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THE EFFECT OF γ-RAYS ON SEMI-SYNTHETIC PENICILLIN POWDERS

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SUMMARY

Gamma-irradiation in the dry state of the semi-synthetic penicillins, mecillinam and nafcillin sodium, results in minimal degradation even following a 10 Mrad radiation dose, suggesting the feasibility of their radiation sterilization. Carindacillin sodium and epicillin are more susceptible to radiation-induced damage.

INTRODUCTION

Increasing application of the ionizing radiation to the sterilization of pharmaceuticals has prompted this study into the effect of gamma-irradiation on a number of the semi-synthetic penicillin powders. The rationale for the choice of this particular group of pharmaceuticals is based on their known susceptibility to hydrolysis at elevated temperatures (Lynn, 1966), thus eliminating sterilization of injectables by conventional methods such as autoclaving. The necessary practice of sterilizing powders for injections by techniques involving costly and highly demanding aseptic processes, makes sterilization by irradiation most desirable.

Detailed reports of studies of the effects of high energy radiation on members of the rapidly increasing group of semi-synthetic penicillins are few (Sekules et al., 1975; Fleurette et al., 1975; Dziegielewski et al., 1973; Jacobs, 1977a, 1979). Because of the destructive nature of ionizing radiation and the difficulty in predicting its radiolytic effect, particularly in more complex molecules, it is necessary to analyze each compound individually for molecular damage in order to determine the feasibility of its radiation sterilization.

The present investigation, following one on the γ-irradiation of amoxycillin, carbenicillin, flucloxacillin, methicillin and phenethicillin (Jacobs, 1979), is aimed at studying the effect of different doses of gamma-radiation on 4 of the semi-synthetic penicillins selected for their quite different chemical structures (see Fig. 1) and hence their differing

microbiological activities (Lynn, 1968). They have been irradiated in the dry-state and, by means of pertinent chemical and microbiological tests, our findings have been related to the feasibility of their radiation-sterilization.

MATERIALS AND METHODS

Penicillins

The semi-synthetic penicillins tested were carindacillin sodium (Pfizer, G.F.R.), epicillin (Squibb, U.S.A.), mecillinam (Leo, Denmark) and nafcillin sodium (Wyeth, U.S.A.). Their chemical structures are depicted in Fig. 1. They were tested without any further purification.

Irradiations

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 2.5 and 10 Mrad doses (i.e. 1.56×10^{20} and 6.24×10^{20} eV/g, respectively) checked by periodic dosimetric determinations using a ferrous sulphate dosimeter ($G_{Fe^{3+}} = 15.4$ (Spinks and Woods, 1976)) and routinely confirmed by means of

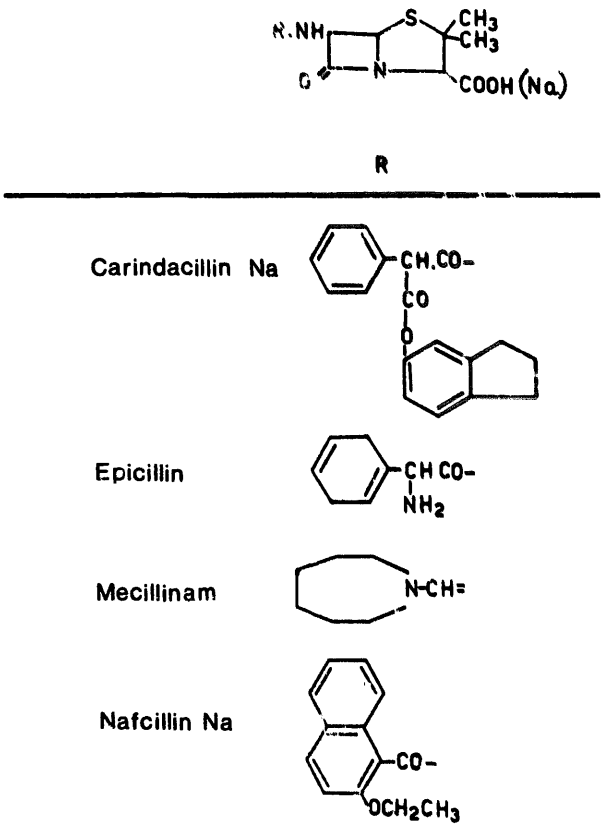

Fig. 1. The chemical structures of the semi-synthetic penicillins used in the present study.

a clear Perspex HX dosimeter (Berry and Marshall, 1969). A 2.5 Mrad dose is that recommended by several pharmacopoeias for sterilization purposes (for review, see I.A.E.A., 1975). Whilst the 10 Mrad is far in excess of that used for this purpose, the testing of drugs having received this exaggerated dose is useful for indicating the type of radiolytic decomposition that the penicillin might be expected to undergo at lower radiation levels.

Chemical analyses

Melting point determinations were made with a Thomas Unimelt apparatus.

Chemical assays were undertaken using the methodology of Bundgaard and Ilver (1972) which is based on the spectrophotometric measurement of penicillenic acid mercuric mercaptide of the penicillins. Determinations of the content of iodine absorbing substances (I.A.S.) was by the methodology of the British Pharmacopoeia, 1973.

Specific optical rotation was determined using a 100 mm microcell in a Perkin Elmer 141 polarimeter.

TLC examination was carried out on 1% solutions of the penicillins in aqueous methanol (70% v/v) using pre-coated silica-gel plates (Polygram Sil N-HR/UV_{254}, Mackery Nagel), with the mobile phase being either an equal part mixture of acetone and methanol, or a mixture of isopropanol and methanol (30 : 70 parts by volume respectively), or a 1.5% v/v solution of strong ammonia in methanol. Each solvent 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 0.1% ninhydrin spray reagent (Merck).

NMR spectral determinations, using a Brucker WP 60 spectrometer, were carried out in a fashion similar to that described by Wilson, Avdovich and Hughes (1974) using D_2O as solvent with 3-trimethylsilyl-propionic acid, sodium salt as the internal reference.

Microbiological assay

The microbiological assays of the antibiotics were carried out as previously described (Jacobs, 1977b).

Sterility testing

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 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 onto Difco Sabouraud dextrose Agar (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 (1973) were used. The rinsing of the filter with saline solution ensured no antibiotic residue than might otherwise interfere with microbial growth. This was ascertained by deliberately contaminating penicillin solutions with small inocula of *Staphylococcus aureus* (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 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 the semi-synthetic penicillin powders deliberately contaminated with 10^6 spores of the radiation resistant *Bacillus pumilus* E601 (ATCC 27142), prior to irradiation.

RESULTS

The preliminary screening of the semi-synthetic penicillins for radiation-induced damage was carried out by the determination of melting points. Our findings, summarized in Table 1, show there to be no change in nafcillin sodium and mecillinam, even following a 10 Mrad dose. Changes are observed in epicillin and carindacillin sodium even at 2.5 Mrads.

Chemical assay results of the irradiated antibiotics are summarized in Table 2. These reveal that up to a 10 Mrad radiation treatment there is no decomposition of nafcillin sodium, epicillin and mecillinam. Carindacillin sodium does undergo some radiolysis (~4%) at the maximum dose level tested. Following the 2.5 Mrad dose, some slight breakdown is also apparent.

Content of iodine-absorbing substances, which is indicative of cleavage of the β-lactam ring of the penicillins was generally less than 0.1% of the total penicillin content. In the case of epicillin and carindacillin sodium, following a 10 Mrad treatment, this value increased to 0.5%.

Specific optical rotation measurements are summarized in Table 3. With mecillinam and nafcillin sodium there is a small drop in value (~3%) in the 10 Mrad-treated samples. With epicillin and carindacillin sodium there is a rise in optical rotation, although in the case of carindacillin sodium this is only apparent at the 10 Mrad dose level.

Table 4 presents R_f values obtained by TLC examination of the irradiated and unirradiated drugs. Some change is apparent in all compounds at the 10 Mrad dose level.

No change could be noticed in the NMR spectra of the irradiated drugs except that irradiated epicillin (10 Mrads) exhibited some change in the intensity of the peaks,

TABLE 1

MELTING POINT VALUES (°C) FOLLOWING IRRADIATION TREATMENT

Semi-synthetic penicillin	Dose (Mrads)		
	0	2.5	10
Carindacillin sodium	197	191	184
Epicillin	204	199	196
Mecillinam	165	165	165
Nafcillin sodium	166	165	165

TABLE 2

CHEMICAL ASSAY VALUES (MEAN ± S.D.) FOR IRRADIATED PENICILLINS

Semi-synthetic penicillin	Dose (Mrads)		
	0	2.5	10
Carindacillin sodium	(100)	98.8 ± 0.1	96.2 ± 1.6
Epicillin	(100)	103.1 ± 2.3	99.5 ± 0.6
Mecillinam	(100)	102.8 ± 0.1	100.0 ± 0.1
Nafcillin sodium	(100)	101.0 ± 1.9	98.4 ± 2.1

TABLE 3

SPECIFIC OPTICAL ROTATION MEASUREMENTS FOR AQUEOUS SOLUTIONS OF IRRADIATED PENICILLINS

Concentration (%)	Dose (Mrads)		
	0	2.5	10
Carindacillin sodium	180	180	315
Epicillin	216	242	242
Mecillinam	294	294	286
Nafcillin sodium	200	200	195

TABLE 4

R_f VALUES OBTAINED FROM TLC EXAMINATION OF SOLUTIONS OF IRRADIATED PENICILLINS

	Dose (Mrads)		
	0	2.5	10
Carindacillin sodium	0.71	0.71	0.76
Epicillin	0.88, 0.64	0.88, 0.64	0.67
Mecillinam	0.47, 0.32	0.47, 0.32	0.47
Nafcillin sodium	0.73	0.73	0.75

assigned, on the basis of standard spectra established by Wilson et al. (1974), to the two β-lactam ring protons, H-5 (a single proton doublet) and H-6 (a single proton quartet). Changes in intensities of these peaks may be as a result of cleavage of the β-lactam ring or epimerization at C-6 giving the *trans*-β-lactam system (Demarco and Nagarajan, 1972).

Values for microbiological assay are summarized in Table 5. Considering the large inherent error in such procedures, only carindacillin sodium (10 Mrads) exhibits a real decrease in potency (~15%).

TABLE 5

MICROBIOLOGICAL ASSAY VALUES (% MEAN ± S.D.) FOR IRRADIATED PENICILLINS USING A TWO-DOSE CYLINDER METHOD WITH *Staphylococcus aureus* (TEVA 29) AS THE TEST ORGANISM

Semi-synthetic penicillin	Dose (Mrads)		
	0	2.5	10
Carindacillin sodium	(100)	96.9 ± 5.0	84.8 ± 1.8
Epicillin	(100)	99.5 ± 5.0	101.6 ± 2.5
Mecillinam	(100)	98.4 ± 0.5	101.0 ± 0.3
Nafcillin sodium	(100)	98.7 ± 0.9	99.3 ± 0.8

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 2.5 Mrad radiation dose.

DISCUSSION

Carindacillin sodium

Our results indicate that carindacillin sodium undergoes some radiolysis following the 2.5 Mrad dose and quite extensive radiolysis at the 10 Mrad dose level. Translated into numerical terms, G [1] (-carindacillin sodium), based on the per cent change observed in the various assays and calculated, for improved accuracy, at the 10 Mrad dose, is around 16.0. Whilst breakdown following the 10 Mrad dose has been detected by the analytical techniques adopted (except NMR), breakdown following the 2.5 Mrad dose was detected by the same techniques (with the exception of TLC) but to a lesser extent. The TLC results apparently point to poor sensitivity of the technique or at least to the procedures adopted for the present study.

The pronounced breakdown obtained on irradiation of this drug has prompted an analysis and identification of the radiolysis products, which is currently being undertaken. Since carindacillin is the indanyl ester of 6-[D-2-carboxy-2-phenylacetamido] penicillanic acid (in fact, carbenicillin) removal of the indanyl grouping may be likely with the release of carbenicillin. Preliminary, although circumstantial, evidence for such a reaction is the lower pH of solutions of irradiated carindacillin. Whilst the pH of a 10% aqueous solution of the drug is 6.7, that of the irradiated (2.5 Mrads) antibiotic is 6.1 (Jacobs, unpublished data), suggesting, possibly, the presence of an additional carboxyllic

[1] G value denotes the number of molecules changed for each 100 eV of radiation energy absorbed.

acid grouping. However, it may have been anticipated that such cleavage of the molecule may have caused some NMR spectral changes. It is unlikely that cleavage of the β-lactam ring has occurred as this would have been detected in the test for iodine-absorbing substances as well as by NMR spectroscopy.

Epicillin

Whilst melting point determinations and specific optical rotation measurements show that epicillin undergoes some change following even a 2.5 Mrad dose, the other results do not support such a conclusion. Differences in the sensitivities of the analytical procedures adopted may help account for our findings. At the 10 Mrad dose level, however, changes are more apparent, although the microbiological and chemical assays still reveal no breakdown. Interestingly, only the irradiated epicillin (10 Mrads) revealed change in the NMR spectrum, which has been accredited to epimerization at C-6 giving the *trans*-β-lactam system (Demarco and Nagarajan, 1972). However, such epimerization is often accompanied by sharp drops in both specific optical rotation and microbiological activity (Johnson et al., 1968). Whilst there is a change in the microbiological assay, there is a *rise* in specific optical rotation.

The chemical structure of epicillin is very similar to that of ampicillin, except that epicillin has a 1,4-cyclohexadienyl substituent, instead of a phenyl substituent, in the 2 position of the acetamido side-chain. Previous studies on the irradiation of ampicillin sodium, under identical conditions to those described here (Jacobs, unpublished data), show that this antibiotic undergoes radiolysis. For example, there is a 7% drop in the microbiological potency and a 25% increase in the UV absorbance.

Mecillinam

Our results indicate that this penicillin is the most radiation-stable of the 4 antibiotics examined in this study. The only indication of instability is the specific optical rotation measurements of the 10 Mrad-irradiated drug. The marked stability of this compound no doubt stems from its unusual structure. Whilst the other 3 compounds are substituted 6-aminopenicillanic acids, mecillinam has a 6β-amidinopenicillanic acid structure.

Nafcillin sodium

This member of the semi-synthetic penicillins shows no breakdown at the 2.5 Mrad dose level, and negligible breakdown following a 10 Mrad dose. Any products of radiolysis were certainly in too small a concentration to the detected by TLC examination.

The absence of contaminants in samples deliberately inoculated with 10^6 spores of *Bacillus pumilus* is somewhat anticipated from the known D_{10} value [2] for this organism when irradiated in air-dried conditions in air. Using a D_{10} value of 0.17 Mrads (Ley and

[2] D_{10} value is defined as the radiation dose that will reduce a given microbial population by a factor of 10.

Tallentire, 1965), a 1 Mrad dose should give an inactivation factor of approximately 10^6.

In conclusion, the results of our tests indicate that mecillinam and nafcillin sodium may be safely irradiated at the commonly employed sterilization dose of 2.5 Mrads. The fact that radiolysis is negligible even at the 10 Mrad dose level adds much support to this conclusion. The other two antibiotics tested, carindacillin sodium and epicillin, display some breakdown and loss of potency at the 2.5 Mrad dose. Whilst the loss of potency particularly in the case of epicillin may be within limits generally acceptable for antibiotics, nevertheless, it would have to be proven, because of the nature of the sterilization process, that any radiolysis products are not toxic. Only when this has been established, might it be possible to safely subject samples of carindacillin and epicillin to radiation doses not exceeding 2.5 Mrads.

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