N_3^- \rightarrow OH^- + N_3^{\bullet} \quad G(N_3^{\bullet}) = 5{,}8.10^{-7} \text{ mol J}^{-1}$$

or

$$^{\bullet}OH + Br^- \rightarrow OH^- + Br^{\bullet}$$

$$Br^{\bullet} + Br^- \rightarrow Br_2^{\bullet -} \quad G(Br_2^{\bullet -}) = 5{,}8.10^{-7} \text{ mol J}^{-1}$$

Absorption spectra

Absorption spectra, recorded 200 ns after the pulse (2 ms for the radicals from KBr solutions), were determined for the three studied cephalosporins (Figs. 2–4). The different working conditions described before were used. The molar extinction coefficients were calculated after determination of the absorbed doses and by using the radiochemical yields mentioned previously.

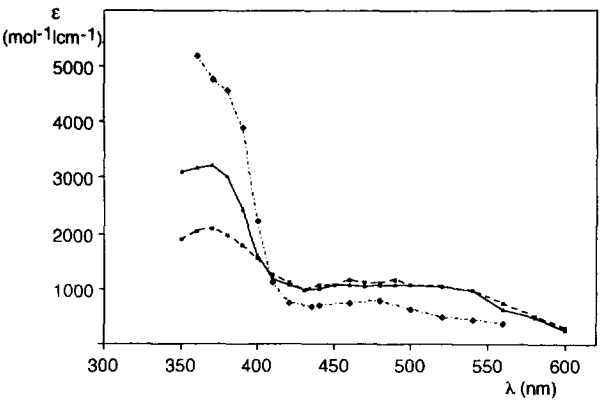

Fig. 4. Differential absorption spectra of 1×10^{-2} M cefotaxim in aqueous solution (—), in 0.1 M NaN_3 aqueous solution (· – ·), and in 0.2 M KBr aqueous solution (- - -). All these solutions were N_2O-saturated.

Table 1. Rate Constants of the Solvated Electron Reactions on Cephalosporins in Terbutanol 1% Aqueous Solutions, Argon-Saturated

Compound	k_1 (mol^{-1} L s^{-1})
Cefotaxim	$9.8 \times 10^9 \pm 0.5 \times 10^9$
Cefuroxim	$9.9 \times 10^9 \pm 0.5 \times 10^9$
Ceftazidim	$1 \times 10^{10} \pm 0.05 \times 10^{10}$

Kinetic analysis of radicals formation

Reaction of the solvated electron on cephalosporins (RH). The hydrated electron acts as a nucleophile. This reaction can be written:

$$e_{aq}^- + RH \xrightarrow{k_1} \text{radical anion} \quad \text{(reaction 1)}$$

This reaction was followed by monitoring the decay of the solvated electron at 700 nm. Due to the high concentration of the solute (RH) in comparaison with the initial e_{aq}^- concentration, the hydrated electrons decay with a pseudo first-order kinetic with a rate constant $k = k_1 [RH]$. A plot of k against the solute concentration gives a straigt line with a slope equal to k_1. Table 1 summarizes the results obtained for the different solutes studied. The rate constants for the three cephalosporins are very close to the value obtained for cephalosporin C, cephaloridine, and cephalothin.[9]

Reaction of hydroxyl radical. The hydroxyl radical can add itself to unsaturated bonds, abstract hydrogen atoms, or transfer an electron.[13] These reactions can be symbolized:

$$^{\bullet}OH + RH \xrightarrow{k_2} {}^{\bullet}RHOH \quad \text{(reactions 2)}$$

$$^{\bullet}R + H_2O$$

$$^{+\bullet}RH + {}^{-}OH$$

Because the hydroxyl radical absorbs only weakly in the U.V., we observed the growth of the oxidated specie(s) to deduce the bimolecular rate constant k_2 of reactions 2. For the same reasons as before, the reaction followed a pseudo first-order kinetic. Table 2 summarizes the results obtained.

Reactions of azide radical and dibromine radical anion on sodium cefotaxim. $N_3^{\bullet}$ (ref. 14) and $Br_2^{\bullet -}$ (ref. 15) can transfer an electron and $Br_2^{\bullet -}$ can also add itself to unsaturated bonds. These reactions can be symbolized:

Table 2. Rate Constants of the Hydroxyl Radical Reactions on 10^{-3} M Cephalosporins in Aqueous Solutions, N_2O-Saturated

Compound	λ (nm)	k_2 (mol^{-1} L s^{-1})
Cefotaxim	450	$9.8 \times 10^9 \pm 0.9 \times 10^9$
Cefuroxim	350	$1.3 \times 10^{10} \pm 0.1 \times 10^{10}$
	370	$1.5 \times 10^{10} \pm 0.1 \times 10^{10}$
	500	$7.5 \times 10^9 \pm 0.7 \times 10^9$
Ceftazidim	385	$7.9 \times 10^9 \pm 0.8 \times 10^9$
	520	$6.5 \times 10^9 \pm 0.6 \times 10^9$

$$N_3^\bullet + RH \xrightarrow{k_3} {}^{\bullet+}RH + N_3^- \quad \text{(reaction 3)}$$

$$Br_2^{\bullet-} + RH \xrightarrow{k_4} {}^{\bullet+}RH + 2\,Br^- \quad \text{(reactions 4)}$$

$$RHBr^\bullet + Br^-$$

The bimolecular rate constants (k_3 and k_4) were obtained by the same method as those for the hydroxyl radicals reactions. Tables 3a and 3b summarize the results obtained for the reactions of $N_3^\bullet$ and $Br_2^{\bullet-}$ on 10^{-2} M sodium cefotaxim. Other concentrations were used (5×10^{-3} M in 0.1 M NaN_3 solution, 10^{-3} M in 0.02 M NaN_3 solution and 4×10^{-3} M in 0.1 M KBr solution), but the same values of the rate constants were found.

Kinetic of the decay phases of the radical products. The rate constants of the disappearance of the transient species from reactions 1, 2, 3, and 4 were determined. The decay showed a second-order kinetic law. The rate constants were measured from the slope of the straight line when the inverse of optical density was plotted as a function of time. The slope is equal to $\frac{2k}{\varepsilon l}$. These rate constants are summarized in Tables 4a, 4b, and 4c.

Table 3a. Rate Constants of $N_3^\bullet$ Reactions on 10^{-2} M Sodium Cefotaxim

k_3 (mol^{-1} L s^{-1})	λ (nm)
$1.6 \times 10^9 \pm 0.2 \times 10^9$	390
$1.7 \times 10^9 \pm 0.2 \times 10^9$	450
$1.7 \times 10^9 \pm 0.2 \times 10^9$	500

Table 3b. Rate Constants of $Br_2^{\bullet-}$ Reactions on 10^{-2} M Sodium Cefotaxim

k_4 (mol^{-1} L s^{-1})	λ (nm)
$1.6 \times 10^8 \pm 0.2 \times 10^8$	500
$1.5 \times 10^8 \pm 0.1 \times 10^8$	520

Oxygen and superoxide radical reactions on radicals.

Oxygen. The rate constants of the transient species disappearance were measured without oxygen (in argon-saturated solutions) and in the presence of oxygen (in aerated solutions and in oxygen-saturated solutions in which the oxygen concentrations are 2.5×10^{-4} M and 1.25×10^{-3} M, respectively). An increase of the rate constants means that the oxygen reacts on the radical products. The rate constant of the oxygen reaction on the transient species (k_5) was calculated with the help of computer simulation. The direct determination of k_5 value was not possible because the rate decay of the radicals with themselves is of the same order of magnitude as the rate of the reaction with O_2. The kinetic is a mixture of first- and second-order processes. The program MACKSIM, for mass action kinetics simulation, developed at Atomic Energy of Canada,[16] was used. The decay of the radicals was simulated with the reactions:

$$R^\bullet + R^\bullet \rightarrow R_2$$

$$R^\bullet + O_2 \xrightarrow{k_5} RO_2$$

Those rate constants are summarized in Table 5.

The experimental results of the radical decay from aerated 10^{-2} M sodium cefotaxim solution and oxygen-saturated 10^{-2} M sodium cefotaxim solution are shown in Figures 5a and 5b, respectively. Simulated results are reported on the same graphs. The correlation is very good for $k(R^\bullet + O_2)$ equal to 6.10^6 $mol^{-1}Ls^{-1}$.

Superoxide radical. The same experiments were realised at lower cephalosporins concentrations (10^{-3} M instead of 10^{-2} M). In this case, most of the e_{aq}^- react on oxygen to form superoxide radical.

$$e_{aq}^- + O_2 \xrightarrow{k_6} O_2^{\bullet-} \quad k_6 = 2 \times 10^{10}\ mol^{-1}\ L\ s^1$$

The superoxide radical can react on the radicals formed by reactions 2 of hydroxyl radicals on the solutes.

$$O_2^{\bullet-} + P^\bullet \xrightarrow{k_7}$$

where $P^\bullet$ are the radicals formed by reactions 2.

The rate constants k_7 of this reaction were determined and are summarized in Table 6.

DISCUSSION

Absorption spectra

All the radicals produced from the three cephalosporins had similar absorption spectra—a flat absorption

Table 4a. Disappearance Rate Constants of the Transient Species Formed From Cephalosporins in 1% Aqueous Solutions, Argon-Saturated

Compound	Concentration (M)	λ (nm)	ε (mol^{-1} L cm^{-1})	2k (mol^{-1} L s^{-1})
Cefotaxim	4×10^{-3}	450	100	$3.1 \times 10^8 \pm 0.3 \times 10^8$
Cefuroxim	4×10^{-2}	500	400	$1.1 \times 10^9 \pm 0.1 \times 10^9$
Ceftazidim	4×10^{-3}	390	1900	$2.8 \times 10^8 \pm 0.3 \times 10^8$
		500	1200	$1.1 \times 10^9 \pm 0.1 \times 10^9$

Table 4b. Disappearance Rate Constants of the Transient Species Formed From Cephalosporins in N_2O-Saturated Solutions

Compound	Concentration (M)	λ (nm)	ε (mol^{-1} L cm^{-1})	2k (mol^{-1} L s^{-1})
Cefotaxim	1×10^{-2}	390	2400	$2.1 \times 10^8 \pm 0.2 \times 10^8$
		450	1100	$3.4 \times 10^8 \pm 0.3 \times 10^8$
		500	1100	$4.2 \times 10^8 \pm 0.4 \times 10^8$
	1×10^{-3}	450	1200	$2.8 \times 10^8 \pm 0.3 \times 10^8$
Cefuroxim	1×10^{-3}	370	1300	$9.5 \times 10^7 \pm 0.9 \times 10^7$
		500	300	$5.5 \times 10^7 \pm 0.6 \times 10^7$
Ceftazidim	1×10^{-3}	385	2200	$1.6 \times 10^8 \pm 0.2 \times 10^8$
		520	1200	$2.0 \times 10^8 \pm 0.2 \times 10^8$

Table 4c. Disappearance Rate Constants of the Transient Species Formed From Sodium Cefotaxim in 0.1 M NaN_3 Aqueous Solution N_2O Saturated and in 0.2 M KBr Solutions N_2O-Saturated

Conditions	Concentration (M)	λ (nm)	ε (mol^{-1} L cm^{-1})	2k (mol^{-1} L s^{-1})
0.1 M NaN_3 solution				
N_2O-sautrated	1×10^{-2}	450	700	$1.1 \times 10^8 \pm 0.1 \times 10^8$
	5×10^{-3}	390	4300	$3.4 \times 10^8 \pm 0.3 \times 10^8$
		450	500	$1.6 \times 10^8 \pm 0.2 \times 10^8$
0.2 M KBr solution				
N_2O-saturated	1×10^{-2}	390	1800	$9.5 \times 10^7 \pm 0.9 \times 10^7$
		450	1100	$1.7 \times 10^8 \pm 0.2 \times 10^8$
		500	1100	$2.7 \times 10^8 \pm 0.3 \times 10^8$

band near 500 nm and an intense one at lower wavelength. Radicals formed from sodium cefuroxim showed quite different spectra.

The same spectrum was observed after the hydroxyl radicals reaction on 10^{-3} M or 10^{-2} M cefotaxim aqueous solutions (see Figs. 3, 4)—all hydroxyl radicals reacted on the solute. The same effect was observed for azide radicals.

The absorption spectrum of the radicals produced by $N_3^\bullet$ reaction can be attributed to an electron transfer reaction[14] (see Fig. 4). The reactions of the other oxidizing radicals ($^\bullet OH$ and $Br_2^{\bullet -}$) formed radicals showing different absorption spectra (see Fig. 4)—the decrease of the absorption band centered at 360 nm and an increase at higher wavelength. The addition of $^\bullet OH$ and $Br_2^{\bullet -}$ to an unsaturated bond in competition with an electron transfer could explain these differences.

Table 5. Rate Constants of the Oxygen Reaction on the Transient Species Formed From Cephalosporins

Compound	λ (nm)	k_5 (mol^{-1} L s^{-1})
Cefotaxim	450	$6 \times 10^6 \pm 2 \times 10^6$
Cefuroxim	500	$5 \times 10^6 \pm 2 \times 10^6$
Ceftazidim	385	$8 \times 10^6 \pm 2 \times 10^6$
	520	$1 \times 10^6 \pm 2 \times 10^6$

Reduction by the solvated electron

The electron reaction on the solutes is fast, and it is likely that e_{aq}^- adds itself to similar positions of the three cephalosporins. The sulphur center of the 7-aminocephalosporanic acid (see Fig. 1) could be proposed because a fast reaction ($k_1 \approx 10^{10}$ mol^{-1} L s^{-1}; see Table 1) and a similar absorption spectra were observed for the three compounds. But, a mixture of radicals was probably observed because the rate constant of recombination of the radical anions from cephalosporins depended on the wavelength (see Table 4a).

Oxidization by $^\bullet OH$, $N_3^\bullet$, *and* $Br_2^{\bullet -}$

The hydroxyl radicals can react by hydrogen abstraction, addition on a double bond, or electron transfer.[13] The hydroxyl radical is a stronger oxidant than $N_3^\bullet$ ($E^\circ_{^\bullet OH/OH^-} = 1.91$ V (ref. 17), $E^\circ_{N_3^\bullet/N_3^-} = 1.35$ V

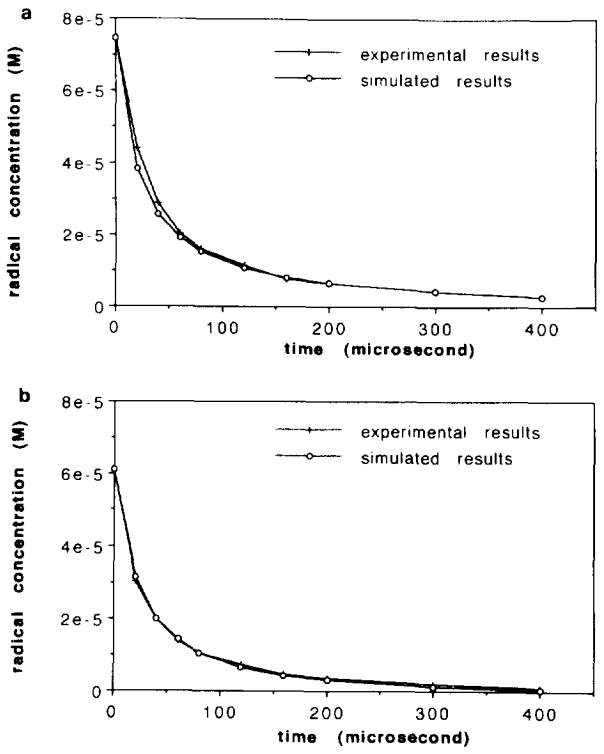

Fig. 5. Experimental results (−+−) and simulated results (−○−) by the program MACKSIM of the reaction $R^{\bullet} + R^{\bullet} \rightarrow R_2$ ($k = 3.10^8$ $mol^{-1}Ls^{-1}$) in competition with the reaction $R^{\bullet} + O_2 \xrightarrow{k_5} RO_2$ ($k_5 = 6.10^6$ $mol^{-1}Ls^{-1}$) in (a) aerated 10^{-2} M cefotaxim solution and in (b) oxygen saturated 10^{-2} M cefotaxim solution.

(ref. 18)) and because $N_3^{\bullet}$ oxidised the cephalosporins, it is believed that part of the absorption is due to an electron transfer from the cephalosporins to the hydroxyl radical. The difference between the spectrum observed with $N_3^{\bullet}$ and $^{\bullet}OH$ (Fig. 4) can be attributed to the addition of hydroxyl radical to a double bond in a ring or to a hydrogen abstraction.

Reactions of oxygen and superoxide radicals

The solute concentration determines the proportion *of* e_{aq}^- that reacts on the oxygen.

$$e_{aq}^- + RH \xrightarrow{k_1} R^{\bullet -}$$

$$e_{aq}^- + O_2 \xrightarrow{k_6} O_2^{\bullet -}$$

$$\frac{[R^{\bullet -}]}{[O_2^{\bullet -}]} = \frac{k_1[RH]}{k_6[O_2]}$$

In 10^{-2} M cephalosporins solutions, it was mainly the oxygen that reacted on the transient organic radicals. It was not possible to distinguish among these radicals experimentally because the reactions of e_{aq}^- and $^{\bullet}OH$ gave the same absorption spectra. The rate constant of this reaction was quite slow (10^6–10^7 mol^{-1} L s^{-1}) (see Table 5). In 10^{-3} M cephalosporins solutions, superoxide radicals were formed and could react on the radicals formed by the solute oxidation. The observed rate constant was faster (10^9 mol^{-1} L s^{-1}) (see Table 6).

$$RH^{\bullet -} + O_2 \rightarrow \text{slow } k \cong 10^6 - 10^7 \text{ mol}^{-1} \text{ L s}^{-1}$$

$$P^{\bullet} + O_2 \rightarrow \text{slow } k \cong 10^6 - 10^7 \text{ mol}^{-1} \text{ L s}^{-1}$$

$$P^{\bullet} + O_2^{\bullet -} \rightarrow \text{fast } k \cong 10^9 \text{ mol}^{-1} \text{ L s}^{-1}$$

The decay of the radicals followed a second-order kinetic as expected because the concentration of the radicals and of $O_2^{\bullet -}$ were equal. On the other hand, the recombination of the radicals with themselves was one order of magnitude slower than the reaction with $O_2^{\bullet -}$. There was no experimental evidence that $O_2^{\bullet -}$ could react with cephalosporins at least under the pulse radiolysis experimental conditions. In the same way, in pulse radiolysis, the rate of the reaction between the radicals and $O_2^{\bullet -}$ was faster than the rate with O_2. The reverse situation would be observed in gamma radiolysis, because the concentration of the radicals would be several orders of magnitude lower.

CONCLUSION

Radicals such as $^{\bullet}OH$ and $O_2^{\bullet -}$, physiologically or radiotherapeutically produced, could react on administrated cephalosporins. Moreover, radiation processing like radiosterilization also led to the formation of free radicals. Pulse radiolysis combined with kinetic spectroscopy is well appropriated to gather basic informations about such radical reactions. This technique gives free radicals in homogeneous solution in a determined amount and allows the direct observation of their reaction with substrates. Quantitative data like rate constant values can be obtained.

This study reveals that in aqueous solutions, both

Table 6. Rate Constants of the Superoxide Radical Reaction on the Transient Species Formed From Cephalosporins

Compound	λ (nm)	k_7 (mol^{-1} L s^{-1})
Cefotaxim	450	2.2×10^9
Cefuroxim	370	1.8×10^9
	500	1.7×10^9
Ceftazidim	385	2.2×10^9

the hydroxyl radical and the hydrated electron react fast with the cephalosporins to form free radicals. The reaction of the radicals formed either by oxidation with hydroxyl radicals or by reduction by hydrated electrons with molecular oxygen is comparatively slow, but they react fast with the superoxyde ion. To study the mechanism of reaction, the analysis of the stable final products from radical–radical combination reaction will be undertaken. These final products will be compared with those obtained after the irradiation of the solid cephalosporins.

REFERENCES

1. Schorderet, M. et collaborateurs. *Pharmacologie: Des concepts fondamentaux aux applications thérapeutiques* (Slatkine ed.). Genève: Slatkine; 1988.
2. Zeegers, F.; Crucq, A.-S.; Gibella, M.; Tilquin, B. Radiolysis and radiosterilization of drugs. *J. Chim. Phys.* **90:**1029–1040; 1993.
3. Gibella, M.; Crucq, A.-S.; Tilquin, B. Détection R.P.E. de l'irradiation de médicaments. *J. Chim. Phys.* **90:**1041–1053; 1993.
4. Miyazaki, T.; Kaneko, T.; Yoshimura, T.; Crucq, A.-S.; Tilquin, B. Electron spin resonance study of radiosterilization of antibiotics: Ceftazidime. *J. Pharm. Sci.* **83:**68–71; 1994.
5. Ferradini, C. Apports de la chimie des rayonnements à la chimie bioradicalaire. *J. Chim. Phys.* **88:**873–887; 1991.
6. Jacobs, G. P. The radiation–sterilization of cephalosporins. *Int. J. Appl. Radiat. Isotopes* **31:**91–95; 1980.
7. Jacobs, G. P. Stability of cefazolin and other new cephalosporins following gamma irradiation. *Int. J. Pharmaceut.* **17:**29–38; 1983.
8. Jacobs, G. P.; Wills, P. A. Recent developments in the radiation sterilization of pharmaceuticals. *Radiat. Phys. Chem.* **31:**685–691; 1988.
9. Phillips, G. O.; Power, D. M.; Robinson, C.; Davies, J. V. Interactions of bovine serum albumin with penicillins and cephalosporins studied by pulse radiolysis. *Biochim. Biophys. Acta.* **295:**8–17; 1973.
10. Marignier, J. L.; Hickel, B. Pulse radiolysis measurements of the soluation rate of benzophinone anion in liquid alcoal: Effect of temperature. *J. Phys. Chem.* **88:**5375–5379; 1984.
11. Hickel, B. La radiolyse pulsée en chimie: Instrumentation et perspectives. *J. Chim. Phys.* **85:**9; 1988.
12. Ferradini, C.; Pucheault, J.; Masson, eds. *Biologie de l'action des rayonnements ionisants* Paris: Masson; 1983.
13. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. Rate constants for reactions of radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **17**(2):516; 1988.
14. Alfassi, Z. B.; Schuler, R. H. Reaction of azide radicals with aromatic compounds: Azide as a selective oxidant. *J. Phys. Chem.* **89:**3359–3363; 1985.
15. Bahnemann, D.; Asmus, K. D.; Willson, R. L. Free radical induced electron oxidation of the phenothiazine, chlorpromazine and promethazine. *J. Chem. Soc. Perkin Trans.* **II:**1661–1668; 1983.
16. Boyd, A. W.; Carver, M. B. Computed and experimental product concentrations in the radiolysis of water. *Radiat. Phys. Chem.* **15:**177–185; 1980.
17. Klaning, U.; Sehested, K.; Holeman, J. Standard Gibbs energy of formation of the hydroxyl radical in aqueous solution: Rate constants for the reaction $ClO_2^- + O_3 \rightleftharpoons O_3^- + ClO_2$. *J. Phys. Chem.* **89:**760; 1985.
18. Alfassi, Z. B.; Harriman, A.; Huie, R. E.; Mosseri, S.; Neta, P. The redox potential of the azide lazidyl couple. *J. Phys. Chem.* **91:**2120; 1987.