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Stability of cefazolin and other new cephalosporins following gamma irradiation

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Summary

Gamma irradiation of cefazolin and other newer cephalosporins in the dry state results in minimal degradation of cefazolin sodium, cefadroxil monohydrate, ceforanide, cefotaxim, and CGP 9000 dihydrate, even following a 50 kGy (5 Mrad) radiation dose, suggesting the practical feasibility of their radiation sterilization. Cephradine monohydrate, however, is particularly susceptible to radiation-induced damage.

Introduction

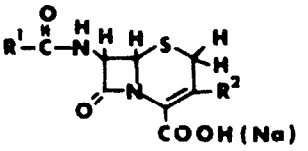
The hydrolytic susceptibility of cephalosporins especially at high temperatures, obviates sterilization of their parenteral products by conventional methods such as autoclaving. The sterilization of powders of their injectables by gamma irradiation seems a desirable alternative to the necessary practice of utilizing highly demanding aseptic processes. The high penetrability of gamma rays, concomitant with only a very small rise in temperature of the irradiated material makes radiation treatment applicable to the drug in its final package often without removal from its transport container.

An earlier study of the effect of gamma rays on 4 members of the cephalosporin group of the β-lactam antibiotics indicated varying degrees of susceptibility to radiolysis when the antibiotics were irradiated in the dry state (Jacobs, 1979, 1980). The introduction into clinical use of several newer cephalosporins prompted us to investigate their irradiation stability with the aim of determining the feasibility of their radiation sterilization. The destructive nature of ionizing radiation and the

difficulty in predicting its radiolytic effect, particularly in more complex molecules, makes it necessary to analyze each compound individually for radiation damage to determine the feasibility of its radiation sterilization.

Materials and Methods

The cephalosporins tested were cefazolin sodium (Eli Lilly, IN, U.S.A.), cefradoxil monohydrate (Mead Johnson, IN, U.S.A.), ceforanide, previously known as



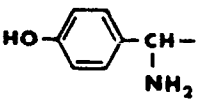
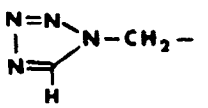
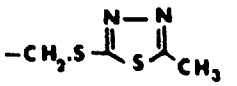
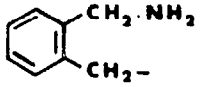
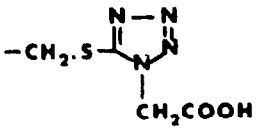
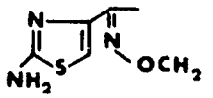
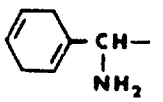
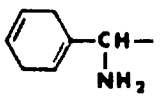
	R^1	R^2
CEFADROXIL		$-CH_3$
CEFAZOLIN Na		
CEFORANIDE (BL-S786)		
CEFOTAXIM (HR 756)		$-CH_2O.CO.CH_3$
CEPHRADINE		$-CH_3$
CGP 9000		$-OCH_3$

Fig. 1. Chemical structure of the cephalosporins used in the present study.

BL-S786 (Bristol Laboratories, NY), cefotaxim, also known as HR-756 (Hoechst AG, F.R.G.), cephradine monohydrate (E.R. Squibb and Sons, NJ, U.S.A.), and CGP 9000, supplied as the dihydrate (Ciba-Geigy AG, Switzerland). These compounds, all of pharmaceutical grade, were tested without any further purification; their chemical structures are depicted in Fig. 1.

The ^{137}Cs γ-irradiation source and irradiation vessels are as described elsewhere (Jacobs and Melumad, 1976).

Routinely, 5 g samples of the drugs were γ-irradiated at ambient temperature with 25 and 50 kGy radiation doses (i.e. 1.56×10^{20} and 3.12×10^{20} $eV \cdot g^{-1}$, respectively) checked by periodic dosimetric determinations using a ferrous sulphate dosimeter ($G_{Fe^{3+}} = 15.4$ (Spinks and Woops, 1976)) and routinely confirmed by means of a clear Perspex HX dosimeter (Berry and Marshall, 1969). The rationale for the choice of these doses has been presented earlier (Jacobs, 1977).

The analytical techniques adopted for detection of products of radiolysis included change in melting point, microbiological and chemical assays, UV spectrophotometric absorption, specific optical rotation, thin-layer and high-performance liquid chromatography, pH of aqueous solutions, and sterility testing. At least two tests were routinely performed on the 25 and 50 kGy samples immediately following irradiation, as well as on unirradiated controls; however, our limited quantity of ceforanide only allowed us to test this compound at the lower radiation level.

Melting point determinations were made with a Thomas Unimelt apparatus.

UV spectrophotometric determinations, using a Pye Unicam SP 1800 spectrophotometer with 10 mm matched quartz cells, were carried out on aqueous solutions (pH 6.5) of the irradiated drugs at the appropriate λ_{max} and concentration.

Chemical analyses were undertaken using the iodometric assay of the British Pharmacopoeia 1973.

Specific optical rotation was determined on 0.5% w/v aqueous solutions of the irradiated and unirradiated cephalosporins using a 100 mm microcell in a Perkin Elmer 141 polarimeter.

TLC examination was carried out on 1% solutions of the cephalosporins in aqueous methanol (70% v/v) using precoated silica-gel plates (Polygram Sil N-HR/UV_{254}; Mackery Nagel), with the mobile phases being an equal-part mixture of acetone and methanol; a mixture of isopropanol and methanol (30 : 70 parts v/v, respectively); a 1.5% v/v solution of strong ammonia in methanol; or chloroform. Each solvent (of analytical grade components) was tested separately with each antibiotic. Detection was under UV light at 254 nm followed by spraying with either a 1% aqueous solution of potassium permanganate or a 0.1% ninhydrin spray reagent (Merck, F.R.G.). The rationale for the use of chloroform, a non-polar solvent, with the polar antibiotics was essentially to enable detection of any radiolysis products that might be masked by the excess of unchanged antibiotic.

In an effort to determine the sensitivity of the TLC methodology adopted, solutions of mixtures of antibiotics having similar R_f values were prepared and tested. It was generally found that one antibiotic at a 1% w/w concentration in another (concentrations in solution were 0.01% and 1%, respectively) could not be detected; it could be detected at a 2.0% concentration. The testing of such mixtures

is justified, because any predominant radiolysis product would be structurally similar to the parent compound and presumably have a similar R_f value. Other radiolysis products with different R_f values might be detectable even at a concentration of less than 1%.

HPLC analysis of cefazolin sodium was carried out using a Water Associates 244 liquid chromatograph equipped with a reverse phase 30-cm Bondapack Phenyl Column at ambient temperature, a Model 440 fixed UV detector (254 nm), and a Schoeffel SF770 variable wavelength detector, connected in series, and set a 270 nm. The mobile phase was 0.01 M ammonium acetate in a 30% v/v aqueous methanol solution; 50 μl samples were eluted at a flow rate of 1.8 ml · min^{-1}.

The pH of the 5% w/v aqueous solutions of the irradiated cephalosporins was determined using a PHM 64 Research pH meter (Radiometer, Denmark). Specific optical rotation and pH measurements could not be carried out on CGP 9000 dihydrate and ceforanide because of their low water solubility.

The microbiological assay of the irradiated cephalosporins was carried out using a two-dose cylinder plate method with Difco Antibiotic Medium 1, seeded, whilst molten, with 0.1 ml of an overnight culture of *Staphylococcus aureus* (Teva 29) (kindly supplied by Teva Pharmaceutical Industries, Jerusalem, Israel). The choice of antibiotic concentrations of 10 and 100 μg · ml^{-1} was based on the determination of a linear relationship between concentration and diameter of zone of inhibition over this range of concentrations. Following 18 h incubation at 37°C, diameters of zones of inhibition of bacterial growth were measured.

Sterility testing was by a membrane filtration technique in which 20-ml aliquots of 1% aqueous solution of the drug followed by 4 similar aliquots of saline (0.9% w/v) were passed through a membrane filter (25 mm diameter) having a mean pore diameter of 0.22 μm (Millipore type GSWP), using a Millipore Swinex apparatus (code SX0002500) attached to a 20-ml disposable syringe. Immediately following filtration, each membrane was cut in two, with one half aseptically introduced into 50 ml of Brewer thioglycollate medium (for detection of aerobic and anaerobic bacteria (Difco, MI, U.S.A.)) and the other half onto Sabouraud dextrose agar (for detection of fungi and moulds (Difco)). All manipulations were undertaken in a laminar air-flow cabinet. Incubation of the media was at 32°C for the thioglycollate and 25°C for the Sabouraud, both for 14 days. The usual media controls, as stipulated in the United States Pharmacopoeia XIX, were used. The rinsing of the filter with saline solution ensured that no antibiotic residue which might otherwise interfere with microbial growth was present. This was ascertained by deliberately contaminating cephalosporin solutions with small inocula of *Staphylococcus aureus* Teva 29 (100 organisms/ml) prior to filtration. In the absence of rinsing, no growth was apparent, whereas membranes which had been through the rinsing process showed bacterial contamination. Sterility testing for each set of condition was carried out in duplicate.

The efficacy of the radiation sterilization process was assessed by sterility testing, described above, of 1-g aliquots of the cephalosporin powders deliberately contaminated with 10^6 spores of the radiation resistant *Bacillus pumilus* E601 (ATCC 27142), prior to irradiation.

Results

Melting points of cefazolin sodium and cefadroxil monohydrate (Table 1) were virtually unchanged with increasing radiation doses. There were changes, however, in CGP 9000 dihydrate and cephradine monohydrate. In the case of cefotaxim, none of the samples had melted at 360°C. A 5% w/w mixture of either cephradine (m.p. 195°C) or another cephalosporin, cephalexin (m.p. 192°C), in cefadoxil (m.p. 212°C), gave melting points of 204 ± 1°C and 203 ± 1°C, respectively, suggesting that when degradation products are chemically similar to the parent compound and have melting points close to that of the parent compound, the presence of such products at a 5% (or even lower) concentration can readily be detected using melting-point determinations.

UV absorbance measurements (Table 1) indicate a marked change in cephradine monohydrate alone. It is generally assumed that a change in UV absorbance indicates an alteration in the O=CNC=C linkage of the parent molecule (Sabath et al., 1965). An increase in the absorbance of cefotaxim, indicating the radiation-induced formation of a chromophore with an extinction coefficient greater than that for cefotaxim, is noteworthy.

The chemical assay results suggest negligible changes in potency for 5 of the 6 compounds tested. The sixth compound, cephradine monohydrate, however, undergoes a 10% loss of potency at the 25 kGy radiation dose level, and a 30% decrease at the 50 kGy level.

From Table 1 there appears to be no change in the stereochemistry of cefadroxil monohydrate, even following the higher dose of radiation. There were, however, slight changes in cefazolin sodium, cefotaxim, and cephradine monohydrate. Likewise, only cefazolin sodium and cephradine monohydrate displayed any significant reduction in pH of aqueous solutions.

With the exception of cephradine monohydrate, no products of radiolysis could be detected, by thin-layer chromatography, in any of the irradiated compounds, seemingly suggesting that the concentration of radiolysis products is not greater than 2% (Table 1). In the case of cephradine monohydrate, and additional spot (R_f 0.48) was observed under UV light (254 nm), clearly indicating radiolysis of this compound.

Repeated HPLC analysis of cefazolin indicated a 99.5% recovery for the 50 kGy-irradiated sample, based on comparison of the areas of irradiated and unirradiated cefazolin peaks. Only a single radiolysis product peak (see Fig. 2) could be detected, and computation of its area relative to that of the unirradiated cefazolin peak gave values of 0.03 and 0.04% for the 25 and 50 kGy doses, respectively. Irradiated samples were only monitored at two wavelengths in the UV range, and, therefore, it is possible that some degradation products escaped detection, either because they absorbed at other wavelengths (including the visible range), or not at all; the radiolysis product peak that was observed was not necessarily detected as its λ_{max}. This could account for the disparity between the value for percentage recovery and that for percentage of radiolysis products at (say) the 50 kGy dose level, in addition to the disparity due to the inherent error in HPLC analysis.

TABLE 1

DATA FOR SELECTED CEPHALOSPORINS IRRADIATED AT TWO DIFFERENT DOSES

	Dose (kGy)	m.p. (C) [a]	UV absorbance [b] $E^{\%}_{1cm}$	Chemical assay ± S.D. (%)	Specific optical rotation	Δ pH [e]	R_f values	Microbiological potency ± S.D. (%)
Cefazolin sodium	0	174	244	100	−16°	(4.6)	0.70	100
	25	171	244	98.4 ± 0.4	−15°	−0.4	0.70	91.7 ± 6.2
	50	171	244	98.7 ± 0.6	−14°	−0.4	0.70	94.5 ± 2.8
Cefadroxil monohydrate	0	212	220	100	+155°	(4.9)	0.62	100
	25	211	218	101.3 ± 0.1	+155°	−0.1	0.62	101.3 ± 1.4
	50	210	216	99.0 ± 0.1	+155°	−0.2	0.62	97.1 ± 1.5
Ceforanide [c]	0	224	210	100	–	–	–	100
	25	222	210	100.0 ± 1.0	–	–	–	95.8 ± 3.5
Cefotaxim	0	[d]	338	100	+52°	(5.1)	0.75	100
	25	[d]	342	100.0 ± 0.3	+52°	−0.2	0.75	104.9 ± 1.5
	50	[d]	346	99.7 ± 0.2	+51°	−0.2	0.75	103.0 ± 3.8
Cephradine monohydrate	0	195	198	100	+16°	(4.7)	0.53	100
	25	188	178	87.7 ± 6.1	+16°	−0.3	0.53	89.0 ± 2.9
	50	188	178	71.0 ± 1.0	+15°	−0.7	0.53, 0.48	67.0 ± 0.1
CGP 9000 dihydrate	0	185	182	100	–	–	0.62	100
	25	182	182	100.2 ± 1.4	–	–	0.62	101.3 ± 3.4
	50	180	184	100.4 ± 1.1	–	–	0.62	96.7 ± 0.7

[a] Mean values with a maximum S.D. of ± 1°C

[b] Means of ≠ 2 determinations with 1.5%

[c] Tested only at 25 kGy

[d] Not melted at 360°C

[e] Represents change in pH of aqueous solutions. The numbers in parenthesis are actual pH values for the unirradiated drugs.

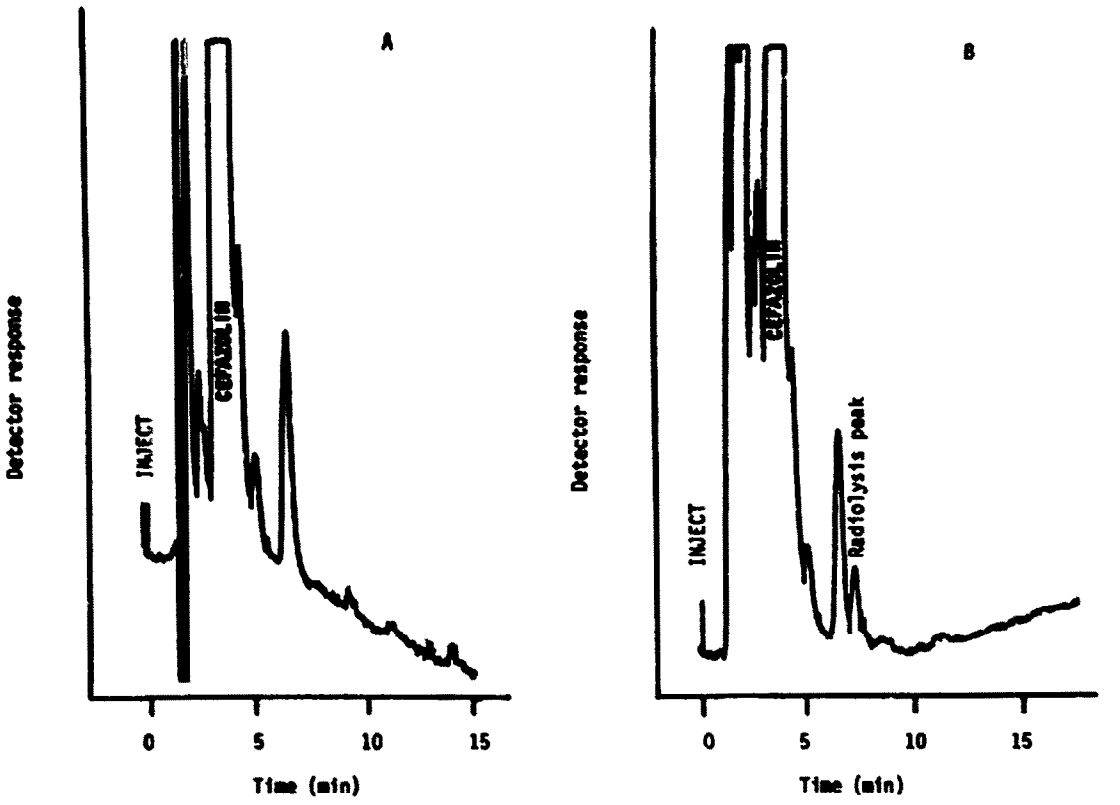


Fig. 2. Chromatograms of (A) unirradiated (1.71 mg·ml^{-1}) and (B) 5 Mrad irradiated (1.38 mg·ml^{-1}) cefazolin. The mobile phase was 0.01 M ammonium acetate in 30% v/v methanol at 1.8 ml·min^{-1} at 270 nm, 0.04 AUFS.

Table 1 includes the results of our microbiological assays. Considering the large experimental error encountered in such assays (usually $\pm$ 4%), our results suggest that there is no decomposition of cefotaxim, even following a 50 kGy radiation dose, and that at the 25 kGy dose there is no decomposition of cefadroxil monohydrate or CGP 9000 dihydrate. These two compounds, on the basis of this assay, undergo slight radiolysis (approximately 3%) following a 50 kGy radiation dose. On the other hand, there is a slight decrease in the potency of ceforanide and cefazolin sodium, even at the lower dose level. With cephradine monohydrate, the loss of potency is significant (approximately 10%) even after a 25 kGy dose, which reflects values obtained by chemical assaying (see above).

Sterility testing indicated that both irradiated and unirradiated samples of the antibiotics were free of bacterial and fungal contaminants. Whilst bacterial growth did occur in unirradiated samples deliberately contaminated with approximately 10^6 spores of the radiation-resistant *Bacillus pumilus* E601 (ATCC 27142), no growth was apparent in similarly contaminated samples which subsequently received a 25 kGy radiation dose.

Discussion

Some 8 analytical methods have been used to detect radiation-induced degradation of cephalosporins, with each technique usually being able to detect a change in a specific moiety of the irradiated molecule. Thus, for example, despite a marked decrease in the UV absorbance of cephradine, the stereochemistry of this compound following irradiation is hardly affected. Examination of all the generated data together can generally give an indication of the extent of degradation. Interestingly, the analysis of cefazolin by HPLC, generally considered one of the more sensitive techniques available for detection of degradation, certainly indicates that any degradation inferred from the other data is, in fact, minimized by the HPLC results. HPLC analysis of other β-lactam antibiotics (Jacobs, unpublished data) shows that these data are always consistent with those generated using other analytical techniques, a factor which greatly increases confidence in the results presented here.

In discussing the above results, the percentage change in chemical assays has been used to estimate G(-cephalosporin) values. A G-value is defined as the number of molecules produced or changed for each 100 eV of radiation energy absorbed. On the assumption of a linear relationship between radiation dose and the number of molecules decomposed, then G-values for the same molecule irradiated under similar conditions, but with different radiation doses, should theoretically be similar and independent of the dose. In practice, however, these values, as demonstrated by our data in Table 2, are within the same order of magnitude.

G-Values cannot be calculated from measurements if there is no direct relationship between the measured effect (e.g. melting point) and the concentration of degradation products, nor if a small absolute change in the measured parameter (e.g. ± 1°C in the optical rotation measurements) may yield markedly different percentage changes for different drugs because of the absolute differences in the measured parameter between the different control (unirradiated) drugs (e.g. optical

TABLE 2

PERCENTAGE CHANGE IN POTENCY OF IRRADIATED CEPHALOSPORINS (Δ%), ON THE BASIS OF CHEMICAL ASSAYING, AT EACH DOSE LEVEL, AND THE CORRESPONDING G(-CEPHALOSPORIN) VALUE[a]

	Dose (kGy)				Mean G(-)
	25 kGy		50 kGy		
	Δ%	G(-)	Δ%	G(-)	
Cefadroxil monohydrate	1.3	13	1.0	5	9
Cefazolin sodium	1.6	13	1.3	5	9
Ceforanide	0	< 1	–	–	< 1
Cefotaxim	0	< 1	0.3	1	1
Cephradine monohydrate	12.3	130	29.0	152	141
CGP 9000 dihydrate	0.2	2	0.4	2	2

[a] G-value = number of molecules changed for each 100 eV of radiation energy absorbed.

rotation values of [+]16° and [+]155° for cephradine and cefadroxil, respectively). The large inherent errors associated with the microbiological assay results has obviated their use in computing mean percentage changes.

Cefazolin sodium

The data indicate that gamma radiation only slightly reduces the potency of cefazolin (mean *G*(-cefazolin) of 9). Consideration of the HPLC recovery of 99.5% reduces the *G*-value for the 5 Mrad data to 3, and the mean value to 8.

Cefadroxil monohydrate

On the basis of the data presented above, cefadroxil monohydrate is virtually unaffected at the 25 kGy dose. Slight decomposition is discernible, however, at the 50 kGy dose level. These conclusions are illustrated by the relatively low mean *G*-value of 9. It is noteworthy that cephalexin, with a chemical structure that very closely resembles that of cefadroxil (the former has the p-OH grouping on the 7-amino side-chain), has been found to be similarly affected by γ-rays as reflected by a *G*(-cephalexin) value of about 11 (Jacobs, 1980).

Ceforanide

The limited data prevent any definitive conclusion to be reached concerning irradiated ceforanide. However, on the basis of these data, it would appear (*G*(-ceforanide) of < 1) that it is quite stable at the 25 kGy radiation dose.

Cefotaxim

No significant breakdown of cefotaxim could be detected using any of the analytical techniques adopted, even following the 50 kGy radiation dose. This is reflected in a low mean *G*(-cefotaxim) value of 1. Whilst we have previously suggested (Jacobs, 1980) that an acetoxymethyl substituent in the C-3 position of the dihydrothiazine ring may contribute to irradiation susceptibility, the present finding with cefotaxim negates such a thesis. It would appear that the C-7 substituent stabilizes this compound.

Cephradine monohydrate

This member of the cephalosporins is radiation labile and undergoes very significant degradation at the 25 kGy dose level, as illustrated by the high *G*(-cephradine H_2O) of 130. The close correlation between the microbiological and chemical assay values is certainly noteworthy. Structurally, this compound is similar to both cefadroxil H_2O and cephalexin with *G*-values of 8 and 11, respectively. Its unique feature is the partially saturated ring structure in the C-7 substituent.

CGP 9000 dihydrate

Despite the decrease in melting point with increasing radiation dose, the other data seem to support a conclusion that this compound is relatively resistant to radiolysis. A low *G*(-CGP 9000) value of 2 reflects the relatively low radiolytic breakdown.

It has been suggested by Dziegielewski et al. (1973) that compounds in the form

of salts or esters are generally less susceptible to radiolysis than are the free acids, which, in turn, are more stable than their respective hydrates. If this is the case, then certainly other factors must play a very significant role in affecting susceptibility to irradiation, as illustrated by our data. Cefazolin sodium, the only salt examined, is, on the basis of *G*-values, less stable than the two free acids, ceforanide and cefotaxim, and the hydrated compound, CGP 9000. It is, therefore, hardly credible that the radiolytic decomposition of cephradine monohydrate (mean *G*(-cephradine H_2O) of 141) is as a result of its water content. It is worth recalling that cephaloridine monohydrate, one of the cephalosporins previously examined (Jacobs, 1980), displayed a low *G*-value of 3 calculated at a 50 kGy dose level. No doubt, the partially saturated ring structure of cephradine makes a very significant contribution to its radiation susceptibility.

In conclusion, our results indicate that cefazolin sodium, cefadroxil monohydrate, ceforanide, cefotaxim, and CGP 9000 dihydrate may be safely irradiated at the commonly employed sterilization dose of 25 kGy (2.5 Mrad). However, it would have to be established that the approximate 1% decomposition observed did not result in the formation of any toxic products. Our results also indicate that the radiation sterilization of cephradine monohydrate does not appear to be feasible.

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