Cement with Antimitotics

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There are two causes of failure in the surgical treatment of metastatic tumors : firstly, local recurrence of the tumour is not always prevented, even after extra-tumoral surgical exeresis and systemic chemotherapy, and secondly, failure of osteosynthesis after surgery. For these reasons, we thought that it would be helpful to provide local chemotherapy during and immediately after surgery, for instance, by adding an antimitotic to the acrylic cement used to replace the bone loss or to seal reconstruction prostheses. It was thought that the antimitotic would be likely to be released into the surrounding tissues in the same way as many antibiotics. Diffusion into the surrounding tissues is well established for numerous antibiotics [1–6].

We performed a number of experiments [7–9] 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 such 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 [10–12] with pharmacological data from patients. It was possible to envisage using cement/drug mixtures to treat orthopaedic complaints calling simultaneously for mechanical consolidation of the bone [13] and *in-situ* release of a drug: one example would be the strengthening of bone with cement after resection of a bone tumour [14] plus the local release of antimitotic drugs from the implant.

Cement was the first vehicle to be studied for the purpose of releasing local chemotherapy. Methyl polymethacrylate (P.M.M.A.) fulfils the two following criteria: it has good biocompatibility, since the system has to remain *in situ* throughout the rest of the patient's life; it is not biodegradable, so that it provides mechanical support for bone which has been weakened by the surgical exeresis of a neoplastic site.

Many antimitotic drugs are available; for our first investigations we used methotrexate and cisplatine. Methotrexate 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.

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Study of the Release of Methotrexate (MTX)

The first study investigated the *in-vitro* release of antimitotics included in acrylic cement. After confirming that this release does actually occur, starts rapidly and is maintained over a prolonged period, two further studies were then carried out *in vivo*: one in dogs suffering from spontaneous osteosarcoma, in order to investigate the release of the antimitotic from the cement into the plasma, the systemic safety and the local activity of the antimitotic-loaded cement following exeresis of the neoplasm.

The second study was conducted in laboratory rats with implanted osteosarcomas. This type of tumour was used so that a large number of animals with tumours could be studied and divided into uniform groups. Under these experimental conditions, it was possible to monitor the progress of the tumours left *in situ* as well as the histopathological changes brought about by the local action of antimitotics released from implants.

- Kinetic profile of the release of MTX from implants

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 in the elution fluid was measured before each change. These test pieces were made from a mixture of 500 mg methotrexate 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 methotrexate. Methotrexate elution was evaluated daily for 15 days and then weekly for 6 months for six specimens, the results being given as an average of the 6.

The release profiles from implants containing 1 % w/w have shown that methotrexate is released more rapidly during the first 2 h and 10 % of the load is released within the first 18 h. The rate of release then slows. Implants immersed in an extraction medium which is changed regularly, continue to release methotrexate for 6 months; the quantities released initially being greater the greater the initial load.

- The release of methotrexate from acrylic cement

This has been investigated *in vivo* in dogs with spontaneous sarcoma. 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 cisplatine. In experiments at the National Veterinary School of Maisons-Alfort, we used dogs with spontaneous osteosarcoma. This is a malignant tumour [15, 16] 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 [17, 18]. Simple resection of the tumour rapidly leads to local relapse, and even after amputation 85 % of dogs die within 7 months of diagnosis [15, 19, 20]. The loss of substance resulting from the exeresis of the tumour was compensated using freshly prepared methotrexate-loaded cement. The dose of methotrexate received ranged from 1.6 to 16 mg/kg. Two hours after being implanted, plasma levels of methotrexate ranged from 0.08 to 0.02 µmol/l (1 µmol of methotrexate = 0.455 mg). After 24 h, the plasma levels were between 0.1 and 0.02 µmol/l and by the third day were no longer detectable. Toxic effects were observed on day 4 in the 3 animals which had received a dose of more than 200 mg of methotrexate. The other animals, which had received a dose of between 100 and 150 mg, did not display any signs of toxicity. The survival curve of the animals in this group seemed to be better than that of the animals which underwent surgery without adjuvant treatment, where 85 % of the animals had died within 7 months.

The Efficacy of Methotrexate-Loaded Implants

This was investigated using the experimental model of osteosarcoma in the rat [21, 22]. Using implants equivalent to 1.5 mg of active constituent, tumour

growth was temporarily slowed and the survival time of the animals significantly prolonged.

These experiments have shown that the rise in temperature which accompanies the polymerisation of the cement does not destroy MTX and, like antibiotics, MTX can be released from the cement. Migration probably occurs as a result of diffusion; the cement constitutes a network of pores and micro-fissures which makes it accessible to the liquid medium in which it is immersed. This liquid penetrates into the system and dissolves the crystals of MTX which then diffuse into the surrounding medium. This mechanism is certainly the dominant one at work during the early stages of MTX release. It probably accounts for the initial peak which characterises the kinetics of MTX release. It is logical to suppose that the outer layers of the cement are more accessible to the liquid medium than the inner layers.

Study of the Release of Cisplatin

Cisplatin is one of the antimitotics which would be suitable for mixing with cement and it has the following characteristics : it is often used to treat primary bone tumours; in the context of bone metastases from visceral tumours, it is generally used in multiple-drug therapy of tumours which are characterised particularly by being radioresistant and resistant to other antimitotics [23–27]: hence the appeal of a local cisplatin-based therapy, which has the advantage of being radiosensitising [28].

Cisplatin takes the form of a whitish-yellow crystalline powder. It has no melting point as it decomposes without melting at 270 °C. Cisplatin has a solubility in water at room temperature of 1 mg/ml.

In the solid state, cisplatin is relatively stable. In contrast, in solution it forms *mono-aquo* and *di-aquo* derivatives by the successive shedding of chloride ions. Cisplatin is most stable in solution at an acid pH and in the presence of chloride ions, which prevent a shift in the reaction equilibrium towards the formation of degradation products. It should also be noted that cisplatin has a chemically inert structure with few reactive groups. Differential thermal analysis did not provide further information in this regard, as cisplatin decomposes without melting at around 270 °C and parasite peaks from PMMA superimpose on the cisplatin peak at these temperatures. X-ray diffraction did however allow us to demonstrate that there is no difference between the spectrum of the physical mixture and that of the cisplatin implants. Moreover, this hypothesis was supported by a very simple experiment in which an accurately weighed 5 % cisplatin implant was dissolved in methylene chloride, a solvent for the polymer but not the cisplatin. The cisplatin crystals were sedimented and extracted by a 9 p. 1000 solution of sodium chloride in a separating funnel. Assay of this solution revealed that it contained all of the active ingredient present in the implant. The very slow release of cisplatin does not therefore appear to be due to a chemical bond with the polymer, but rather to the fact that a large proportion of cisplatin is trapped in the matrix.

The mixture was prepared as follows: during the first step, the active constituent, cisplatin, was mixed with the polymer. A predetermined weight of polymer was placed in a porcelain mortar. A known quantity of cisplatin was then added in small fractions. In the second step, the monomer was added, depending on the quantity of polymer taken, the volume of polymer being that recommended by the manufacturer. The constituents were then thoroughly mixed for 4 min to form a homogeneous paste.

This paste was then poured into the barrel of a stoppered syringe. The mixture was then expelled by the pressure of the piston into polyethylene moulds measuring 6.7 mm (inside diameter) by 10.3 mm in height (cylindrical mould, Prolabo, Paris [France]). The implants were left in the moulds for 24 h, to allow complete polymerisation to take place, and then tipped out and kept in darkness and at room temperature. The *in-vitro* release of cisplatin was investigated by placing the implants in a release medium with the following composition : sodium chloride: 9 g. distilled water, q.s.p.: 1000 ml; 1 N hydrochloric acid, q.s.p. pH = 4. After weighing, the implants were placed in the release medium at 37 °C and stirred in darkness. Samples were taken at regular intervals and an equal volume of fresh medium added to replace the reaction mixture removed. “Sink”

conditions were maintained, i.e. the concentration of cisplatin in the release medium was never more than one-tenth of the saturation concentration (i.e. 100 mg of cisplatin per litre).

The *in-vitro* release data obtained from implants containing various loads of cisplatin (from 1 to 20 % w/w) are shown as a function of time : the quantities released were related to the initial concentration of cisplatin in the implants. For instance, after 90 days, the implants with the highest load had released about 12 % of cisplatin, whereas implants containing 1 % had released only 3 % under these experimental conditions. It should also be noted that the release was incomplete from all the implants and never reached 100 % of the initial load.

In the case of pure PMMA films, diffusion experiments have shown that cisplatin in solution had great difficulty in crossing even a thin membrane. Cisplatin therefore appears to be unable to cross pure PMMA. In the case of PMMA/MMA films, we found that up to a certain thickness, cisplatin was readily able to diffuse across the membrane. This diffusion can be accounted for by the structure of the polymer, which is not a uniform matrix, but a layer of spheres of PMMA which are linked to one another by the polymerised monomer. In solution, cisplatin must be able to diffuse into the relatively less compact zone between the spheres, which may have defects of structure and cohesion. However at thicknesses from 133 µm, diffusion is slower, as if a thicker layer of spheres impedes the diffusion of the active constituent. These findings should be interpreted in the light of the structure of the cement viewed under electron microscopy, which reveals areas of regular polymer and defects, fissures which doubtless permit the diffusion cisplatin.

Clinical Experience

The clinical research was done at the Henri Mondor hospital and has confirmed the experimental data obtained in animal studies. It provided the basis of the protocol for clinical use. During surgery, a dose of 100 mg of MTX mixed with a complete dose of cement (46 g of polymer and 20 ml of monomer) was administered, followed by an intramuscular administration of folinic acid between 72 and 86 h later. This was well tolerated by the patient. The local concentration of MTX found in the drains within the first few hours may reach levels 10,000 times greater than the plasma concentration and remained 100 times greater than the plasma concentration for the next 3 days if the drain was kept in place. Systemic distribution of this local chemotherapy was observed, as can be seen from the blood levels of MTX. The release and diffusion of MTX from the cement was continued well beyond 10 days (when MTX could still be assayed in one patient), since urinary excretion continued for at least 3 weeks.

In the time of cisplatin, which has a lower rate of diffusion from the cement than methotrexate, a dose of 200 mg of cisplatin mixed with one packet of cement was used without any postoperative adverse haematological or renal effects being observed.

If further developments in the investigations we have initiated [7–9] confirm these early findings, this method of local neoplastic chemotherapy could offer an adjuvant therapy which is likely to be easier to handle. There is of course no question that this therapy could offer a substitute for systemic chemotherapy or radiotherapy when these therapies are indicated. Several other studies [29–32] have confirmed the experimental data of the diffusion of antimitotics from cement.

References

1. Buchholz HW, Engelbrecht H. Uber die Depotwirkung einiger Antibiotica bei Vermischung mit dem Kunstharz Palacos. Chirurg. 1970;41:511–5.
2. Carlsson AS, Josefsson G, Lindberg L. Revision with gentamicin impregnated cement for deep infections in total hip arthroplasties. J Bone Joint Surg Am. 1978;60-A:1059–64.
3. Elson RA, McGechie DB. Antibiotics and acrylic bone cement. J Bone Joint Surg [Br]. 1976;58-B:134.
4. Elson RA, Jephcott AE, McGechie DB, Verettes D. Antibiotics, loaded acrylic cement. J Bone Joint Surg [Br]. 1977;59-B:200–5.
5. Fischer L-P, Gonon G-P, Carret J-P, Vulliez Y, de Mourgues G. Association methacrylate de methyle (Ciment acrylique) et antibiotique: etude

bacteriologique et mecanique. Rev Chir Orthop. 1977;63:361–72 (Eng. abstr.).
6. Marks KE, Nelson CL, Lautenschlager EP. Antibiotic-impregnated acrylic bone cement. J Bone Joint Surg Am. 1976;58-A:358–64.
7. Hernigou P, Thiery JP, Benoit J, et al. Release of antimitotic drugs from acrylic cement and plaster. Eur Surg Res. 1987;19 Suppl 1:25.
8. Hernigou P, Thiery JP, Benoist M, et al. Etude experimentale sur l'osteosarcome d'une chimiotherapie locale diffusant a partir de ciment acrylique chirurgical et de platre. Rev Chir Orthop. 1987;73:517–25.
9. Hernigou P, Thiery JP, Benoit J, et al. Methotrexate diffusion from acrylic cement. Bone Joint Surg [Br]. 1989;71-B:804–11.
10. Hernigou P, Brun P, Thiery JP, et al. Antimitotic loaded acrylic cement. In: Langlais F, editor. Limb salvage. Berlin: Springer; 1991.
11. Hernigou P, Brun B, Autier A, et al. Osteosarcoma in adolescent and young adults. Boston: Kluwer Academic Publishers; 1993.
12. Hernigou P, Brun B, Astier A, et al. Diffusion of methotrexate from acrylic surgical cement. Cancer Treat Res. 1993;62:231–5.
13. Harrington KD, Sim FH, Enis JE, et al. Methylmethacrylate as an adjunct in internal fixation of pathological fractures: experience with three hundred and seventy-five cases. J Bone Joint Surg Am. 1976;58-A:1047–55.
14. Strube HD, Komitowski D. Experimental studies of the treatment of malignant tumors with bone cement. In: Enneking WF, editor. Limb salvage in musculoskeletal oncology. London: Churchill Livingstone; 1987. p. 459–69.
15. Brodey RS, Abt DA. Results of surgical treatment in 65 dogs with osteosarcoma. J Am Vet Med Assoc. 1976;168:1032–5.
16. Ling GV, Morgan JP, Pool RR. Primary bone tumors in the dog a combinated clinical, radiographic and histologic approach to early diagnosis. J Am Vet Med Assoc. 1974;165:55–67.
17. Misdorp W, Van der Heul RO. Tumours of bones and joints. Bull World Health Organ. 1976;53:265–82.
18. Owen LN. Cancer chemotherapy. Vet Ann. 1979;19:204–11.
19. Parodi AL. L'osteosarcome (sarcome osteogenigne) chez le chien. Chirurgie. 1970;96:75–80.
20. Pool RR. Bone and cartilage. In: Moulton JE, editor. Tumors in domestic animals. 2nd ed. Berkeley: University of California Press; 1978. p. 89–149.
21. Klein B, Pals S, Masse R, et al. Studies of bone and soft-tissue tumours induced in rats with radioactive cerium chloride. Int J Cancer. 1977;10:112–9.
22. Thiery JP, Perdereau B, Gongora R, Gongora G, Mazabraud A. Un modele experimental d'osteosarcome chez le rat: 11. L'osteosarcome greffable du rat. Sem Hop Paris. 1982;58:1686–9.
23. Baum ES, Gaynon P, Greenberg L, Krivit W, Hammond D. Phase II study of cis-dichlorodiammineplatinum (II) in childhood osteosarcoma: Children's cancer study group report. Cancer Treat Rep. 1979;63:9–10, 1621–1627.
24. Tsuchiya H, Tomita K, Yasutake H, Takagi Y, Katsuo S, et al. Intraarterial cisplatin and caffeine with/without doxorubicin for musculoskeletal high-grade spindle cell sarcoma. Oncol Rep. 1994;1:27–36.
25. Jaffe N, Keifer R, Robertson R, Cangir A, Wang A. Renal toxicity with cumulative doses of Cis-diamminedichloroplatinum-II in pediatric patients with osteosarcoma. Effect on creatinine clearance and methotrexate excretion. Cancer. 1987;59:1577–81.
26. Litterst CL, LeRoy AF, Guarino AM. Disposition and distribution of platinum following parenteral administration of cis-dichlorodiammineplatinum (II) to animals. Cancer Treat Rep. 1979;63(9–10):1485–92.
27. Straw RC, Withrow SJ, Douple EB, Brekke JH, Cooper MF, et al. Effects of Cis-diamminedichloroplatinum II released from D, L-polylactic acid implanted adjacent to cortical allografts in dogs. J Orthop Res. 1994;12:871–7.
28. Withrow SJ, Thrall DE, Straw RC, Powers BE, Wrigley RH, et al. Intra-arterial cisplatin with or without radiation in limb-sparing for canine osteosarcoma. Cancer. 1993;71:2484–90.
29. Janmin L. Experimental observations on acrylic bone cement containing antitumor drugs. Natl Med J China. 1989;69:143.
30. Marshall GJ, Kirchen ME, Lee JH, Menendez LA. The effect of methotrexate eluted from bone cement on giant cell tumour lines in vitro. Trans Orthop Res Soc. 1990;15:537.
31. Wang HM, Galasko CS, Crank S, Oliver G, Ward CA. Methotrexate loaded acrylic cement in the management of skeletal metastases. Clin Orthop. 1995;312:173–86.
32. Guan WY, Yi WT, Zhi MY, Zhen SS. Experimental research on the use of an antineoplastic drug with a bone implant. Int Orthop. 1990;14:387–91.