Acrylic cement added with antiblastics in the treatment of bone metastases

ULTRASTRUCTURAL AND IN VITRO ANALYSIS

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An increased long-term survival of patients with malignant tumours also increases the possibility of the development of skeletal metastases and pathological fractures. The management of bone metastases includes the removal of gross disease and the administration of local adjuvants. We have investigated the possibility of adding antiblastic drugs to acrylic cement.

Cylinders of acrylic cement were manufactured containing three different antiblastic drugs, methotrexate, cisplatin and doxorubicin.

We performed in vitro analysis on MCF-7 human breast cancer cells in order to evaluate the biological effect of the mixtures and surface analysis of the acrylic cement-cisplatin cylinders using energy-dispersive x-ray analysis (EDAX). All drugs were released in an active form from the cement. Each drug had a different effect on cell viability. Doxorubicin had the greatest effect on breast cancer cells. Surface analysis showed that antiblastic drugs were present in the form of granules.

These results confirm the potential of antiblastic-loaded cement as a possible adjuvant in the local treatment of bone metastases.

Further studies should be undertaken to determine whether the release of antiblastic drugs from cement is elution or if they are only released from the surface.

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Acrylic cement is now widely used in orthopaedic surgery to provide anchoring for prostheses. Another more recent application is the filling of bone cavities in the management of skeletal neoplasms. Cement may also be used as a spacer in the two-stage treatment of septic loosening of implants. Particular use has been made of the ability of antibiotics to be slowly eluted from cement after arthroplasty.

Recently, the possibility of adding chemotherapeutic agents such as methotrexate to cement for the treatment of skeletal metastases has been suggested. Wang et al measured the in vitro diffusion of methotrexate and the in vivo effects on rabbits. We have evaluated the technical aspects of adding selected drugs to cement and determining their effect on cell culture.

Materials and Methods

Methylmethacrylate was obtained in the form of a powder and a liquid monomer (Surgical Simplex P, Howmedica). The preparation included 40 g of powder and 20 ml of monomer. The following drugs were added: 50 mg of cloridrate doxorubicin powder (Adriblastine; Pharmacia & Upjohn), 100 mg of methotrexate powder (Methotrexate; Lederle), or 50 mg of cisplatin powder (Citoplatinum; Rhône-Pulenc Rorer).

Manufacture of implantable cylinders. The antiblastic-containing cement mixtures were made by mixing the powders of the individual drugs with cement powder and adding the liquid monomer under vacuum using dedicated instrumentation (Simplex Enhancement Mixer, Howmedica). After mixing for 100 seconds, the vacuum was removed. The mixture was then poured onto the dedicated instrument in accordance with ASTM F-451-959 to make cylinders by compression. The control cylinders were made of cement without drugs using the same process. The cylinders were 10 mm in length and 4 mm in diameter (Fig. 1).

Surface analysis. The cylinders were coated with gold palladium and examined using SEM microscopy (SEM 515 Philips) and energy-dispersive x-ray analysis (EDAX). To confirm the presence of the antiblastic drugs on the surface of the cylinder, a qualitative analysis with EDAX was performed on cement-cisplatin mixtures in order to detect platinum, which is only contained in cisplatin and is not present in cement.
Cell cultures. All tests were performed 24 hours after the manufacture of the cylinders in order to avoid the theoretical cytotoxic effect of the exothermic reaction of polymerisation of the cement. The MCF-7 breast cancer cell line was obtained from the American Type Culture Collection and cultured according to the instructions of the supplier in RPMI medium (Gibco) supplemented with 10% heat inactivated fetal bovine serum. Two different sets of experiments were performed as follows. 

First set of experiments. Tests were performed at 24 hours and 15 days after manufacture of the cylinders. All materials were maintained in a sterile environment during the period between preparation and tests. Two cylinders of each mixture were incubated with 4 ml of medium for 24 hours. The medium was then added to the cells and the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma) test was performed 48 hours later. 

Second set of experiments. Two cylinders of each mixture were incubated with 4 ml of medium and the medium was changed every day for 15 days. At each time point, medium was collected and frozen at -80°C. At the end of the experiments, the collected media were thawed and added to cells. 

Cell proliferation assay. The effects of different media on the proliferation of MCF-7 cells were evaluated using the MTT test which is widely used for this type of experiment. Briefly, MCF-7 cells were seeded at a concentration of $2.5 \times 10^4$ cells/well in 24 wells of a multiwell plate and left for 36 hours to obtain good adhesion on the substrate. The medium was then replaced with fresh medium or with medium collected after incubation with cylinders of cement alone or those enhanced with the drugs. After 48 hours, the medium was removed and the cultures incubated with medium containing 1 mg/ml MTT for two hours at 37°C. The medium was then discarded and 500 ml acid-isopropanol (0.04 N HCl in isopropanol) were added to each well to stop the cleavage of the tetrazolium ring by dehydrogenase enzymes which convert MTT to an insoluble purple formazan in living cells. Plates were then agitated at room temperature for about 15 to 20 minutes and the level of the coloured formazan derivative determined on a multiscan reader at a wavelength of 540 nm (reference wavelength 630 nm). Each experiment was repeated in triplicate. The toxic effect was expressed as the percentage of survival of cells compared with the control.

Results

Surface analysis. SEM showed that cylinders without drugs had a beaded surface (Fig. 2). By contrast, those containing drug powders had granules of powder in the cement (Fig. 3). EDAX for platinum (cement with cisplatin mix-
First set of experiments

Cell cultures. Cell survival altered 24 hours after cell seeding with medium extracted from the cylinders. There was a different effect with each drug. Cell survival was 61.2 ± 2.6% with methotrexate, 51 ± 5.7% with cisplatin and 11.9 ± 2.6% with doxorubicin compared with cells incubated with medium collected from control cylinders. Using the cylinders 15 days after manufacturing, the results were similar, 49 ± 4.22% of cell survival for methotrexate, 51.3 ± 3.8% for cisplatin, and 14.2 ± 1.9% for doxorubicin (Fig. 5).

Discussion

The aim of our study was to evaluate the elution of chemotherapeutic drugs from cement in the management of patients with bone metastases requiring surgery which includes the implantation of cement for structural support. The drugs which were chosen are commonly used in the treatment of tumours which may have associated metastases in bone. Thus, methotrexate is widely used in the treatment of metastatic bone tumours, cisplatin for lung tumours and doxorubicin for cancer of the breast. We verified that cylinders manufactured with the addition of these drugs had an acceptable mechanical compressive strength according to ASTM F451-95 (data not shown). SEM showed that drug powders could be identified on the surface of the cylinder. EDAX showed platinum on the surface of the cylinder (Fig. 4).

Second set of experiments

Cell cultures. When medium collected at 24 hours was added to cells, survival was 51.6 ± 4.1% for methotrexate, 68.2 ± 4.6% for cisplatin and 10.4 ± 0.4% for doxorubicin. After the daily change of culture medium, the following observations were made. At 48 hours, cell survival was 55.5 ± 5.5% for methotrexate, 86.1 ± 2.5% for cisplatin and 46.4 ± 2.3% for doxorubicin. At three days, cell survival was 60.9 ± 5.1% for methotrexate, 88.1 ± 7.4% for cisplatin and 77.6 ± 4.7% for doxorubicin. At seven days, cell survival was 74.7 ± 3.5% for methotrexate, 95.1 ± 2.5% for cisplatin and 93.1 ± 8.5% for doxorubicin. Finally, at 15 days, cell survival was 90.5 ± 4.8% for methotrexate, 98.8 ± 8.5% for cisplatin and 98.6 ± 6.4% for doxorubicin. The controls showed no toxic effect on cell viability (Fig. 6).
firmed that the platinum-containing drug was present within the cylinders and that the drug was the only source of the platinum (Fig. 4). Cell culture medium incubated with cylinders for different periods of time and under different conditions, was added to cultures of exponentially growing breast cancer cells, and cell survival was evaluated using the MTT test, which is widely used to evaluate the in vitro cytotoxicity of candidate biomaterials. Only cells which are alive and metabolically active have the capability to transform MTT into formazan salts. The first set of experiments showed that the selected drugs all had significant biological effects on MCF-7 breast cancer cells and that cell inhibition was the same if the cylinders were used immediately or if there was a delay of 15 days. Thus the cylinders retained their chemotherapeutic activity with the passage of time.

In the second set of experiments, the test was closer to the clinical situation in that there was a continuous change of tissue fluid in contact with the bone tumour and the cement. In fact, the biological inhibitory effect on MCF-7 breast cancer cells became progressively smaller, so that medium eluted after 15 days of exposure to cylinders showed no toxic effects in the presence of doxorubicin and cisplatin. There was a toxic effect in the presence of methotrexate, which was, however, significantly lower than that obtained in the first days of elution.

We did not study the possible role of heat polymerisation on drug activity. However, other authors have reported no changes in the stability of these three drugs. Both our sets of experiments showed a toxic effect of medium incubated with cylinders on cell culture. Thus, it can be assumed that drugs contained in the cylinders are metabolically active, and even if heat polymerisation affects the drug in the cement, it does not impair its cytotoxicity. Two issues remain unanswered and require further study. First, we were unable to demonstrate conclusively that the drugs are released from the cylinders. It is possible that only drug particles on the surface of the cylinders are released and contribute to the observed cytotoxic effect. The duration of release which, although it declines, lasts for a few days seems to suggest release from inside the cylinders, and not only from the surface. However, independently of the mechanism involved, our experiments have shown the feasibility of this approach in the treatment of osteolysis in bone metastases. Secondly, it is not clear how long the chemotherapeutic effect of the implanted drugs will last in a clinically significant concentration. We can only acknowledge that we have shown the release of doxorubicin and that it produced a marked toxic effect within 24 hours of implantation, while the response to methotrexate was more stable with the passage of time. There are two possible explanations for this. The half-life of doxorubicin may be only 24 hours in water and its release from within cement may be limited once that on the surface of the cylinder has been released. We did not address the issue of what happens to particles of the drug which are trapped within the cement and whether they are eventually released. It may well be that both mechanisms are present since macromolecules of the drugs may be trapped within the cylinder and may effectively be released into the surrounding environment only after the particles on the surface of the cylinders have been released.

In conclusion, we have shown that when antiblastic drugs are included in cement they remain metabolically active. The duration and extent of their effect were found to vary between drugs but were potentially clinically relevant and not affected by the time interval between manufacture and implantation of the cement.

Studies are ongoing to verify that the compressive, tensile and fatigue properties of drug cement composites are the same as those for cement alone, before they can be included as part of routine treatment for metastatic lesions in bone.

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References


