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Research paper

Cisplatin delivery by biodegradable polymer implant is superior to systemic delivery by osmotic pump or i.p. injection in tumor-bearing mice

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The use of biodegradable polymer implants to deliver cisplatin was compared with delivery by systemic injection and by osmotic pump. Drug levels in the tumor were found to be higher than those in the blood and kidney when the drug was delivered using the polymer implant. In contrast, for the other two delivery methods blood and kidney cisplatin levels were greater than those in the tumor. It has been previously shown that tumor response, in terms of growth delay, was greatest when drug was delivered by polymer implant and least when treatment was by osmotic pump. [© 1998 Lippincott Williams & Wilkins.]

Key words: Biodegradable polymer, cisplatin, drug delivery, RIF-1.

Introduction

The use of polymeric slow-release drug devices implanted at the treatment site has been suggested for prolonged release and localization of drugs to a particular site in the body.[1] One particular class of polymers, the polyanhydrides, has been used with some success for localizing drugs at tumor sites. One such polyanhydride based polymer is bis(*p*-carboxyphenoxy)propane sebacic acid (CPP:SA; in a 20:80 ratio) which has been extensively studied[2] and its degradation products found to be benign.[3] In experimental studies this co-polymer has been used as a vehicle for direct intratumoral delivery of taxol[4] and of buthionine sulfoximine (BSO) to potentiate the effects of 4-hydroperoxycyclophosphamide,[5] both studies being done in a rat model of malignant glioma. Clinically, phase I–III trials have been conducted of CPP:SA (20:80) for the delivery of BCNU intra-cranially in patients with malignant glioma.[6]

Our group has used the same polymer to produce a cisplatin-containing implant for the intratumoral delivery of the drug in the mouse fibrosarcoma, RIF-1. Using the polymer implant system, we were able to increase the dose of cisplatin delivered to the tumor 4-fold compared to that delivered systemically without any adverse effects or observable systemic toxicities in the mouse.[7] Tumor growth delay (TGD), which was used as the endpoint in these studies, was significantly increased for mice treated with cisplatin-polymer implant compared to that for control mice which received either no treatment or cisplatin delivered by systemic injection.[7] Studies examining the interaction, in terms of tumor control, between radiation and concurrent delivery of cisplatin using the biodegradable polymer implants have also been completed and are published elsewhere.[8]

Initial findings on the distribution of cisplatin when the drug was delivered either systemically or via the polymer implants have been presented.[13] In this paper, we compare levels of cisplatin found in the blood, in the tumor and, since nephrotoxicity is an important side effect of chemotherapy involving cisplatin, in the kidney at intervals after the drug was delivered via intratumoral polymer implant, osmotic pump implanted in the i.p. cavity or i.p. injection.

Materials and methods

Tissue culture

The procedures for maintaining the RIF-1 cell line and growing tumors have been published previously.[7] Briefly, cells were passaged using standard tissue

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culture techniques in α modification of MEM media supplemented with 10% fetal bovine serum and 1% antibiotic-mycotic (all supplied by Gibco/BRL, Burlington, Ontario, Canada). Tumors were initiated by s.c. injection of 2×10^5 cells into the backs of C3H mice (female, 6 weeks old, 20 g; Charles River). Tumors appeared within 10 days and reached a volume of 94–130 mm^3 within 3 weeks.

Polymer implants

The CPP:SA polymer was synthesized according to the procedure published in the literature. Briefly, 1,2-bis(*p*-carboxyphenoxy)propane (CPP) was first synthesized from *p*-hydroxybenzoic acid and 1,3-dibromopropane in a basic aqueous medium. The prepolymer of sebacic acid and CPP were then prepared by reaction with acetic anhydride. The final polymer product was prepared from a mixture of the prepolymers (molar ratio of 80:20, SA:CPP) in a polycondensation reaction carried out at about 200–220°C *in vacuo*. The crude product was purified by precipitation ($CHCl_3$/petroleum ether) and finally washed with diethyl ether before being dried under vacuum.

Cisplatin administration

Biodegradable polymer implant. The procedure for making the cisplatin-polymer implants has been described. Essentially, the polymer was mixed (17%w/w) with cisplatin (Sigma, St Louis, MO) and ground into a homogenous, fine powder. The mixture was heated to 70–80°C and extruded through a 'combi-tip' (Eppendorf, Madison, WI). The polymer-cisplatin rods were cooled and stored in a desiccator until required. The dimensions of the rods used were 8×0.5 mm. For implantation the mouse was anesthetized and the rod inserted into the tumor after the skin has been pierced by a hypodermic needle. The total dose of cisplatin delivered was 25–30 mg/kg over 8–10 days.

Intraperitoneal injection. Cisplatin dissolved in saline was injected i.p. (7 mg/kg) in a volume of 100 μl (the LD_{50} for cisplatin in C3H mice is 10 mg/kg[9]).

Osmotic pump. Cisplatin dissolved in saline (1.56 mg/ml) was loaded into osmotic pumps (Alzet Pumps, Palo Alto, CA) of maximum volume 100 μl. The total dose of cisplatin delivered over 7 days was 0.156 mg (6.5–7.5 mg/kg). The pumps were loaded under sterile conditions and placed in the i.p. cavity of the mouse using standard sterile surgical procedures. No adverse effects were seen in the mice and surgical wounds healed within 24 h. The pump was constructed from a semi-permeable and rigid material shaped into a sealed tube with a pore at one end. Mouse body fluids diffuse through the walls into the pump and displace the drug solution forcing it out of the pore. The pumps used were designed to release drug continuously for 7 days.

Experimental design

When tumors reached the treatment size (100 mm^3), mice were divided into groups of three for treatment by polymer implant, osmotic pump or i.p. injection. Mice treated with the polymer implant were sacrificed at 1, 2, 4, 8 and 12 days, while mice given cisplatin systemically were sacrificed after 1, 2, 4 and 8 days. Mice implanted with the osmotic pumps were sacrificed at days 4 and 8. It was not possible to extend the study times for the groups treated by injection or with the osmotic pump for longer than 8 days because the tumors were not controlled by the treatment and grew to a size which required that the mouse be sacrificed.

Determination of Pt

The procedure has been published previously.[7] Briefly, mice were sacrificed at intervals, and their blood, tumor and kidneys harvested. The blood samples were centrifuged to collect the serum which was analyzed directly. Tumor and kidney samples were digested enzymatically in a buffered solution and the appropriate dilutions made prior to analysis for platinum (Pt). Atomic absorption spectroscopy (AAS) was used to determine the level of Pt present in the samples (details published elsewhere[7]) and these results were used as an index of the amount of drug in the sample. Analytical results were expressed as μg of Pt per g of tissue (or ml of blood) per mg of cisplatin administered.

Results

Tumor response was assessed using TGD, which was defined as the time required for the tumor to grow to four times its volume at the time of treatment (five to six mice per experimental group). The time for untreated control tumors to reach the endpoint was 5.7 ± 0.7 days.[7] The longest TGD was seen when the cisplatin-containing polymer implants were used (16.1 ± 1.9

days). The difference between this and the increase in TGD produced by i.p. injection of cisplatin (9.5 ± 1.7 days) was extremely significant ($p < 0.0001$). Tumors treated with cisplatin delivered by osmotic pump showed no increase in TGD over the control growth.[8]

The Pt levels found in tumor tissue, kidney and blood at intervals following i.p. injection are shown in Figure 1. Elimination of Pt from blood and tissue has been reported to be biphasic with an initial rapid phase during the first hour after injection followed by a slow phase over the next 7 days.[10] Figure 1 shows the second phase of Pt elimination between 1 and 8 days after injection. During this period the clearance half-lives of Pt for serum, kidney and tumor were 55, 67 and 38 h, respectively. Lowest Pt levels were seen for the tumor and by 8 days after injection Pt was not detectable in tumor tissue.

Figure 2 shows the level of Pt in serum and kidney (Figure 2a) and in tumor (Figure 2b) following cisplatin-polymer implant. The Pt level in the tumor is significantly higher than that in the kidney at all sampling times, and remains relatively constant between 24 and 96 h at around 20 μg Pt/g tissue. In other terms, the level of cisplatin maintained in a tumor of 0.5 g over this 4 day period is equivalent to approximately 2% of that present in the initial polymer implant. Between 4 and 8 days after implant there is a dramatic increase in the amount of Pt found in the tumor to 194 μg Pt/g tissue/mg cisplatin administered at 8 days. For an 0.5 g tumor this implies that, at the time of sampling, 20% of the cisplatin of the polymer implant was present in tumor tissue. The very high level of Pt seen in the tumor at 192 h after polymer implant may be attributable to reduction in polymer implant size to a point at which drug release is no longer determined by degradation of the polymer but by dissolution and leakage of the drug from what is left of the polymer infrastructure. In *in vitro* studies we have observed that degradation of the polymer surface eventually leads to increasingly connected pore structures and below a certain critical volume the interconnected pore network becomes the dominant pathway for drug loss.[7] A similar model has been described by Chui *et al.* to describe the degradation of poly(*dl*-lactide-*co*-glycolide).[11] Another factor which may contribute to the increase of the concentration of the drug in the tumor at longer times after polymer implant, is the reduction in tumor volume which occurs as tumors are being treated with the implant.

The distribution of Pt in serum and kidney of animals in which cisplatin-polymer is implanted in the tumor is related to events which take place in the tumor. Serum levels of Pt are highest at 48 h post-implant and then decline to a point where Pt cannot be detected in serum. Levels of Pt in the kidney increase at each sampling time after polymer implant to approximately 12 μg/g tissue/mg cisplatin adminis-

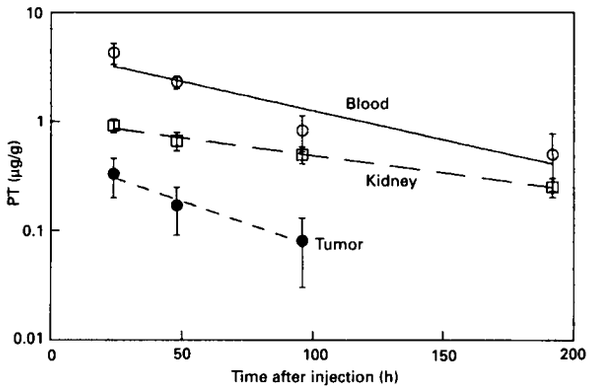

Figure 1. Pt levels in serum, kidney and tumor following i.p. injection of cisplatin (7 mg/kg) to tumor-bearing mice.

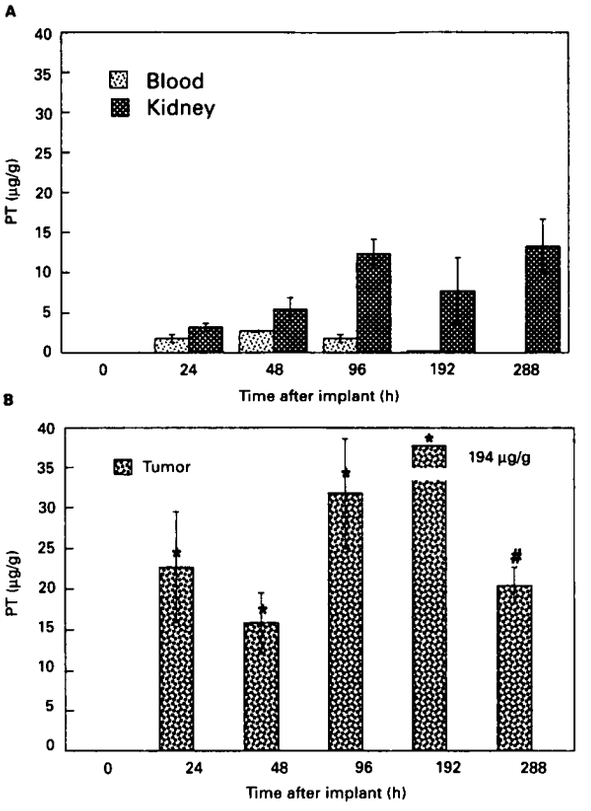

Figure 2. Pt levels in tumor tissue, kidney and serum when cisplatin is delivered by implanted cisplatin–polymer. (A) Levels in serum and kidney. (B) Levels in tumor tissue. *Pt levels in tumor very significantly greater than Pt levels in the kidney ($p < 0.01$). #Pt levels in tumor significantly greater than Pt levels in the kidney ($p < 0.05$).

tered by 4 days after implant and remain around this level for up to 12 days after implant. The levels in the kidney presumably increase as drug is cleared from the tumor which is acting as a reservoir for cisplatin.

Figure 3 shows the distribution of Pt after cisplatin is released by the i.p. implanted osmotic pump at 4 and 8 days after implantation of the pump. Compared with administration by injection Pt levels were higher in all compartments with particularly high levels in the kidney, comparable to those seen at the same time following cisplatin-polymer implant. Tumor levels of Pt were higher than those seen after i.p. injection but much lower than those seen following polymer implant.

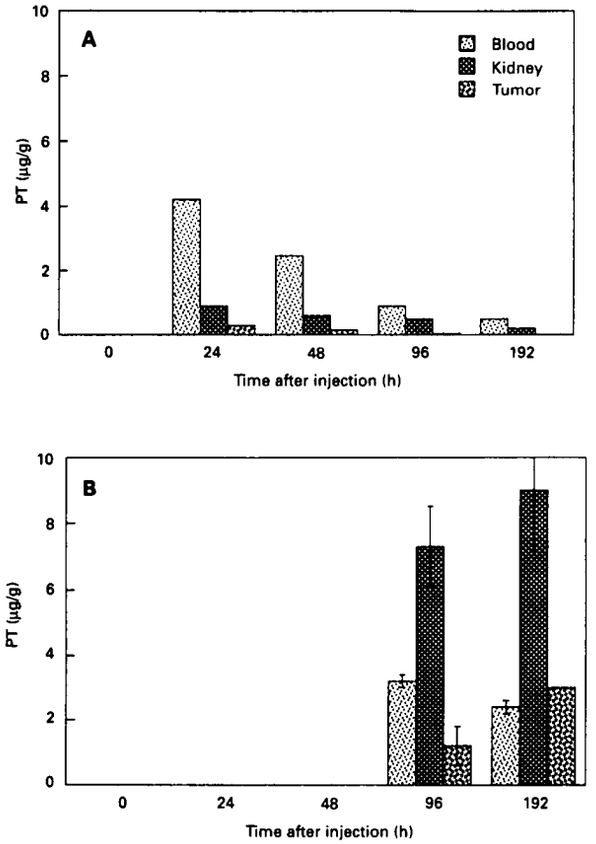

Figure 3. Comparison of Pt levels in serum, kidney and tumor tissue when cisplatin is delivered by systemic administration. (A) Intraperitoneal injection (B) Intraperitoneally implanted osmotic pump.

Discussion

We have investigated the levels of cisplatin present in the tumor, kidney and blood for the maximum drug load which could be delivered by each method. The three methods used in this study cannot, however, be compared directly because of the difference in drug loading and method of administration. The polymer implants carry the most cisplatin (0.5–0.6 mg per mouse, equivalent to 20–24 mg/kg for a 25 g mouse). This is released directly in the tumor as the polymer matrix degrades slowly in the tumor environment. For systemic delivery the drug doses were limited by the relative insolubility of cisplatin in aqueous solution and, in the case of the osmotic pump, by the volume (100 µl). The two methods deliver similar amounts of drug into the peritoneal cavity, but in one case delivery is instantaneous whereas the osmotic pump releases drug over 7 days. Characteristics of the three drug delivery methods and the time at which maximum levels of Pt are detected in kidney and tumor are shown in Table 1.

When the drug is delivered via the polymer implant, Pt levels in the tumor are significantly higher than those found in the blood or kidney (Figures 2a and b). Drug levels in the tumor remain relatively constant for sampling times of 1, 2 and 4 days but there is a dramatic increase observed at 8 days followed by decreasing levels over the next 96 h. We hypothesize

Table 1. Doses of cisplatin administered and maximum levels of Pt measured in tissue

Mode of administration	Pt: distribution and rate of release	Dose/mouse (mg)	Dose (mg/kg)	Pt (µg/g tissue/mg cisplatin administered) ± SD	
				Kidney	Tumor
Cisplatin–polymer implant	intratumoral; released over 12 days	0.68	27.7	13.2 (12)[a] ± 3.4	192 (8) ± 9.0
Intraperitoneal injection	systemic; rapidly distributed	0.175	7.0	0.9 (1) ± 0.13	0.3 (1) ± 0.13
Intraperitoneal osmotic pump	systemic; released over 7 days	0.156	6.2	9.1 (8) ± 1.8	3.0 (8) ± 0.6

[a]Numbers in parentheses: day on which maximum level was observed.

that during the first several days drug release is due to degradation of the polymer matrix from the surface resulting in diffusion of the drug out into the surrounding tissue. When the implant is reduced below a certain critical size the rate of drug release is no longer dependent on the rate of polymer degradation and occurs by passive leakage of the drug from the remains of the polymer infrastructure. The subsequent decrease in Pt levels occurs as drug is cleared from the tumor which may, by this time, have shrunk to only 30% of its original volume. The implant is completely degraded by 12 days and no traces of it can be found in the excised tumors.

During and after the dissolution of the polymer a high level of cisplatin is released into the tumor and subsequently distributed systemically. At this time a relatively high level of Pt is observed in the kidney, exceeding that observed for systemic or osmotic pump delivery of cisplatin. This is a disturbing observation since the aim of implanting the polymer for drug delivery is (i) to increase the level of drug in the tumor, which is realized, and (ii) to avoid normal tissue toxicity by limiting the amount of drug in tissues other than the tumor. It seems likely, however, that this problem is a function of the experimental model rather than being inherent to the polymer-based drug delivery system. Adaptation of this technology for clinical application will require the use of much larger polymer implants than those used in the mouse with the possibility of varied geometries. In this case, the surface:volume ratio of the implant would be a factor which modulates the rate at which drug is released in addition to the proportions of the constituent co-polymers. As described above, degradation of the polymer from the surface eventually reduces the polymer to a critical size below which the drug leaks out passively through the pores which are now interconnected with each other and with the outside. In the case of a large human tumor implanted with proportionally sized polymer implants this critical size will not be attained until very late in treatment and the 'burst' of drug released will be small in relation to body size and so extensively diluted in the blood as to make little or no difference to the level of cisplatin retained by the kidney and other organs. Under these circumstances it is unlikely that kidney or other toxicities will be a problem following intratumoral implant of cisplatin-polymer. We are currently investigating the relationship between polymer size and geometry and the rate of drug release.

For osmotic pump-implanted mice Pt levels in the tumor were higher at 96 and 192 h than those found in tumors treated with systemic injections analyzed at the same time points. Tumor response in terms of increase in TGD was greater for treatment by injection than by osmotic pump despite the lower levels of Pt found in the tumor at the time of sampling. Presumably, following i.p. injection there is a short period of tumor exposure to high levels of cisplatin which is more effective in terms of cell kill than is a prolonged exposure to lower levels obtained with the osmotic pump. By 96 h after osmotic pump implant when appreciable levels of Pt are detected in tumor tissue the tumor has grown to an extent such that it cannot be controlled. This suggests that systemic slow delivery methods, such as the osmotic pump, are likely to be more effective against slow growing tumors. At 4 and 8 days after osmotic pump implant kidney levels of Pt are high and comparable with levels seen at the same times following cisplatin-polymer implant. Thus, under these experimental conditions the osmotic pump has an unfavorable therapeutic ratio combining no effect on tumor response with high drug accumulation in normal tissue. Douple *et al.*[12] have reported significant therapeutic effects when MTG-B (a murine mammary adenocarcinoma) tumors were treated using pumps with a capacity of 200 μl to deliver cisplatin continuously although systemic toxicity was higher than expected. This suggests that an increase in the amount of cisplatin delivered by the osmotic pump could improve the anti-tumor activity but that normal tissue toxicity, as suggested by our results, would be dose limiting.

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References

1. Langer RJ. New methods of drug delivery. *Science* 1990; **249**: 1527-33.
2. Leong KW, Brott BC, Langer R. Bioerodible polyanhydrides as drug-carrier matrices. Characterization, degradation, and release characteristics. *J Biomed Mater Res* 1985; **19**: 941-55.
3. Leong KW, D'Amore P, Marletta M, Langer R. Bioerodible polyanhydrides as drug carrier matrices. II Biocompatibility and chemical reactivity. *J Biomed Mater Res* 1986; **20**: 51-64.
4. Brem H, Piantadosi S, Burger PC, *et al.* Placebo-controlled trial of safety and efficacy of intra-operative controlled delivery by biodegradable polymer of chemotherapy for recurrent gliomas. *Lancet* 1995; **345**: 1008-12.

5. Walter KA, Cahan MA, Gur A, *et al.* Interstitial taxol delivered from a biodegradable polymer implant against experimental malignant glioma. *Cancer Res* 1994; **54**: 2207–12.
6. Sipos EP, Witham TF, Ratan R, Baraban J, Brem H. Buthionine sulfoximine potentiates the antitumor effect of 4-hydroperoxycyclophosphamide when delivered locally in the intracranial 9L-glioma model. *Proc Am Ass Cancer Res* 1995; **36**: 308.
7. Yapp DTT, Lloyd DK, Zhu J, Lehnert SM. Tumor treatment by sustained intra-tumoral cisplatin: effects of drug alone and combined with radiation. *Int J Radiat Oncol Biol Phys* 1997; **39**: 497–504.
8. Yapp DTT, Lloyd DK, Zhu J, Lehnert SM. Potentiation of radiation treatment by intra-tumoral delivery of cisplatin using a biodegradable polymer implant on a mouse tumor model (RIF-1). *Int J Radiat Oncol Biol Phys* 1998; in press.
9. Twentyman PR, Brown JM, Gray JW, Franko AJ, Scoles MA, Kallman RF. A new mouse tumor model system (RIF-1) for comparison of end-point studies. *J Natl Cancer Inst* 1980; **64**: 595–604.
10. Johnsson A, Olsson C, Nygren O, Nilsson M, Seiving B. Cavallin-Ståhl E. Pharmacokinetics and tissue distribution of cisplatin in nude mice: platinum levels and cisplatin-DNA adducts. *Cancer Chemother Pharmacol* 1995; **37**: 23–31.
11. Chiu LK, Chiu WJ, Cheng Y-L. Effects of polymer degradation on drug release a mechanistic study of morphology and transport properties in 50:50 poly(*dl*-lactide-*co*-glycolide). *Int J Pharmacol* 1995; **126**: 169–78.
12. Douple EB, Wills ML, Jones EL. Radiopotentiation in a murine tumor (MTG-B) by continuous infusion platinum. In: Rotman M, Rosenthal CJ, eds. *Concomitant continuous infusion chemotherapy and radiation*. New York: Springer-Verlag 1985: 191–6.

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