THE SUSTAINED RELEASE OF ANTIMICROBIAL DRUGS FROM BONE CEMENT

AN APPRAISAL OF LABORATORY INVESTIGATIONS AND THEIR SIGNIFICANCE

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The release of gentamicin sulphate, sodium fusidate and diethanolamine fusidate from Palacos and CMW cements was studied using elution and serial plate transfer tests. Further tests were made to assay the drug remaining in the cement after antibacterial activity could no longer be detected by the above methods, to detect the sustained slow release of the residual drug, and to ascertain the mechanism of release. The results confirmed that the release of gentamicin sulphate could be detected for longer from Palacos cement than from CMW cement, but the opposite was true for sodium fusidate. Little difference was found in the case of diethanolamine fusidate. Comparison of elution and serial plate transfer tests, and of results of elution in buffers of different pH, demonstrated that the test method employed had a significant effect on the results, and the omission of details of methodology from some publications made comparison and evaluation of results difficult.

Varying quantities of residual drug were found in cement from which antibacterial activity could no longer be demonstrated; further tests for sustained, slow release showed that the antibiotic did not remain fixed in the cement but was released at a rate too slow to be detected in the elution and serial plate transfer tests. It is concluded that antibiotics are released from the cement by a process of diffusion, but tests to determine the mechanism of diffusion were unhelpful. The theory of diffusion of drugs through solid matrices, and the clinical implications of the experimental findings, are discussed.

The incidence of deep infections after arthroplasty of the hip has been reported as being between 0.7 per cent (Ring 1974) and 3.5 per cent (Tachdjian and Compere 1957). Symptoms may present within a month of operation or months or years later. Some workers suggest that both early and late infections are contracted during operation (Charnley and Eftekhar 1969) but there are several convincing reports of later infections which are presumably due to haematogenous spread (D’Ambrosia, Shoji and Heater 1976) after pneumococcal pneumonia (Mallory 1973) or infections of the urinary tract (Hall 1974; Irvine, Johnson and Amstutz 1974). There is also experimental evidence that bone cement may become infected during bacteraemia (Elson et al. 1977b). Prophylaxis should therefore continue indefinitely. This has been attempted by mixing antibiotics into the bone cement (Buchholz and Engelbrecht 1970; Buchholz and Gartmann 1972).

Conflicting reports have been published of the mechanism by which drugs are released from the cement, particularly whether they can diffuse through the cement or are removed only from its surface, and of the duration of release. There has been no standard method of testing cement impregnated with antibiotic and, as a result, inconsistent results have been reported. We have therefore made a controlled comparison of different antibiotics to test the pattern and duration of release from bone cement.

METHODS

Production of cement samples. Palacos cement (Mulzer and Company Ltd) and CMW cement (CMW Laboratories Ltd) were mixed according to the manufacturers’ instructions. Antimicrobial substances were mixed with the polymer powder before addition of the monomer. After thorough mixing and kneading, a portion of the cement was placed in a stainless steel mould and pressure applied while the mould was incubated at 37 degrees Celsius for 10 minutes. The mould produced standard test discs, 20 millimetres in diameter and 1 millimetre thick.

Special slipper-shaped test pieces of impregnated cement were also made using an open-ended steel trough. Before the cement had hardened, a circular pit five millimetres in diameter and four millimetres deep was made at one end of each test piece. The cement, still in the
tough, was then incubated at 37 degrees Celsius for 10 minutes.

**Antimicrobial substances tested.** The drugs tested were gentamicin sulphate (Nicholas Laboratories Ltd), sodium fusidate and diethanolamine fusidate (Leo Laboratories Ltd). The drugs were added to the polymer powder to give a concentration of one per cent by weight rather than the active base. No adjustment was made for potency. Since some of the monomer is lost by evaporation during mixing (Charnley 1970), the weights of the cement at various stages of processing were determined and the results indicated that the final concentration of a drug added to the mixture would be 73 per cent of its original concentration in the polymer powder.

**Elution in buffer.** Standard test discs containing antimicrobial drugs were immersed in 10 millilitres of phosphate-buffered saline (PBS), pH 7.4, in separate sealed tubes and incubated on a tissue culture roller drum at 37 degrees Celsius for 18 hours. After incubation, the discs were removed from the tubes, blotted dry and planted onto the surface of a diagnostic sensitivity test agar plate (DST Oxoild Ltd) which had previously been seeded with a stock strain of *Staphylococcus albus*. One face of each disc was marked to ensure that the same face was tested for activity on each occasion. The plates were incubated overnight at 37 degrees Celsius and the diameters of the zones of inhibition were noted. The discs were then removed from the plate, and returned to the tubes containing fresh phosphate-buffered saline. The process was repeated until antibacterial activity could no longer be detected, which was considered to be when the zone of inhibition exceeded the diameter of the disc by less than one millimetre.

In order to test the effect of pH of the eluting fluid, a further series of discs were tested using the same technique but in Sørensen's buffers of pH 6, 7 and 8. All the tests were carried out in triplicate.

**Layered plate test for slow release.** When the discs of impregnated cement eluted in phosphate-buffered saline at pH 7.4 showed no more activity, they were washed well in several changes of PBS and blotted dry. They were then placed onto a separate DST agar plate which had not been inoculated, and were overlaid with 10 millilitres of cooled DST agar to which a broth containing a culture of *Staphylococcus albus* had been added to give a viable count of approximately 10⁶ colony-forming units per millilitre, as determined by the Miles-Misra method. One plate from each trio was incubated immediately, and the other two were refrigerated for three days and seven days respectively before incubating. After incubation, the zones of inhibition of growth in the upper layer of the agar were measured.

**Serial plate transfer.** Discs of bone cement containing antimicrobial drugs, and having one face marked as above, were planted onto the surface of seeded DST agar plates and incubated overnight at 37 degrees Celsius. After the inhibition zones had been measured, the discs were transferred to fresh seeded plates and re-incubated. The process was repeated until antibacterial activity could no longer be detected.

**Reinstatement of antibacterial activity.** Discs which showed no more activity after elution with PBS pH 7.4 were cut in half diametrically and placed on seeded DST agar plates so that the cut edges were in contact with the agar surface. The plates were incubated and any zone of inhibition noted.

**Diffusion of drugs through cement.** Attempts to demonstrate diffusion of antibiotics through cement were made using two different techniques: the "slipper" method and a method using thin films of cement.

**Diffusion through films of cement.** Thin films of untreated Palacos cement were prepared by placing the dough between sheets of aluminium foil and rolling in a RAPRA mill (H. W. Wallace and Company Ltd). The thickness of the sheets ranged from 0.10 to 0.18 millimetres with a mean of 0.13 millimetres. After cross-linking, the foil was removed and the sheet cut into one-centimetre squares with a scalpel. Squares showing cracks or holes were discarded. Mixtures of bone cement and drugs, weighing approximately one gram, were pressed manually into the centre of each square and allowed to solidify. The squares were then placed onto seeded DST agar plates and incubated, taking care that the cement containing the drugs did not come into contact with the agar. A zone of inhibition was considered to be present when the ring around the square of thin cement was continuous and more than two millimetres wide. When no zone was present the squares were transferred to further plates and this was repeated until zones appeared or for two weeks. The day of incubation on which zones of inhibition first occurred was recorded. Great care was taken to ensure that the squares were handled with forceps by their edges, and the central portion containing the drug was not allowed to come into contact with the agar or forceps.

The "slipper" method. Slipper-shaped pieces of cement impregnated with antibiotics were made as described and planted onto seeded DST agar plates. Fifty microfiltrates of a one per cent solution of antibiotic in phosphate-buffered saline were added carefully to the wells in the test pieces. Five test pieces of Palacos and CMW cement were used for each of the three antibiotics. The plates were incubated overnight at 37 degrees Celsius and examined for zones of inhibition of bacterial growth. The method used was the same as that described by Medcraft and Gardner (1974) except that, in the present study, more drugs were tested and both Palacos and CMW cements were used.

After the results had been recorded, the residual drug solution was removed from the wells without removing the test pieces from the plates, and replaced with 50 microfiltrates of a two per cent aqueous solution of gentian violet. The plates were then re-incubated and examined the next day.

**Assay for residual antibiotics.** Discs of cement which had been tested by serial plate transfer until they ceased to show antibacterial activity were inverted and the marked face tested until that too showed no more activity. They were then dried at 37 degrees Celsius for 24 hours, weighed, and the drug extracted with chloroform. In the case of gentamicin sulphate, which is insoluble in chloroform, the viscous suspension resulting from disruption of the discs by chloroform was extracted with PBS. Chloroform extracts containing the fusidates were evaporated to dryness at 37 degrees Celsius and the residue redissolved in one millilitre of PBS. The extracts were then assayed microbiologically. The efficiency of extracting the drugs using this technique was determined by repeating the process with discs of impregnated cement which had not been eluted and whose drug concentration had been calculated. The weight of active drug remaining in the discs was calculated from the results of the assays of the extracts and efficiency of extraction.

**RESULTS**

The effects of eluting Palacos cement in buffers of different pH are shown in Table I. The effect of pH was only slight in the case of gentamicin sulphate and sodium fusidate but diethanolamine fusidate showed a progressively shorter duration of activity with rising pH.

When the duration of activity using the elution method at pH 7.4 and the serial plate transfer method was compared, a four-fold increase by the latter method was found in the case of Palacos cement containing gentamicin sulphate (increased from 3 to 13 days) and sodium fusidate (increased from 5 to 19 days); whereas the duration of activity of cement containing diethanolamine fusidate was the same whichever method was used.

**Table I. Periods of activity of Palacos cement containing drugs after elution in buffers of different pH**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Duration of detectable activity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6</td>
</tr>
<tr>
<td>Gentamicin sulphate</td>
<td>5</td>
</tr>
<tr>
<td>Sodium fusidate</td>
<td>5</td>
</tr>
<tr>
<td>Diethanolamine fusidate</td>
<td>11</td>
</tr>
</tbody>
</table>
Table II. Determination of the amounts of active drugs removed on testing by serial plate transfer until no more activity could be demonstrated

<table>
<thead>
<tr>
<th>Cement + antibiotic</th>
<th>Amount of active drug</th>
<th></th>
<th>Amount removed (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (micro-grams)</td>
<td>Remaining (micro-grams)</td>
<td></td>
</tr>
<tr>
<td>Palacos + gentamicin sulphate</td>
<td>1800</td>
<td>1693</td>
<td>6.0</td>
</tr>
<tr>
<td>Palacos + sodium fusidate</td>
<td>2880</td>
<td>1743</td>
<td>39.4</td>
</tr>
<tr>
<td>CMW + sodium fusidate</td>
<td>2880</td>
<td>1636</td>
<td>43.2</td>
</tr>
<tr>
<td>Palacos + diethanolamine fusidate</td>
<td>2670</td>
<td>1588</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Table III. Zones of inhibition around discs of treated Palacos cement, which had previously been eluted until no further antibacterial activity was shown, tested by the layered plate method

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Zone of inhibition (millimetres)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days*</td>
</tr>
<tr>
<td>Gentamicin sulphate</td>
<td>1</td>
</tr>
<tr>
<td>Sodium fusidate</td>
<td>1</td>
</tr>
<tr>
<td>Diethanolamine fusidate</td>
<td>1</td>
</tr>
</tbody>
</table>

*One of the three samples was incubated immediately and the other two were incubated after refrigeration for 3 and 7 days respectively.

Five days. Serial plate transfer tests showed that the activity of CMW cement containing sodium fusidate persisted for 50 days, CMW with gentamicin sulphate eight days, and CMW with diethanolamine fusidate seven days.

The amount of each drug released from the cement by the time no more activity could be demonstrated is shown in Table II. Ninety-four per cent of the gentamicin sulphate and approximately 60 per cent of the fusidates originally introduced remained in the cement.

Discs which had been eluted until no more activity could be demonstrated showed renewed activity which increased with time when tested by the layered plate test (Table III). The effect of testing the cut edges of a bisected disc of cement after antibacterial activity had apparently ceased is shown in Figure 1; all discs tested showed renewed activity from the cut edge.

Attempts to demonstrate diffusion of drugs from impregnated cement through thin films of untreated cement over 14 days were uniformly unsuccessful, although diffusion through slipper-shaped pieces of cement produced inhibition zones in two out of 15 cases with Palacos cement and six out of 15 cases with CMW cement. However, tests with dye indicated that this effect may have been due to siphoning of the drug solution out of the wells by capillary faults in the surface of the cement (Figs 2 and 3).

DISCUSSION

There is now ample evidence that when antibiotics are mixed into bone cement they are subsequently released, but reports of the duration of release vary considerably from one publication to another.

Wahlig and Dingledein (1979), using an elution method, found that gentamicin sulphate was released for over five years from Palacos cement; Elson et al. (1977a), also using an elution method, found that the drug was released from Palacos cement for up to 24 days when the eluting fluid was left unchanged for the last two weeks of this period. Levin (1975) used an elution method but assayed the activity of the cement rather than the eluting fluid, as in the elution method described here. He demonstrated activity for only 10 days in the case of cement containing gentamicin sulphate, but he tested Simplex, not Palacos, cement. A version of the serial plate transfer test was used by Marks, Nelson and Lautenschlager (1976), who demonstrated antibacterial activity for 13 days for gentamicin sulphate in Simplex cement. The different findings reported may well be due to the use of different test systems. We have compared an
elution system with a serial plate transfer method. The periods of release of gentamicin and sodium fusidate were approximately four times as long using the latter method. Also, technical details such as the method of agitation, if any, and the type of eluting fluid used have often been omitted from previous reports. Distilled water, bacteriological broth, saline and diluted serum have all been used, often with no apparent regard to pH. Using phosphate-buffered saline, we have shown that, in the case of diethanolamine fusidate, the duration of activity is affected by the eluting fluid. Our method involved constant gentle rolling on a tissue culture drum, whereas some workers have either shaken their containers occasionally by hand or have not applied any form of agitation, in which case one face of the sample of cement would remain in contact with the wall of the vessel and release of drugs from this face would be affected. We feel that proper comparison of results is impossible in the absence of standardised or fully described methods of testing.

The mechanism by which drugs are released from cement has been in doubt. Some workers state that the drug diffuses through the matrix of the cement (Medcraft and Gardner 1974; Elson et al. 1977a), others that it is released through holes and pores in the cement (Marks et al. 1976), while others hold that diffusion through the cement substance is unlikely (Wroblewski 1977; Hughes et al. 1980).

Even though the discs of cement tested here eventually ceased to show antibacterial activity, they still contained a large proportion of the drug originally introduced, and antibacterial activity could be restored by exposing the cut surface of a disc on a seeded agar plate. The results of the layered plate test (Table III) show that the residual drug is not fixed in the cement, but that its rate of release has fallen so low that it cannot be detected by serial plate transfer and elution tests. These results support those of Wahlig and Dingledein (1979) in respect of the long term release of gentamicin sulphate. Our results suggest that if a sufficiently sensitive assay method were used, slow release of the drug could be demonstrated for some time, and that since more than twice as much gentamicin sulphate remained in the cement compared with the fusidates, its slow release should continue for longer. We consider that the release of drugs over such a period cannot be due solely to their release from drug particles at the cement surface, and the proportion of drugs found to be released supports this.

Studies on the diffusion of agents through matrices (Higuchi 1961; Baker and Lonsdale 1974; Flynn 1974) indicate that certain factors are important in predicting the rate of release. These include the molecular weight of the drug, the molecular weight and degree of crosslinking of the polymer, the solubility of the drug in the polymer, and the relative solubilities of the drug in the polymer and in the medium outside the matrix. The molecular weights of the drugs used here were similar, ranging from 538 to 605. The molecular weight and degree of crosslinking of the polymer is determined by processing conditions, and as far as possible, these were kept constant. These factors should not, therefore, account for the different rate of release for the drugs tested. However, the starting materials of Palacos and CMW cements differ in that the polymer powder is much finer for Palacos cement. How this would account for the shorter periods of activity of gentamicin sulphate in CMW cement, but a longer period for sodium fusidate, is not clear. The solubility of the drug in the crosslinked matrix is difficult to determine experimentally, but may be inferred indirectly from the solubility of the drug in a solvent in which the polymer is soluble (Baker and Lonsdale 1974). Gentamicin sulphate is insoluble in chloroform, but soluble in water, whereas diethanolamine fusidate is more soluble in chloroform than the sodium salt, but poorly soluble in water. Thus slower rates of diffusion through the matrix, and therefore longer periods of activity, would be expected for gentamicin sulphate and, to a lesser extent, for sodium fusidate.

Matrices consisting of drugs and inert vehicles may be classified according to the mechanism by which the drug is released. Flynn (1974) has described two types of sustained mechanism of release. A Type I matrix consists of particles of the drug suspended in a solid polymer, and release is by dissolution of the particles to form solvent-filled capillaries in the matrix; the drug does not diffuse through the substance of the polymer. In a Type IIb matrix, the drug diffuses through the entire matrix rather than through formed capillaries. If a Type I system only were involved in release of drugs from cement, then much of the drug would be in the form of enclosed particles and these would remain incarcerated in the cement, but if a Type IIb system operated then all of the drug should eventually be released.

Medcraft and Gardner's experiments (1974) were repeated in order to determine whether, as was claimed, antibacterial drugs could diffuse through blocks of bone cement. We found that the results reported previously (Medcraft and Gardner 1974) could be explained by demonstrating capillary flow of dye over the surface of the blocks, and the eccentricity of zones of inhibition and inconsistency of the results suggest that this, rather than diffusion, was responsible for the phenomenon. This would tend to discount the role of large pores in the mechanism of release, but tells us little about which diffusion system is operating. It is possible that the system requires priming, so that the thin-film barrier also contains the drug, before diffusion will occur; this would also suggest that the Type I system was operating. If the Type I system applied, then those drugs most soluble in water, such as gentamicin sulphate, would be released most rapidly, whereas if the Type IIb system applied the quickest release would be from those drugs which are soluble in chloroform.

The results reported here suggest that diffusion of
some kind is the means of release of drugs from bone cement, probably by a Type IIb system, and that the
drugs tested diffused at varying rates through the
substance of the cement. The finding that, after antibac-
terial activity can no longer be demonstrated at the
cement surface in vitro, the antibiotic remaining in the
cement is released eventually, has important clinical
implications. The dissipation of antibiotic after release
from the cement is likely to be much slower when the
cement is surrounded by bone than when it is immersed
in elution fluid, as suggested by the difference between
the elution and serial plate transfer results. Therefore
prophylactic concentrations of antibiotic may persist at
the interface between bone and cement for longer periods
than the results of some experiments in vitro would
suggest.

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