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# THE RADIATION STERILIZATION OF CEFOXITIN SODIUM

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SUMMARY

The effect of 25 and 50 kGy γ-radiation doses on cefoxitin sodium, a member of the cephamycin group of β-lactam antibiotics, has been assessed by different chemical and microbiological analytical techniques. The remarkable radiation stability of this compound suggests its suitability for radiation-sterilization.

## INTRODUCTION

The effect of γ-irradiation on β-lactam antibiotics has been the subject of several of our earlier studies. Our attention has been focused on two classes of this group of antibiotics, the semi-synthetic penicillins (Jacobs, 1979a, 1980a, 1981) and the cephalosporins (Jacobs, 1979b, 1980b), in order to assess the feasibility of their radiation-sterilization. With the discovery of another family of β-lactam antibiotics, the cephamycins (Nagarajan et al., 1971; Stapley et al., 1972) we have been prompted to extend our studies and investigate their radiation susceptibility. The cephamycins are similar in structure to the cephalosporins, except that they possess a methoxy group in place of H in the 7-position on the β-lactam ring. They differ from cephalosporins in that they are produced not by fungi but by actinomycetes. One particular compound belonging to this new family of antibiotics and currently under clinical investigation is cefoxitin sodium

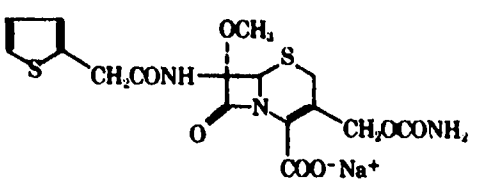


Fig. 1. Chemical structure of cefoxitin sodium.

(see Fig. 1). Cefoxitin sodium, the sodium salt of 3-(hydroxymethyl)-7α-methoxy-8-oxo-7-[2-(2-thienyl)acetamido]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate carbamate (ester) is chemically unique in that the cephem nucleus is substituted with a 3-carbamoyloxymethyl group and, as stated above, a 7α-methoxy group. It is active against Gram-positive and Gram-negative bacteria (Onishi et al., 1972) and is resistant to destruction by bacterial β-lactamase (Onishi, 1974; Neu, 1974).

The present paper is concerned with the effect of γ-irradiation on cefoxitin sodium powder for injection in order to assess the feasibility of its radiation-sterilization, particularly in view of its susceptibility to hydrolysis (Oberholtzer and Brenner, 1979) and hence, elimination of its sterilization by more conventional methods.

## MATERIALS AND METHODS

### *Cefoxitin sodium*

Cefoxitin sodium, whose chemical structure is depicted in Fig. 1, was kindly donated by Merck Sharp and Dohme Research Laboratories, Penn., U.S.A. It was tested without any further purification.

### *Irradiations*

The $^{137}Cs$ γ-radiation source and irradiation vessels are as previously described (Jacobs and Melumad, 1976). Routinely 5 g samples of the drug were γ-irradiated at ambient temperature with 25 and 50 kGy [1] (that is, $1.56 \times 10^{20}$ and $3.12 \times 10^{20}$ eV $g^{-1}$, respectively) checked by ferrous sulphate (Spinks and Woods, 1976) and Perspex dosimetry (Berry and Marshall, 1969). The rationale for the choice of these doses has been previously outlined (Jacobs, 1977).

### *Chemical analyses*

Melting point determinations, U.V. spectrophotometry and specific optical rotation measurements were carried out as described elsewhere (Jacobs, 1979a). TLC examination was undertaken as described in our studies on the cephalosporins (Jacobs, 1980b). Chemical assaying was by the iodometric method of the British Pharmacopoeia. pH measurements, using a PHM 64 Research pH Meter (Radiometer, Denmark), were on 5% aqueous solutions of the irradiated compound.

### *Microbiological assay*

The microbiological assay of the irradiated antibiotic was carried out as previously described (Jacobs, 1977) by a two-dose cylinder plate method using Difco Antibiotic Medium 1 seeded, whilst molten, with 0.1 ml of an overnight culture of *Staphylococcus aureus*. The choice of cefoxitin sodium concentrations of 50 and 100 μg $ml^{-1}$ was based on the determination of a linear relationship between antibiotic concentration and diameter of zone of inhibition over this range of concentrations. Following an 18 h incubation at 37°C, diameters of zones of inhibition of bacterial growth were measured.

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[1] The Gray (Gy), the S1 unit for absorbed radiation dose, is equivalent to 1 J $kg^{-1}$ or 100 rads.

*Sterility testing*

Sterility testing was by a membrane filtration technique in which 20-ml aliquots of 1% aqueous solution of the drug followed by 3 similar aliquots of saline (0.9% w/v) were passed through a 25 mm diameter membrane filter having a mean pore diameter of 0.22 μm (Millipore, type GSWP), using a Millipore Swinnex apparatus (code SX0002500) attached to a 20 ml disposable syringe. Immediately following filtration each membrane was cut into two, with one half aseptically introduced into 50 ml of Difco Brewer Thioglycollate Medium (for detection of aerobic and anaerobic bacteria) and the other half into 50 ml of Difco Sabouraud Dextrose Base (for detection of fungi and moulds). 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 by the United States Pharmacopoeia were used. The rinsing of the filter with saline solution ensured no residue of antibiotic that might otherwise interfere with microbial growth. This was ascertained by deliberately contaminating a cefoxitin sodium solution with a small inoculum of *Staphylococcus aureus* (100 organisms per 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 conditions was carried out in duplicate.

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

## RESULTS AND DISCUSSION

Our results, summarized in Tables 1 and 2, show that on the basis of melting point determination, thin-layer chromatography and UV absorbance, there is no decomposition of cefoxitin sodium even following a 50 kGy radiation dose. Any slight change that might have been detected by chemical and microbiological assaying, specific optical rotation and pH measurements is not reckoned to be significant and is within tolerated experi-

TABLE 1

DATA FROM PHYSICAL AND CHEMICAL TESTS CARRIED OUT ON IRRADIATED CEFOXITIN SODIUM

| Dose (kGy) | Melting point ± S.D. (°C) | Chemical assay ± S.D. (%) | S.O.R. [a] ± S.D. | TLC $R_f$ value | UV absorbance at 238 nm | pH [b] ± S.D. |
|---|---|---|---|---|---|---|
| 0 | 260 ± 1 | (100) | (+)195 ± 3° | 0.72 | 0.550 ± 0.005 | 5.0 ± 0.1 |
| 25 | 260 ± 1 | 101.7 ± 1.2 | (+)188 ± 3° | 0.72 | 0.550 ± 0.005 | 4.9 ± 0.1 |
| 50 | 260 ± 1 | 100.0 ± 0.1 | (+)187 ± 3° | 0.72 | 0.550 ± 0.005 | 4.8 ± 0.1 |

[a] Specific optical rotation.
[b] pH of 5% aqueous solutions.

TABLE 2

DATA FROM MICROBIOLOGICAL TESTS CARRIED OUT ON IRRADIATED CEFOXITIN SODIUM

| Dose (kGy) | Microbiological assay ± S.D. (%) | Sterility testing [b] | Sterility testing of of *B. pumilus* contaminated samples [b] |
|---|---|---|---|
| 0 | (100) [a] | (−) | (+) |
| 25 | 97.4 ± 3.6 | (−) | (−) |
| 50 | 100.0 ± 0.4 | (−) | (−) |

[a] Unirradiated compound used as control and taken as 100%.
[b] (−) indicates no bacterial or fungal growth; (+) indicates bacterial or fungal growth.

mental error. Sterility testing indicated that both irradiated and unirradiated samples of the antibiotic were free of bacterial and fungal contaminants. The absence of contaminants in samples deliberately inoculated with $10^6$ spores of *B. pumilus* is proof of the efficacy of the sterilization process. It would therefore appear that cefoxitin sodium may be safely irradiated at the commonly employed sterilization dose of 25 kGy, particularly in view of its stability at the higher dose level tested. However, regulatory authorities would no doubt require suitable toxicological studies to be undertaken before radiation sterilized cefoxitin could be considered for marketing.

The high radiation stability of cefoxitin sodium warrants a comparison to be made with that of the related cephalosporin group of antibiotics. We have previously suggested (Jacobs, 1980b), for example, that the radiation lability of cephalothin sodium and cephapirin sodium may be due to their possession of an acetoxymethyl grouping in the 3-position of the dihydrothiazine ring (compare cefoxitin with a 3-carbamoyloxymethyl group). On the other hand, the fact that cephaloridine and cephalothin sodium, which are affected quite differently by γ-irradiation (Jacobs, 1980b), both possess a 2-(2-thienyl) acetamido substituent in the 7-position of the β-lactam ring suggests that this moiety plays no significant role in radiation stability. Similarly, whether the antibiotic is in the form of the free acid or the sodium salt seems to be of no consequence to its radiation stability as inferred from the radiation stability of cefoxitin sodium, and the radiation lability of cephalothin sodium and cephapirin sodium (Jacobs, 1980b). This is in contrast to the findings of Dziegielewski et al. (1973) who reported that the various penicillinic acids which they examined were more susceptible to irradiation than their salts.

On the basis of these observations one may conclude that the unique chemical structure of cefoxitin sodium confers a high degree of radiation stability, thus affording it the possibility of being radiation-sterilized.

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