METHOTREXATE DIFFUSION FROM ACRYLIC CEMENT

LOCAL CHEMOTHERAPY FOR BONE TUMOURS

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We investigated the possible use of acrylic cement containing chemotherapeutic drugs in the treatment of malignant lesions in bone. The diffusion of methotrexate (MTX) from methylpolymethacrylate implants was studied in vitro: polymerisation of the cement did not destroy the drug; liberation began immediately and about 10% was released by 18 hours. Some release continued for as long as six months.

In vivo experiments on rats with induced osteosarcoma showed that MTX in cement had both local and general effects which were dependent on the dosage. A series of 17 large dogs with spontaneous osteosarcoma were then treated by local resection and cement containing MTX. General chemotherapeutic effects were detectable from 2 hours to 5 days, survival was increased and local recurrence was reduced, but there were four cases of delayed wound healing.

Preliminary studies in human patients confirm the possibility that this method of local chemotherapy could be a useful addition to the treatment of malignant tumours of bone.

The parenteral administration of antimitotic drugs is widely used for primary bone tumours such as osteosarcoma and sometimes for secondary tumours. But even parenteral chemotherapy and wide surgical excision does not always prevent local recurrence or metastasis from bone sarcomas. For these reasons, it is now recommended that chemotherapy starts pre-operatively and is continued as soon as possible postoperatively (Campanacci et al 1980; Rosen et al 1982).

Since cement is often used to fill bony defects or to implant replacement arthroplasties it was thought that the efficacy of treatment might be increased by local chemotherapy from an antimitotic drug added to the acrylic cement. Diffusion into the surrounding tissues is well established for numerous antibiotics (Buchholz and Engelbrecht 1970; Elson and McGeoch 1976; Marks, Nelson and Lautenschlager 1976; Elson et al 1977; Fischer et al 1977; Carlsson, Josefsson and Lindberg 1978; Graham 1978; Hoff, Fitzgerald and Kelly 1981).

We performed a number of experiments to assess acrylic cement as a vehicle for local chemotherapy:
1) Diffusion of antimitotic drugs from acrylic cement was studied in vitro to determine that these drugs were released and were still biologically active after exposure to highly reactive monomer and the exothermic curing reaction.
2) Experiments in vivo were performed on two groups of animals. We tested the effect of this local chemotherapy on experimental osteosarcoma of the rat and on dogs with spontaneous osteosarcoma. General and local tolerance of the antimitotic-loaded cement was assessed.

Finally, we report our preliminary clinical investigations with pharmacological data from patients.

MATERIAL AND METHODS

Many antimitotic drugs are available; for our first investigations we used only methotrexate (MTX). This drug was chosen because its concentration is easy to determine by spectrophotometry, and because there is an
antidote (citrovorum rescue) for adverse effects. We used the acrylic bone cement currently employed by the authors for clinical arthroplasty (Table I).

**In vitro experiments**
These experiments were designed to measure the elution of the antimitotic drug from cement in vitro.

1. **Kinetics of elution.** Small methylpolymethacrylate implants containing MTX were made from the cement. For each, 200 mg of MTX were mixed with 4 g of polymer powder containing benzoyl peroxide and 2 ml of monomer was added. The mixture was poured into moulds before polymerisation giving casts 8.5 mm by 7.5 mm diameter. Each cast weighed 357 mg and had an MTX content of 3.9 mg ± 0.1%.

The implants were then suspended in one litre of phosphate buffer (pH 7.4, 37°C) contained in a covered, round-bottomed flask. This medium was stirred at 70 turns per minute and 1.5 ml aliquots were periodically removed and analysed for MTX content using a plasma emission spectrophotometer (Perkin Elmer, 560, Bois d'Arcy, France). The medium was maintained at a constant volume by adding to the flask the volume of medium which had been removed or had evaporated during the experiment.

2. **Longer-term liberation of methotrexate.** To investigate the release of MTX from a block of acrylic cement implanted into the tissues, cubic test pieces were placed in 32 ml of physiological saline, which was changed every day. The concentration of MTX in the elution fluid was measured before each change. These test pieces were made from a mixture of 500 mg MTX powder, 46.5 g of polymer, and 20 ml of monomer, poured into 2 cm cubic moulds. Each cube weighed about 13 g and contained approximately 100 mg of MTX. Methotrexate elution was evaluated daily for 15 days and then weekly for six months for six specimens, the results being given as an average of the six.

3. **Relationship between the quantity of antimitotic drug and the level of release.** As in experiment 2, cubic 13 g specimens were prepared, but containing 20 mg and 40 mg of MTX. These had surface areas and weights equal to those of that experiment; elution was evaluated daily for 15 days.

4. **To assess the effect of polymerisation of the cement.** Diffusion of antimitotic drug from pellets of plaster was studied and compared with that from cement. The plaster pellets were prepared from a mixture of 15 g of plaster, 1 g of MTX and 400 ml of water, ground in a mortar and sieved before 4 g dicalcium phosphate and 0.2 g magnesium stearate were added. A press was used to give small cylinders of 3.5 mm length and 4 mm diameter weighing 91 mg ± 5, containing 4.88 mg ± 0.28% MTX. These pellets were tested as in experiment 1 above, but, because of early crumbling, could not be used for the longer periods of experiments 2 and 3.

5. **Stability of MTX during polymerisation of the cement.** Solutions of methotrexate extracted from cement and plaster were compared with control specimens by analysis using high pressure liquid chromatography (column Nova Parck, water Milford, Massachusetts, USA).

**In vivo experiments**

**Experimental osteosarcoma in rats.** The cytostatic activity of the eluted MTX was assessed against an experimental osteosarcoma (Allouche et al 1980), induced in the rat by injection of colloidal radioactive Cerium hydroxide (+44Ce) (Fig. 1). Small 100 mm3 fragments of the tumour were grafted subcutaneously into rats weighing 150 g to 250 g, aged between 35 and 45 days. These grafts succeeded in 98% of the experiments and after 15 days, the tumours had reached an average diameter of 3 cm.

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**Table I.** Composition of the acrylic bone cement

<table>
<thead>
<tr>
<th>Monomer 20 ml</th>
<th>Polymer 46.5 g</th>
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<tbody>
<tr>
<td>Methyldimethacrylate</td>
<td>16.91 ml</td>
</tr>
<tr>
<td>N-Butylmethacrylate</td>
<td>2.69 ml</td>
</tr>
<tr>
<td>NN Dimethyl-P-toluidine</td>
<td>0.40 ml</td>
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</table>
The female animals averaged 250 g and the males 300 g at this stage. This tumour is similar to human osteogenic sarcoma in its local aggression, ability to metastasise and calcium metabolism (Klein et al 1977; Mazabraud et al 1982; Thiéry et al 1982).

The 100 rats were divided into four groups: the control group (A) of 20 rats received no treatment, and the natural progress of the tumour was studied; group (B) of 10 rats had a plaster implant with no antimitotic drug placed at the centre of the tumour; group (C) of 10 rats had an acrylic cement implant with no antimitotic drug; and group (D) of 60 rats had a plaster or cement implant containing MTX introduced into the centre of the tumour (Fig. 2).

After operation, the animals were checked daily, and weighed, and the size of the tumour was measured in two perpendicular directions using calipers. The control animals were studied until they died or became moribund. The animals with active implants were followed as long as possible, except that 20 rats were killed between days 10 and 30 for histological study of the early action of the drug.

**Spontaneous osteosarcoma in dogs.** Experiments were conducted to establish the plasma diffusion of MTX, its general tolerance and the local action of cement containing antimitotic drug in conditions similar to those of the management of human malignant bone tumours. We therefore chose an animal with a weight close to that of man, and a spontaneous tumour with an evolution like that of human osteosarcoma, similarly hypervascular because this may influence the diffusion of MTX.

In experiments at the National Veterinary School of Maisons-Alfort, we used dogs with spontaneous osteosarcoma. This is a malignant tumour (Brodey, Sauer and Medway 1963; Ling, Morgan and Pool 1974) with the same aggressive properties as the human type. It affects the very large breeds of dog such as the Saint Bernard (mean weight 70 kg), the mastiff (55 kg) and the boxer (30 kg). It progresses rapidly in the absence of treatment, and death is the rule in a few months (Misdorp and Van der Heul 1976; Owen 1979). Simple resection of the tumour rapidly leads to local relapse, and even after amputation 85% of dogs die within seven months of diagnosis (Parodi 1970; Brodey and Abt 1976; Pool 1978).

![Fig. 2](image1.png)

**Implants containing MTX.**

![Fig. 3](image2.png)

**Radiograph of osteosarcoma of the radius in a dog before and after local resection and internal fixation.**

After general anaesthesia and intubation 17 animals of average weight 50 kg (range 30 to 80 kg) had excision of the tumour at its borders, without any margin of safety. The defect created by the excision of the tumour was filled with freshly-made methotrexate-containing cement, maintained in place by rigid osteosynthesis (Fig. 3). No other treatment was given. The total quantity of MTX received by each dog was about 100 mg in 14, 200 mg in two cases and 500 mg in one. Since the weight of the dogs varied from 30 to 80 kg, the dose per dog ranged from 1.6 mg to 16 mg/kg.

**RESULTS**

**In vitro experiments**

1. The early liberation kinetics of MTX are shown in Figure 4. Release from cement is rapid in the first two hours and reaches approximately 10% of the MTX load at the end of 18 hours. From 18 to 36 hours, release is slow but the concentration of MTX continues to increase very slowly after 36 hours.

2. At 24 hours, the MTX concentration was 120 micromoles per litre (this is 10 000 times the lethal concentration for cells). At the end of the second 24 hour period, the mean concentration was only 15 micromoles per litre and this value fell regularly up to 15 days (Fig. 5). MTX continues to be released very slowly up to six months.

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3. It was shown for MTX that when the shape and the surface area of the cement block are the same, the rate of elution varies with and depends on the concentration of the admixed material (Fig. 6). The blocks with the largest content of MTX gave higher concentrations of MTX for the first 10 days, but after this the difference was no longer statistically significant.

4. The comparative study of liberation of MTX from plaster pellets and acrylic cement showed that the liberation of the antimitotic drugs from plaster was a little more rapid than from cement (see Fig. 5). This difference has also been observed for antibiotics and was shown to be related to the nature of the substrate and not to the destruction of antibiotics by the polymerisation of the cement (Peltier 1961; Varlet and Dauchy 1983).

5. Changes in methotrexate caused by the substrate or by polymerisation was investigated by the study of the wavelength spectrum. On chromatography, no differences were found between the wavelength spectrum of 'pure' MTX, that in pellets of plaster or cement, or that in eluted methotrexate.

**In vivo experiments**

**Local chemotherapy and rat osteosarcoma.** In the control group (A) no tumour regressed spontaneously. The tumour forms a 3 to 4 cm diameter mass by three weeks and 8 to 10 cm in diameter at about two months. The mean survival of the controls is approximately two months, with death generally due to skin ulceration and infection. There was always less than 50% necrosis of the centre of the tumour. The life expectancy of healthy rats, in normal laboratory conditions, is about two-and-a-half years.
The outcome after an implant of plaster or cement without MTX (groups B and C) was identical to that of the controls. After the operation, the animal loses some weight and the growth of the tumour stops for some two days, but then continues and leads to death at the same interval and in the same manner as for the controls. The level of necrosis at the centre of the tumour was comparable with that in the control animals.

The outcome in animals receiving an implant containing MTX (group D) depended on the dose of the drug: 5 mg of methotrexate (10 rats) caused a high mortality (90%) between 4 and 6 days, related to general toxicity with gastrointestinal haemorrhage, and severe nephropathy with necrosis of the epithelium of the convoluted tubules. With an implant containing 1.5 mg MTX (20 rats), tumour growth was temporarily slowed and mean survival extended to 30 days (Fig. 7). Central necrosis of 75% to 90% of the tumour was seen with, usually, some persistence in peripheral zones (Fig. 8).

**Local chemotherapy in dog osteosarcoma**

**General diffusion.** Venous blood samples were taken from day 1 to day 5 after operation. Only two hours after implantation (Fig. 9), MTX is found at a concentration between 0.08 micromoles and 0.02 micromoles per litre (1 mg of MTX = 2.2 micromoles). At 24 hours, the concentration was between 0.1 micromoles and 0.02 micromoles per litre. After the third day, the antimitotic drug could not always be reliably measured in the blood and only exceptionally was it measurable after the fifth day. The limit of detection and measurement in the blood is 0.01 micromoles per litre, but MTX probably continues to be released, because cement blocks, recovered between 2 and 3 months after implantation continued to release MTX in normal saline in vitro.

**Toxic effects.** Toxic effects were observed in three dogs on day 4 with 200 mg or more of MTX mixed with cement in animals weighing less than 50 kg. These toxic effects included gingivitis, glossitis, pharyngitis, anorexia, nausea, vomiting and diarrhoea. An abnormally high MTX concentration was not recorded in the first three days, but in two of the dogs there was a high MTX concentration between the third and sixth days. Toxic effects were reversible in the two dogs which were treated with folic acid, but the one untreated dog died on the sixth day.

In the other dogs of whatever weight, having a dose of 100 to 150 mg, there was no general toxicity and none needed treatment with folic acid. Haematocrit estimations and white cell counts with differential and platelet counts were available throughout for seven dogs and showed no evidence of antimitotic-related anaemia, leukopenia or depression of the platelet count.

**Local action.** Four dogs showed delayed wound healing in the first 15 days, two having septic complications requiring removal of the cement blocks. In all four cases the tumour had been excised from the lower extremity of the radius, and the cement was directly under the skin. There was no delayed wound healing when the cement block was not in direct contact with the skin.

**Tumour growth.** Of the 17 dogs who had a tumour resected, one died from overdosage and four were killed at the request of their owners (two with relapsed tumour and two for other reasons). One dog died after five months from pulmonary metastases and two died before the eighth month without local or pulmonary metastases on autopsy.

Of the 10 surviving dogs at 8 months, two required amputation: one for relapse and one for radial nerve
paralysis without relapse. None of the 10 dogs had pulmonary metastases on clinical examination or radiography. With no general chemotherapy or radiotherapy, dogs having local resection and MTX cement with preservation of the limb had results at least as favourable as after amputation without adjuvant treatment (85% mortality in seven months).

The effect of this treatment in preventing local relapse is even more interesting: local relapse is almost invariable after simple tumour resection, but in our series only three of the 14 dogs had local relapse at 2, 3 and 6 months respectively.

DISCUSSION

We are not yet ready to report our clinical results in detail, but it is of interest to mention the pharmacological data from 14 patients treated by local resection, using cement with MTX to reinforce metal fixation and to fill defects. The 14 patients with primary or metastatic tumours were selected for local chemotherapy because their age made general chemotherapy inappropriate in the opinion of their oncologists.

We were able to confirm the high local concentration of MTX in vacuum drainage (10 000 times the blood concentration), the general chemotherapeutic effect during the first few days and the urinary excretion of MTX for at least 3 weeks (Fig. 10). The release and diffusion of MTX from cement was about the same (Fig. 11).
whatever the site or the shape of the cement implant (4 in the pelvis, 5 in metastatic fractures of the intertrochanteric region, 3 for metastases of the thoracic spine). Local chemotherapy was well tolerated, no patient showed general toxicity, MTX-related anaemia, depressed white cell or platelet counts and there was no change in creatinine clearance. There were wound healing problems in two patients with tumours of the pelvis; one patient needed removal of the cement block.

The clinical application of antimitotic-impregnated acrylic cement constitutes the use of an approved drug for unapproved indications. The use of such local chemotherapy in preventing local recurrence or as adjuvant therapy has not previously been reported and administered in a continuous manner by diffusion from cement, with conventional chemotherapy given sequentially by an intravenous route.

Simple local administration of MTX seems to reduce the risk of local recurrence in the dog. The results appear to be significant although each tumour was spontaneous and therefore different and, for understandable reasons, there were no true controls. This result in dogs may be related to the very high local concentrations of the drug, which were observed in the vacuum drainage from the human patients. Such concentrations cannot be obtained by parenteral administration without general toxic effects.

The influence of such treatment on wound healing

![Graph](image)

**Fig. 11**

Average concentrations of MTX in blood, urine and the vacuum drainage of 14 patients.

its value is arguable (the use of antibiotic-containing cement is still under discussion!); moreover, we have not yet addressed the question of the advantages and complications of clinical use. This paper has examined only the following question: do antimitotic drugs elute from acrylic cement in useful concentrations and over significant periods of time in vitro and in vivo?

Our experiments show that, as for antibiotics, MTX can be released from cement, and that the released MTX is still biologically active. This is proven by the general toxic effects leading to death in some rats and dogs, and the local effect on experimental osteosarcoma in the rat.

It was surprising to discover the mortality produced by the local administration of 200 mg of MTX in dogs weighing under 50 kg, in relation to the known effect of conventional sequential chemotherapy in man. However, it is difficult to compare the effects of a single dose is important. Experimental studies have shown that chemotherapy impairs wound healing, but this has not been significant in clinical trials. Our local chemotherapy caused no such problems in the rat model but skin necrosis was seen four times in 17 dogs and twice in the clinical trials on 14 patients. It is not possible to be certain if these delays in healing were directly related to a local reaction to MTX or to the extensive nature of the operations performed, but they may well be related to side effects of MTX. The systemic blood concentration only two hours after cement implantation gives an early general level of chemotherapy; this may be an advantage in treating primary bone tumours since there is good reason to suspect that micrometastases begin to grow faster immediately after the primary focus has been eliminated (Schabel 1985).

We conclude that it is possible to use acrylic cement.
as a supporting vehicle for the diffusion of MTX for local chemotherapy. Perioperative regional chemotherapy has previously been limited to an anatomical region using intra-arterial perfusion, perfusion after tourniquet, or extra-corporeal circulation. Although these methods have given encouraging results, they have complications which can be serious enough to require amputation. If further studies confirm our initial results, the method of local chemotherapy we have described could complement other therapeutic measures for the treatment of bone tumours.

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REFERENCES


