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Original Contribution

ATTACK OF CEFOTAXIME BY DIFFERENT RADICALS: COMPARISON OF THE EFFECTS

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Abstract—Free radicals are physiological products and can react to administrated drugs. Metabolic transformations by radical mechanisms are, therefore, possible. A fundamental study of radio-induced degradation of aqueous solutions of sodium cefotaxime, a third generation cephalosporin, was realized to describe these possible radical mechanisms. Different radicals produced by the radiolysis method ($^{\cdot}OH$, $N_3^{\cdot}$, $Br_2^{\cdot -}$, $Tbut^{\cdot}$, $e_{aq}^- + {}^{\cdot}OH$, and $e_{aq}^- + Tbut^{\cdot}$) were successively used to induce the radical mechanisms and their effects were compared. All these radicals induce the formation of a same main radiolysis product identified as anticefotaxime. Radical mechanisms induced by $N_3^{\cdot}$, $Tbut^{\cdot}$, and e_{aq}^- include chain reactions to explain the formation of anticefotaxime contrary to those induced by $^{\cdot}OH$ and $Br_2^{\cdot -}$.

Keywords—Radiolysis, Cefotaxime, Radical mechanisms, Free radical, Chain reactions

INTRODUCTION

The present work consists in a fundamental study of the radio-induced degradation of sodium cefotaxime's aqueous solutions, a third generation cephalosporin. The radio-induced degradation of cefotaxime solutions has not been the subject of much work, as shown by a literature survey of this field. Some works deal with the gamma radiation effects on cefotaxime, irradiated in solid phase, to determine the feasability of its radiosterilization.[1–3] No mechanisms are given but only physicochemical changes and loss of antibacterial activity under gamma irradiation.

The knowledge of the radio-induced degradation paths of cefotaxime solutions is of fundamental interest in vivo: free radicals as hydroxyl radicals ($^{\cdot}OH$), aqueous electrons (e_{aq}^-), hydrogen radical ($H^{\cdot}$), superoxide radical ($O_2^{\cdot -}$), are physiological products[4] and can react to administrated cephalosporins. Some metabolic transformations by radical mechanisms cannot a priori be excluded.

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The purpose of this work is to study the reactions of different radicals on cefotaxime to describe the possible radical mechanisms. The radicals were produced by the radiolysis method. This method permits to select radicals, using appropriated captors, to control the rate of their production (dose rate) and the produced quantity (dose). The reactions of the hydroxyl radical, the aqueous electron and of other more selective radicals as azide radicals ($N_3^{\cdot}$), dibromine radical anions ($Br_2^{\cdot -}$), dimethyl-1,1 ethyl radicals (called terbutyl radicals ($Tbut^{\cdot}$)) were studied.

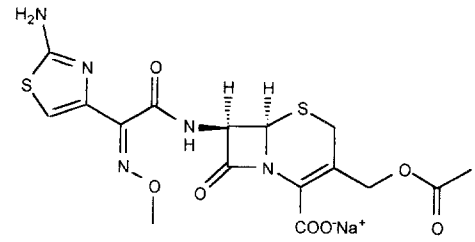
The first part of this work was based on the study of radical intermediates induced by the reactions of the initiator radicals on the cephalosporin molecules by pulse radiolysis.[5] Their absorption spectra and rate constants had been determined. This second part is based on the study of final radiolysis products.

EXPERIMENTAL PROCEDURES

Products

Sodium cefotaxime was obtained from Hoechst. The chemical structure of a sodium cefotaxime molecule is depicted in Fig. 1a. Dimethyl-1,1 ethanol (called ter-

a)



b)

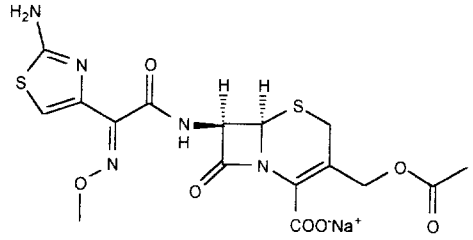


Fig. 1. Chemical structure of (a) sodium cefotaxime and (b) sodium anticefotaxime.

butanol), sodium azide, and potassium bromide proanalysis were used without further purification. All solutions were prepared with tri-distilled water.

Irradiation

Gamma irradiation was performed at room temperature with a ^{60}Co radiation source, at dose rate of 0.20 Gy s^{-1}. The doses were calibrated with the Fricke dosimeter.

Samples preparation

10^{-3} M cefotaxime solutions were irradiated with γ-rays. Different working conditions were used to select successively hydroxyl radicals ($^{\cdot}OH$), azide radicals ($N_3^{\cdot}$), dibromine radical anions ($Br_2^{\cdot -}$), terbutyl radicals (Tbut$^{\cdot}$), and solvated electrons (e_{aq}^-).

1. Oxidizing conditions ($^{\cdot}OH$): solutions were saturated with nitrous oxide (N_2O) to convert e_{aq}^- into $^{\cdot}OH$ radicals.

$$N_2O + e_{aq}^- \xrightarrow{H_2O} N_2 + OH^- + {}^{\cdot}OH$$

$$G({}^{\cdot}OH) = 5.8\ 10^{-7}\ \text{mol J}^{-1} \quad (1)$$

where G is the number of moles transformed by absorption of 1 Joule per kilo of irradiated matter.

2. Azide radicals or dibromine radical anions selection: in N_2O saturated solutions, high concentrations of NaN_3 or KBr were added to transform quantitatively $^{\cdot}OH$ radicals into $N_3^{\cdot}$ or $Br_2^{\cdot -}$ respectively.

$$^{\cdot}OH + N_3^- \rightarrow OH^- + N_3^{\cdot}$$

$$G(N_3^{\cdot}) = 5.8\ 10^{-7}\ \text{mol J}^{-1} \quad (2)$$

or

$$^{\cdot}OH + Br^- \rightarrow OH^- + Br^{\cdot} \quad (3)$$

$$Br^{\cdot} + Br^- \rightarrow Br_2^{\cdot -}$$

$$G(Br_2^{\cdot -}) = 5.8\ 10^{-7}\ \text{mol J}^{-1} \quad (4)$$

3. Terbutyl radicals: in N_2O saturated solutions, 1% terbutanol was added to transform quantitatively $^{\cdot}OH$ radicals into Tbut$^{\cdot}$.

$$^{\cdot}OH + (CH_3)_3COH \rightarrow H_2O + (CH_3)_2^{\cdot}CH_2COH$$

$$G(\text{Tbut}^{\cdot}) = 5.8\ 10^{-7}\ \text{mol J}^{-1} \quad (5)$$

4. e_{aq}^- and Tbut$^{\cdot}$ selection: aqueous solutions were degassed with argon and terbutanol was added to convert $^{\cdot}OH$ into Tbut$^{\cdot}$.

$$G(e_{aq}^- + \text{Tbut}^{\cdot}) = 2.8\ 10^{-7}\ \text{mol J}^{-1} + 2.7\ 10^{-7}\ \text{mol J}^{-1}$$

5. e_{aq}^-+$^{\cdot}OH$ selection: aqueous solutions were degassed with argon.

$$G(e_{aq}^- + {}^{\cdot}OH) = 2.7\ 10^{-7}\ \text{mol J}^{-1} + 2.8\ 10^{-7}\ \text{mol J}^{-1}$$

High-performance liquid chromatography (HPLC)

The irradiated solutions were analyzed by HPLC and compared with a nonirradiated solution. The chromatograph SP 8800 Spectra Physics was equipped with an autosampler cooler model SP 8880 with an injection loop of 20 μl, a variable UV-visible detector (model SP 8450) set at 235 nm, a RP C18 column (5 μm thick particles, 2504 mm, Macherey-Nagel).

The mobile phase was a gradient of methanol in a 0.02 M sodium acetate buffer adjusted to pH 5 (5% of methanol to 30% after 80 min).

RESULTS

The chromatograms of the 10^{-3} M solutions irradiated at different doses (0 to 500 Gy) were superposed in Fig. 2 when $N_3^{\cdot}$ radicals are selected. Cefotaxime is eluted at 32 min and the internal standard (2.5 10^{-3} M

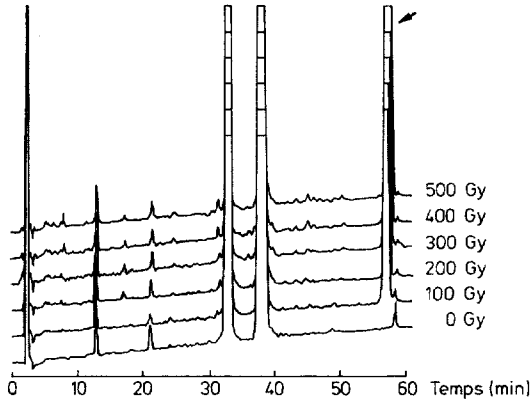


Fig. 2. Chromatograms of 10^{-3} M cefotaxime aqueous solutions with 2.10^{-2} M NaN_3, N_2O saturated ($N_3^{\cdot}$ selection), irradiated at 0 to 500 Gy.

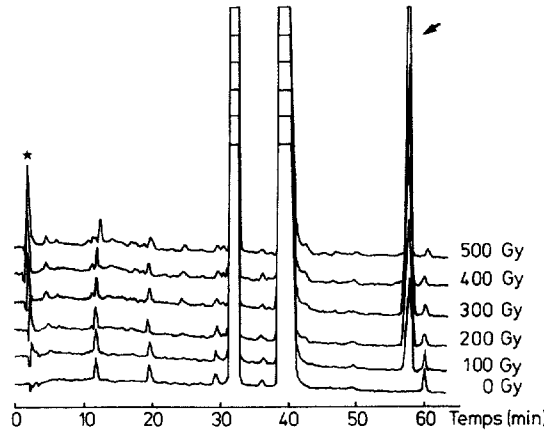


Fig. 4. Chromatograms of 10^{-3} M cefotaxime aqueous solutions, N_2O saturated ($OH^{\cdot}$ selection), irradiated at 0 to 500 Gy.

caffeine) at 38 min. This standard was added after the irradiation to avoid its radiolysis. Only one new product, eluted at 58 min, is detected after irradiation (shown by an arrow in the Fig. 2). This product was identified as the anticefotaxime (see structure in Fig. 1b) by comparison of the retention time with an anticefotaxime reference sample, using different mobile phases, and, by comparison of the absorption spectra recorded with a diode array detector of a Beckman HPLC channel. The absorption spectra normalized at 233 nm of anticefotaxime reference sample and of the main radiolysis product eluted around 60 min were superposed in Fig. 3. The correlation was 0.999.

All the other radicals used to initiate the radical mechanisms ($^{\cdot}OH$, $Br_2^{\cdot -}$, $Tbut^{\cdot}$, and simultaneously e_{aq}^- and $^{\cdot}OH$ or e_{aq}^- and $Tbut^{\cdot}$) also mainly induce the formation of anticefotaxime but in smaller quantities. In these cases, secondary products are also formed. For example, chromatograms of the 10^{-3} M solutions irradiated at different doses (0 to 500 Gy) were superposed in Figs. 4 and 5 when $OH^{\cdot}$ and $Tbut^{\cdot}$ radicals are selected, respectively. The secondary compounds, shown by a star, are different according to the radicals used to initiate the radical mechanisms but are formed in small quantities supposing that their molar absorptivity is similar to one of cefotaxime.

In Figs. 6 and 7, the concentration of cefotaxime or anticefotaxime vs. irradiation doses are expressed. For a determined irradiation dose, the cefotaxime degradation (or anticefotaxime formation) is comparatively smaller with $^{\cdot}OH$ or $Br_2^{\cdot -}$, more important with e_{aq}^- + $^{\cdot}OH$ and still more important with $Tbut^{\cdot}$ or e_{aq}^- + $Tbut^{\cdot}$. The most important degradation is observed with $N_3^{\cdot}$.

The yields of cefotaxime degradation [G(-cefotaxime)] or anticefotaxime formation [G(anticefotaxime)], expressed in Table 1, correspond to the initial slope of the curve of the cefotaxime concentration (or anticefotaxime concentration) vs. dose.

The cefotaxime disappearance or anticefotaxime formation are proportionally smaller when the irradia-

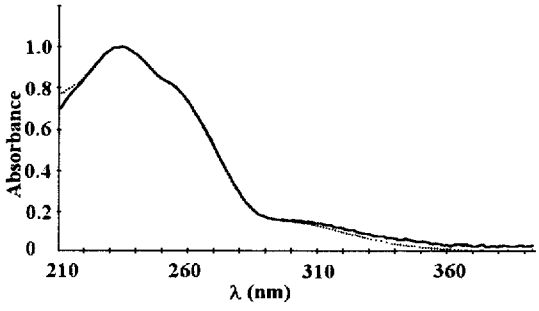


Fig. 3. Superposed normalized spectra (recorded with a photodiode array detector) of a standard solution of anticefotaxime (· · ·) and of the peak eluted around 60 min of the irradiated solutions of sodium cefotaxime (—).

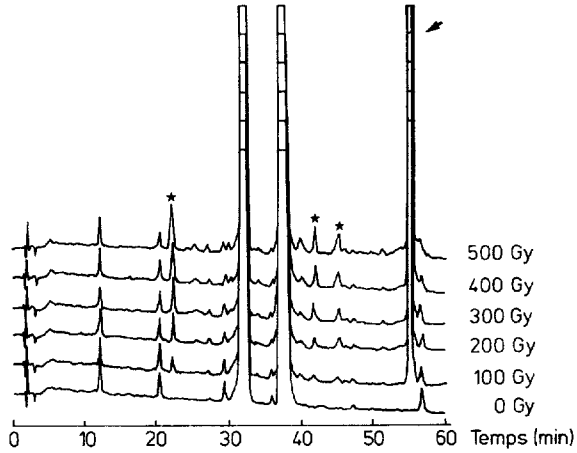


Fig. 5. Chromatograms of 10^{-3} M cefotaxime aqueous solutions with 1% terbutanol, N_2O saturated ($Tbut^{\cdot}$ selection), irradiated at 0 to 500 Gy.

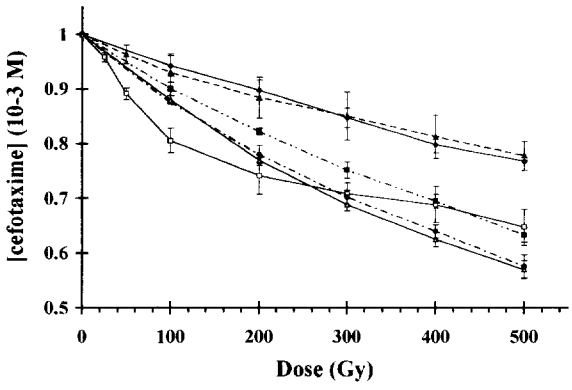


Fig. 6. Cefotaxime concentration vs. irradiation doses. $[cefotaxime]_0 = 10^{-3}$ M. The error bars represent the standard deviation ($n = 4$) (◆ $^{\cdot}OH$; □ $N_3^{\cdot}$; ▲ $Br_2^{\cdot -}$; ● $Tbut^{\cdot}$; ■ $e_{aq}^- + {}^{\cdot}OH$; △ $e_{aq}^- + Tbut^{\cdot}$.

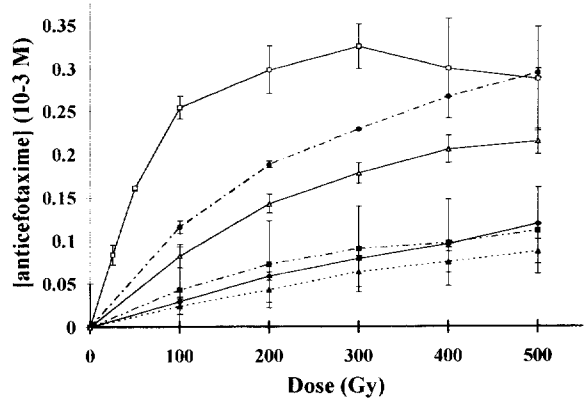


Fig. 7. Anticefotaxime concentration vs. irradiation doses. $[cefotaxime]_0 = 10^{-3}$ M. The error bars represent the standard deviation ($n = 4$) (◆ $^{\cdot}OH$; □ $N_3^{\cdot}$; ▲ $Br_2^{\cdot -}$; ● $Tbut^{\cdot}$; ■ $e_{aq}^- + {}^{\cdot}OH$; △ $e_{aq}^- + Tbut^{\cdot}$).

tion dose is increasing. This dose effect is particularly important with $N_3^{\cdot}$ radicals.

Radical yields of cefotaxime disappearance and anticefotaxime formation vs. the irradiation doses are expressed in Figs. 8 and 9, respectively, for the different initiator radicals.

DISCUSSION

Effects on the irradiation

The main effect of the γ-rays on cefotaxime solutions is the isomerization of the oxime ether leading to the formation of anticefotaxime (see chromatograms in Figs. 2, 4, and 5)). The integrity of the molecule is highly preserved. Lerner et al.[6] has shown that anticefotaxime was also formed by irradiation at 254 nm of cefotaxime solutions. This transformation is of fundamental interest because the configuration is important for the antibiotic activity: cefotaxime is known to be at least 40 to 100 more active than its antiisomer.[6,7] But, the biochemical explanation of the difference between the activity of both isomers is still debated and the radical mechanism of this isomerization has not been studied.

Effect of the radical selection

The efficiency of the isomerization depends on the selected radicals: lower for $^{\cdot}OH$, higher for $N_3^{\cdot}$ (see Figs. 6 and 7). The isomerization yield induced by the different selected radicals does not result from the reaction rate of the primary radicals on the cefotaxime molecule but from ulterior mechanisms because rate constants of the radicals' attack on cefotaxime previously determined[5] were higher for $^{\cdot}OH$ than $N_3^{\cdot}$, $9.3\ 10^9$ and $1.6\ 10^9\ M^{-1}s^{-1}$, respectively.

Yields determination

The radical yields are resumed in Table 1. When the dose effect arises with important irradiation doses, the determination of the yields (initial slope of the concentrations' curve vs. irradiation doses) is more precise. On the other hand, when an important dose effect is observed, the estimation of the slope is more difficult and an error about 10% cannot be excluded.

The analysis of Table 1 shows that $G(^{\cdot}OH)$ and $G(Br_2^{\cdot -})$ are similar to G(-cefotaxime) but higher than G(anticefotaxime): one radical reacts with one molecule of cefotaxime to form anticefotaxime and other final radiolysis products. On the other hand, $G(N_3^{\cdot})$, $G(Tbut^{\cdot})$, $G(e_{aq}^- + {}^{\cdot}OH)$, and $G(e_{aq}^- + Tbut^{\cdot})$ are smaller than G(-cefotaxime) and G(anticefotaxime): $N_3^{\cdot}$, $Tbut^{\cdot}$, and e_{aq}^- induce a cefotaxime degradation yield higher than the yield of their own production. Azide radicals can transform more than three molecules of cefotaxime. A chain reaction of electron transfer could explain this result.

This quantitative approach permits to suggest the following mechanisms to explain the isomerization of the cefotaxime into anticefotaxime induced by the radicals $N_3^{\cdot}$, $Tbut^{\cdot}$, or e_{aq}^- (symbolized by $R^{\cdot}$)

Table 1. Yields of Cefotaxime Disappearance and Anticefotaxime Formation

Selected Radicals	G(radicals) (10^{-7} mol J^{-1})	G(-cefotaxime) (10^{-7} mol J^{-1})	G(anticefotaxime) (10^{-7} mol J^{-1})
$^{\cdot}OH$	5.7	5.1	3
$N_3^{\cdot}$	5.7	22	25
$Br_2^{\cdot -}$	5.7	6.9	3
$Tbut^{\cdot}$	5.7	12	12
$e_{aq}^- + {}^{\cdot}OH$	5.5	9.8	4
$e_{aq}^- + Tbut^{\cdot}$	5.5	12	8

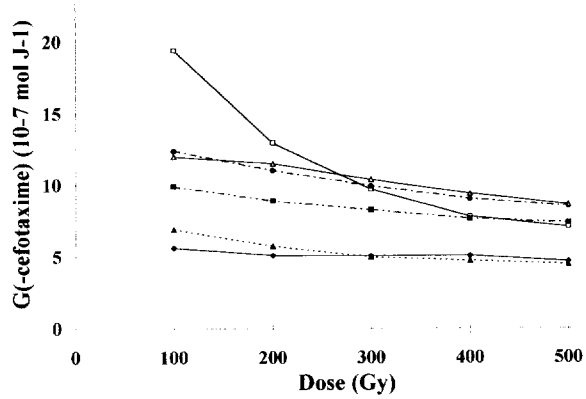


Fig. 8. Radical yields of cefotaxime disappearance. (♦ $^{\cdot}OH$; □ $N_3^{\cdot}$; ▲ $Br_2^{\cdot}$; ● Tbut$^{\cdot}$; ■ $e_{aq}^- + {}^{\cdot}OH$; △ $e_{aq}^- +$ Tbut$^{\cdot}$).

$$R^{\cdot} + \text{cefotaxime} \rightarrow \text{cefotaxime}^{+\bullet} + R^-$$

$$\text{cefotaxime}^{+\bullet} + \text{cefotaxime} \rightarrow \text{anticefotaxime} + \text{cefotaxime}^{+\bullet}$$

$$\text{cefotaxime}^{+\bullet} + \text{cefotaxime}^{+\bullet} \rightarrow \text{non identified final products}$$

The isomerization of the cefotaxime into anticefotaxime induced by the radicals $^{\cdot}OH$ and $Br_2^{\cdot -}$ cannot be explain by an electron transfert chain reaction if radical yields are compared. The following mechanism can be suggested:

$$^{\cdot}OH + \text{cefotaxime} \rightarrow C^{\cdot}$$

where $C^{\cdot}$ is a radical-cation of cefotaxime or a radical formed by the hydroxyl radical addition.

$$C^{\cdot} + \text{cefotaxime} \rightarrow \text{anticefotaxime} + \text{nonidentified products}$$

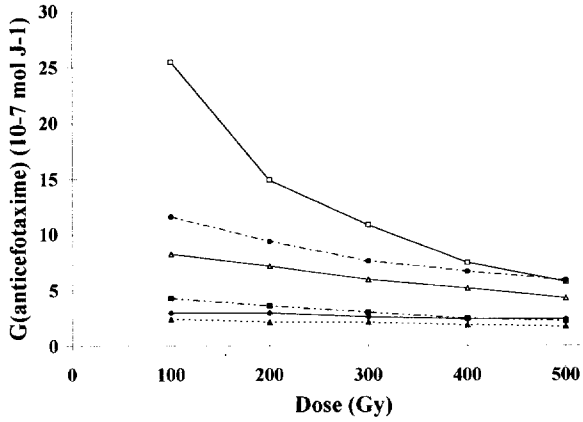


Fig. 9. Radical yields of anticefotaxime formation. (♦ $^{\cdot}OH$; □ $N_3^{\cdot}$; ▲ $Br_2^{\cdot}$; ● Tbut$^{\cdot}$; ■ $e_{aq}^- + {}^{\cdot}OH$; △ $e_{aq}^- +$ Tbut$^{\cdot}$).

in competition with

$$C^{\cdot} + C^{\cdot} \rightarrow \text{final products}$$

Dose effect

The radical yields (number of moles transformed by absorption of 1 Joule per kilo of irradiated matter) decrease when the irradiation doses increase (see Figs. 8 and 9). To expain this dose effect, the following hypothesis can be put forward: the radiolysis product, mainly anticefotaxime, can also be subject to the indirect effect of the ionizing radiations because the evolution of G(-cefotaxime) is similar to this of G(anticefotaxime). Additional reactions could be proposed:

$$R^{\cdot} + \text{anticefotaxime} \rightarrow \text{anticefotaxime}^{+\bullet} + R^-$$

and / or

$$\text{anticefotaxime}^{+\bullet} + \text{anticefotaxime} \rightarrow \text{cefotaxime} + P$$

P symbolizes nonidentified products. To justify the important dose effect observed with $N_3^{\cdot}$, P would correspond to anticefotaxime$^{+\cdot}$ (radical-cation): this reaction would be a chain reaction.

CONCLUSION

The main effect of the irradiation of cefotaxime aqueous solutions is the isomerization of the oxime ether to form anticefotaxime. This isomerization was induced by all the selected radicals ($^{\cdot}OH$, $N_3^{\cdot}$, $Br_2^{\cdot -}$, Tbut$^{\cdot}$, $e_{aq}^- + {}^{\cdot}OH$, and $e_{aq}^- +$ Tbut$^{\cdot}$). The reactions of

these initiator radicals on the cefotaxime molecule are not the important step to explain the isomerization and ulterior mechanisms had to be proposed.

Cefotaxime radical-cation formed by the attack of $N_3^{\cdot}$, Tbut$^{\cdot}$, and e_{aq}^- on cefotaxime would induce chain reactions contrary to those formed by the attack of $^{\cdot}OH$ and $Br_2^{\cdot -}$ on cefotaxime. These chain reactions would produce anticefotaxime and would be in competition with termination reactions and reactions, which justify the dose effect.

In order to strengthen these hypothesis, a simulation program will be used: calculated concentrations of final radiolysis products will be compared with experimentally measured concentrations.

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REFERENCES

1. Jacobs, G. Stability of cefazolin and other new cephalosporins following gamma irradiation. *Int. J. Pharmaceut.* **17:**29–38; 1983.
2. Signoretti, E. C.; Valvo, L.; Fattibene, P.; Onori, S.; Pantalini, M. Gamma radiation induced effects on cefuroxime and cefotaxime. Investigation on degradation and syn-anti isomerization. *Drug Dev. Indust. Pharm.* **20:**2493–2508; 1994.
3. Zegota, H. Z.; Koprowski, M.; Zegota, A. Effects of gamma irradiation on cefotaxime in the solid state. *Radiat. Phys. Chem.* **45:**223–229; 1995.
4. Ferradini, C. Apports de la chimie des rayonnements à la chimie bioradicalaire. *J. Chim. Phys.* **88:**873–887; 1991.
5. Crucq, A.-S.; Tilquin, B.; Hickel, B. Radical mechanisms of cephalosporins: A pulse radiolysis study. *Free Radic. Biol. Med.* **18:**841–847; 1995.
6. Lerner, D. A.; Bonnefond, G.; Fabre, H.; Mandrou, B.; Simeon de Buochberg, M. Photodegradation paths of cefotaxime. *J. Pharm. Sci.* **77:**699–703; 1988.
7. Mirelman, D.; Nuchamowitz, Y. Biosynthesis of peptidoglycan in pseudomonas aeruginosa: Comparison of the inhibitory effects of cefotaxime, its antiisomer and the syn S-oxide compound. *Antimicrobial Agents Chemother.* **17:**115–119; 1980.