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A vanadium/aspirin complex controlled release using a poly(β-propiolactone) film. Effects on osteosarcoma cells

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Abstract—A delivery system for vanadium was developed using poly(β-propiolactone)(PβPL) films. The release kinetics of a complex of vanadium (IV) with aspirin (VOAspi) was evaluated with films prepared from polymers of different molecular weights, as well as with variable drug load. A sustained release of vanadium over 7 days was achieved. The drug release kinetics depends on contributions from two factors: (a) diffusion of the drug; and (b) erosion of the PβPL film. The experimental data at an early stage of release were fitted with a diffusion model, which allowed determination of the diffusion coefficient of the drug. VOAspi does not show strong interaction with the polymer, as demonstrated by the low apparent partition coefficient (approximately 10^{-2}). UMR106 osteosarcoma cells were used as a model to evaluate the anticarcinogenic effects of the VOAspi released from the PβPL film. VOAspi–PβPL film inhibited cell proliferation in a dose-response manner and induced formation of approximately half of the thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, compared to that with free VOAspi in solution. The unloaded PβPL film did not generate cytotoxicity, as evaluated by cell growth and TBARS. Thus, the polymer-embedded VOAspi retained the antiproliferative effects showing lower cytotoxicity than the free drug. Results with VOAspi–PβPL films suggest that this delivery system may have promising biomedical and therapeutic applications.

Key words: Poly(β-propiolactone); diffusion model; vanadium; osteosarcoma cells; cell proliferation; antineoplastic; lipid peroxidation; sustained delivery.

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INTRODUCTION

During the past decade, various controlled release drug delivery systems based on polymeric materials have been developed for biomedical use [1, 2]. Intravenous or oral use of some of these delivery systems could lead to undesirable toxicity or reduced bioavailability of the drug [3]. Implantable drug delivery systems, on the other hand, could be an alternative to optimize the therapeutic profile of specific drugs. However, these devices should fulfill several criteria for biomedical applications, such as biocompatibility, controlled biodegradation of the polymer, lack of local and systemic toxicity, and a sustained delivery of the drugs.

Vanadium compounds have been investigated as alternative therapeutic agents for use in diabetes. However, toxic effects of these agents after chronic use have been reported [4–6]. For these reasons, there is great interest in the synthesis of new vanadium derivatives with different ligands such as vanadium (IV) complexed either with maltol [7] or with aspirin (VOAspi) [8]. In mammals, vanadium is mainly stored in bones and kidney [9]. Using an *in vitro* model of osteoblast-like cells in culture, we have previously shown that vanadium exerts a biphasic effect: while low concentrations stimulate, high doses induce inhibition of osteoblast growth and differentiation, as well as morphological transformations [5, 6, 10–13]. In particular, VOAspi seems to be more potent than uncomplexed vanadyl salt in inhibiting the growth of osteoblast-like cells [8].

In addition, the antineoplastic effects of vanadium have been demonstrated for both *in vitro*, and *in vivo* models [14–16]. In order to avoid systemic toxicity, Jackson *et al.* [14] developed a device for vanadium release from a poly(ε-caprolactone) paste that was effective in the prevention of re-growth of tumors in laboratory animal models. Using these types of devices for delivery, organic–vanadium complex could be conveniently delivered in a controlled fashion, thus resulting in reduced toxicity and better therapeutic effectiveness than a bolus administration.

The poly(β-propiolactone) (PβPL) used in the present study has been synthesized and previously physicochemically characterized in our laboratory [17, 18]. This polymer is structurally similar to those widely used in controlled release systems, such as the polyglycolic and polylactic copolymers that are biodegradable and biocompatible polyesters [3]. However, no information on PβPL is available on its possible biomedical application.

In this study, we have developed a delivery system for VOAspi using PβPL polymeric films. In a series of *in vitro* studies, we have evaluated the delivery kinetics of VOAspi complex from PβPL films of different average molecular weights, as well as the effect of the drug loading in the polymeric films. In addition, we have investigated the effects of controlled release of vanadium on the proliferation, lipid peroxidation, and morphological alterations of UMR106 osteosarcoma cells in culture.

MATERIALS AND METHODS

Materials

Chloroform, methanol, vanadium chloride, and vanadium (IV) oxide sulfate (vanadyl sulfate) were provided by Merck (Darmstadt, Germany), and used without further purification. Tissue culture flasks and multiwell plates were purchased from Corning (Princeton, NJ, USA); Dulbecco's Modified Eagles Medium (DMEM) and trypsin-EDTA were purchased from Gibco (Gaithersburg, MD, USA). Fetal bovine serum (FBS) was obtained from Gen (Buenos Aires, Argentina). A complex of vanadyl with aspirin (VOAspi) was synthesized and characterized as previously described [8]. Briefly, aspirin and $VOCl_2$ in a 2 : 1 ratio were mixed in 96% ethanol, the green oil formed was successively washed with water, and the resulting solid was filtered and dried until constant weight.

Synthesis and characterization of poly(β-propiolactone)

The synthesis and characterization of PβPL have been previously reported [17, 18]. Table 1 shows the relevant properties of the polymer samples used in this study. The changes in the molecular weight and molecular weight distribution were followed by size exclusion chromatography (SEC) in a LKB-2249 instrument at 25°C. A series of μ-Styragel columns, ranging in pore size 10^6, 10^5, 10^4, 10^3 Å, were used with chloroform as an eluent. The polymer concentration was 4–5 mg ml^{-1} and the flow rate was 0.5 ml min^{-1}. The polymer was detected with a differential refractometer detector (Waters R401) and calibration was done by the standard procedure, as described previously [17, 18].

Preparation of the cast films

Drug-containing PβPL films were prepared by solvent casting. A solution of the PβPL polymer in chloroform (5% w/w) was prepared and different amounts (10–30% w/w) of the VOAspi were added to the polymer solution. Aliquots of the clear solution of the drug-containing polymer were poured onto glass Petri dishes (5 cm diameter), and the solvent was allowed to evaporate at room temperature for several days. The resulting films were dried under vacuum until constant weight, and stored in a desiccator until use. The thickness of the films (50–100 μm) was measured using a micrometer.

Table 1.
Physicochemical properties of the PβPL polymers

Sample	Intrinsic viscosity, $[\eta]$ (ml g^{-1})	Viscosity average molecular weight, M_η (g mol^{-1})	Polydispersity index (M_η/M_n)
PβPL-43	55.9	42 700	1.4
PβPL-51	63.1	51 300	2.0
PβPL-112	112.0	112 200	1.4

Infrared spectra of the VOAspi–PβPL films and VOAspi in a KBr pellet were recorded on a Perkin Elmer 580B spectrophotometer. The characteristic infrared bands previously described for the drug [8] were also observed in the VOAspi–PβPL films, indicating that no chemical structural changes in the VOAspi had occurred after its incorporation into the polymeric film.

Vanadium release kinetic studies

Drug release experiments with film samples (5 cm diameter) were carried out in tubes containing 5 ml of 0.1 M phosphate buffer (PBS) (pH 7.4) and 100 μg ml^{-1} bovine serum albumin, at 37°C. At appropriate times (every 15 min during the initial 2 h, then every hour until 5 h, and finally every day for 7 days), the supernatants were removed and replaced by 5 ml of fresh buffer. The time-dependent release of the drug was followed by monitoring the amount of VOAspi present in the supernatant medium, using a CECIL 2000 UV-visible spectrophotometer ($\lambda_{max} = 295$ nm). A linear calibration curve of VOAspi concentration vs absorbance at 295 nm was obtained using VOAspi standards in the range 0–50 μg ml^{-1}. At the end of the drug-release experiments, samples of the films were recovered to evaluate possible changes in the molecular weight distribution of the PβPL polymer.

Water content of PβPL films

The maximum swelling and water absorption capacity of the polymeric films were determined as follows. The PβPL films were weighed (w_0) and then immersed in the PBS buffer at 37°C for predetermined times. After wiping the surface with paper, the films were weighed in the wet state (w). Then the films were dried in vacuum overnight at room temperature and weighed in the dried state. The weight of the final dry film was w_d. The water content of the film was obtained as the difference between w, the weight of the water saturated film and w_0, the weight of the initial dried film. The percentage of swelling of the film is defined as: $\%S_w = 100(w - w_0)/w_0$.

VOAspi–PβPL interaction

The interaction between VOAspi and the PβPL polymer was estimated through the apparent partition coefficient of the drug (K_{app}), according to the procedure of Miyajima [19]. K_{app} was determined from the initial and equilibrium drug concentration in the medium (C_0 and C_e, respectively) after incubation of the drug loaded films in the PBS buffer for 24, 48, or 72 h using Eq. (1):

$$K_{app} = 10(C_0 - C_e)/wC_e, \tag{1}$$

where w is the weight of the film after immersion in the buffer.

Drug release kinetic analysis

The temporal dependence of the VOAspi released at time t, from the film of thickness $2L$ was described by two different unidimensional diffusion models [20]. At the initial time, $t = 0$, the drug concentration was assumed to be constant throughout the film.

Model 1. At $t > 0$, it was assumed that the drug concentration in the liquid near the surface of the film, C_{sup}, was higher than the concentration in the surrounding environment, C_∞ ($C_{sup} > C_\infty$). This condition allowed us to take into account an additional mass transfer resistance from the aqueous layer near the surface of the film.

Model 2. At $t > 0$, it was assumed that $C_{sup} = C_\infty$. This condition represents an ideal situation indicating an instantaneous uniform distribution of the drug throughout the aqueous media.

The cumulative amount of drug released at time t, M_t, in the above two models is mathematically expressed by:

$$M_t/M_\infty = 1 - \sum_{n=1}^{\infty} \frac{2G^2}{\beta_n^2(\beta_n^2 + G^2 + G)} \exp\left(-\frac{\beta_n^2 D}{L^2} t\right), \tag{2}$$

where β_n values are the positive roots of:

$$\beta_n \operatorname{tg}\beta_n = G = L/(D/h), \tag{3}$$

and M_∞ represents the cumulative amount of drug released at infinite time, which is considered as constant. D is the diffusion coefficient of the drug, h is the mass transfer coefficient in the boundary layer, and the ratio D/h is the thickness of the boundary layer. This parameter vanishes in the Model 2 but was a finite value in the Model 1.

Cell culture and incubations

UMR106 rat osteosarcoma-derived cells were grown in 75-cm^2 flasks at 37°C in a humidified 5% CO_2 atmosphere in DMEM supplemented with 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10% (v/v) fetal bovine serum. When 70–80% confluence was reached, cells were sub-cultured using 0.1% trypsin–1 mM EDTA in Ca^{+2}–Mg^{+2}-free PBS [10, 11]. For experiments presented in this study, cells were seeded in six-well plates at a density of 2.5×10^4 cells per well, the medium was replaced by serum-free DMEM and cells were incubated for 24 h with different concentrations of VOAspi, vanadium–PβPL films, or medium alone (basal control), under the conditions described in the legends of figures. Under these conditions, FBS does not interfere with the measurement of VOAspi in the medium. However, the growth factors present in the serum could alter the effect of vanadium

on cell proliferation. Control cells did not show any apparent alterations after being serum starved for 24 h.

Studies of cell growth

In order to determine cell growth, the monolayers were washed twice with PBS, fixed with methanol for 5 min at room temperature, and stained with a solution of Giemsa for 10 min [13]. The plates were then washed with water and the cell number evaluated by counting the stained nuclei in ten fields per well. The mitotic index was defined as the number of mitotic figures per field over the total number of cells per field.

Lipid peroxidation

To measure the extent of lipid peroxidation, the production of thiobarbituric acid reactive substances (TBARS) was evaluated as previously reported [5] by using the method described by Ohkawa *et al.* [21]. Protein content in the cell extract was assessed by the method of Bradford [22]. The lipid peroxide levels were expressed in terms of nmol of malondialdehyde per mg protein, using 1,1,3,3-tetramethoxypropane as the standard.

Statistical analysis

For each experimental condition, at least three separate experiments were performed in triplicate. Data were expressed as the mean ± SEM. Statistical differences were analyzed using a Student's t-test.

RESULTS AND DISCUSSION

VOAspi release kinetics

Figure 1 shows the time course of the percent release of vanadium compound from the PβPL film containing various concentrations of the drug. The time was normalized by dividing the square of the sample half-thickness, L^2, to account for differences in sample thickness when comparing data from multiple samples. The drug release profile of the films containing 10–23% w/w of VOAspi was comparable. However, the film containing 30% w/w drug showed relatively faster initial release of VOAspi. The kinetic analysis shows initially a fast release rate, followed by a constant rate of release over 7 days.

The experimental data were fitted with the diffusion models with three and two parameters (models 1 and 2, respectively). All the data showed the same trend. Within experimental error, both models confirm the results for a diffusion time period of time zero up to 2 h. Figure 2 shows the results obtained with a PβPL-51 film with a half thickness of 50 μm. Model 1 predicts a diffusion coefficient

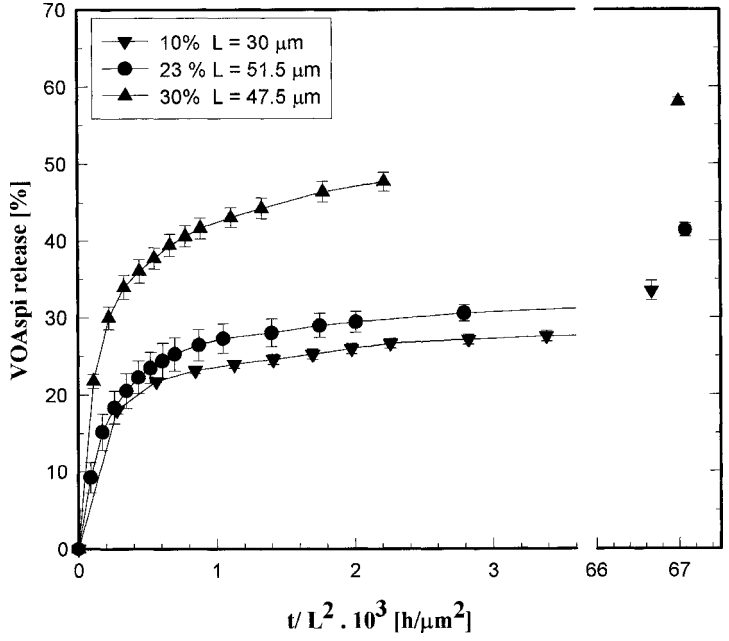

Figure 1. Effect of drug content on VOAspi release from PβPL-51 film at 37 °C.

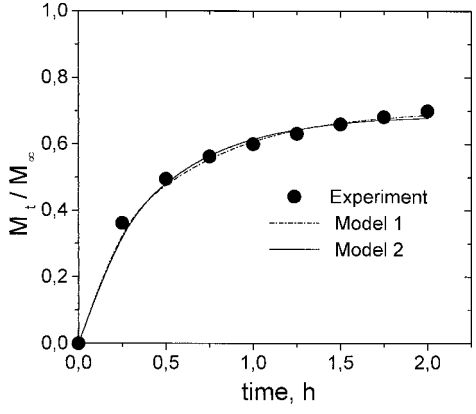

Figure 2. Fractional drug release up to 2 h. Sample: PβPL-51, 30% VOAspi, $L = 50\ \mu$m. Parameters are: $D = 0.48 \times 10^{-10}$ cm^2 s^{-1}, $D/h = 4.8\ \mu$m (model 1); $D = 0.58 \times 10^{-10}$ cm^2 s^{-1} (model 2).

$D = 0.48 \times 10^{-10}$ cm^2 s^{-1} and a thickness of the boundary layer $D/h = 4.8\ \mu$m, i.e. one tenth of the film thickness. A zero value of this layer (Model 2) predicts a higher diffusion coefficient $D = 0.58 \times 10^{-10}$ cm^2 s^{-1}, as expected from physical considerations.

For longer release times, up to 5 h, Model 1 provides a better fit of the experimental data than Model 2 and the corresponding fit parameters are $D = 0.30 \times 10^{-10}$ cm^2 s^{-1}, $D/h = 5.8\ \mu$m (Fig. 3). Nevertheless, at these longer time

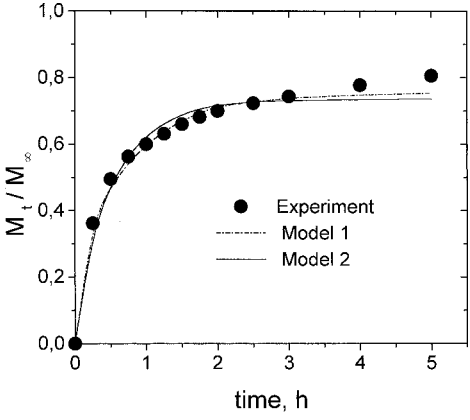

Figure 3. Fractional drug release up to 5 h. Sample: PβPL-51, 30% VOAspi, $L = 50\ \mu$m. Parameters are: $D = 0.30 \times 10^{-10}$ cm^2 s^{-1}, $D/h = 5.8\ \mu$m (Model 1).

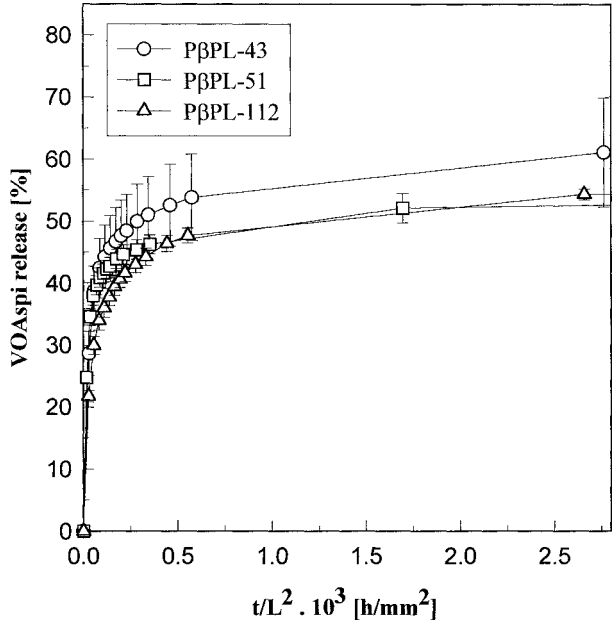

Figure 4. Effect of the molecular weight of PβPL on VOAspi release at 37 °C. The drug concentration was 30% (w/w) in all polymeric preparations.

periods, the one-dimensional model achieved a constant plateau while experimental data showed a monotonic increase of the accumulative release mass, suggesting the existence of complementary effects on the release.

The effect of the molecular weight of PβPL on the drug release profile is shown in Fig. 4. The concentration of drug in all polymer film samples was kept constant at 30% (w/w). No significant effect on the molecular weight of the polymer in the range of 4×10^4 to 1.2×10^5 g mol^{-1} was found on the release kinetics. A similar

Table 2.
Effect of the time of degradation of PβPL-51 on the molecular weight averages and polydispersity index

PβPL-51	0 day	2 days	7 days
Number average molecular weight (M_n)	25 270	23 220	13 730
Viscosity average molecular weight M_η	51 280	48 430	32 080
Polydispersity index (M_η/M_n)	2.0	2.1	2.3

effect was observed by Wang [23] on aspirin release from poly(lactic-glycolic acid) of low molecular weight (approximately 10^3 g mol^{-1}), although, significant dependence of the drug release on the molecular weight of this kind of polyesters has been reported by other investigators [24].

In order to assess the physical and chemical erosion of the PβPL films, the molecular weight distribution of the polymer was analyzed after incubation of the drug loaded films in PBS buffer. The chromatographic analysis (data not shown) demonstrated that for up to the first 2 days of incubation in PBS buffer, no significant changes in the molecular weight distribution of the polymer were observed, suggesting that under these conditions, minimal degradation of the polymer occurred. Table 2 shows the changes in different molecular weight averages and the calculated polydispersity indexes. After the first 2 days, the molecular weights were decreased and the polydispersity index increased, due to the random nature of the hydrolytic degradative mechanism.

Water content

Figure 5 shows the swelling behavior of the unloaded PβPL film at 37°C in PBS buffer pH 7.4, which attained equilibrium after 1.5 h. Although, the water content of PβPL films is lower than the water content of other similar polyesters [25], it is in agreement with other unloaded systems, where there is no osmotic effect [26]. The early-time phase of water transport in the polymer film is shown in the insert of Fig. 5, and verifies a power law behavior [27]:

$$\%S_w = kt^n, \tag{4}$$

where S_w is the percentage of swelling of the film, k is a constant, n is a diffusional exponent that determines the transport mechanism, and t is the time of the swelling. The n exponent was obtained as $n = 0.49 \pm 0.03$, which indicates a Fickian water transport mechanism similar to other hydrophobic polymers [27].

No changes were observed in the dry weight of these film samples (w_d) during the 8 h of the swelling experiment. This observation is in agreement with the size exclusion chromatography analysis displayed in Table 2.

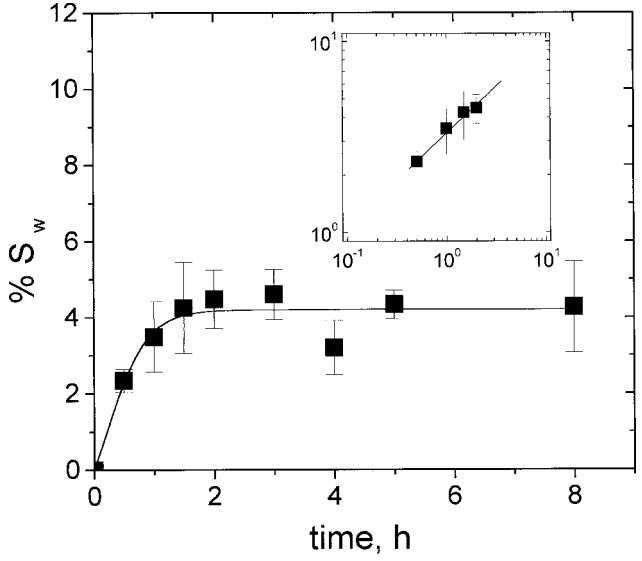

Figure 5. Percentage of swelling of a PβPL sample as a function of time at 37°C. Insert: Log–log graph at initial stage.

VOAspi–PβPL interaction

The possible VOAspi–PβPL interaction was evaluated by measuring the apparent partition coefficient at neutral pH. The K_{app}, obtained after 1 to 3 days of incubation at 37°C in a diluted VOAspi solution, was of the order of 10^{-2}. This result was anticipated from the neutral nature of the VOAspi compound and the fact that no relevant PβPL erosion was observed before 2 days, suggesting a lack of strong drug–polymer interaction.

Effect of VOAspi-released from PβPL films on osteoblast growth

In this study UMR106 osteosarcoma cells were used as a model to evaluate the anticarcinogenic effects of the VOAspi-released from the PβPL film. After overnight incubation in a serum-free media with or without vanadium, cells were fixed, stained with Giemsa, and examined by light microscopy (Fig. 6). The control cells (media alone) showed a polygonal morphology with well stained nuclei and cytoplasms with processes connecting neighboring cells (Fig. 6A). Several mitotic figures were also observed. A solution of free 0.1 mM VOAspi induced a significant decrease in the number of surviving cells, as well as morphological alterations (Fig. 6B). The cytoplasm showed a strong condensation with loss of processes. When the vanadium compound was released in a sustained manner from the 10% w/w VOAspi–PβPL film, a decrease in the cell number was observed with moderate changes in the cell morphology (Fig. 6C). As evaluated in parallel experiments, under this condition, the concentration of VOAspi-released into the culture medium over 24 h was 0.5 mM. However, incubation of the cells with a

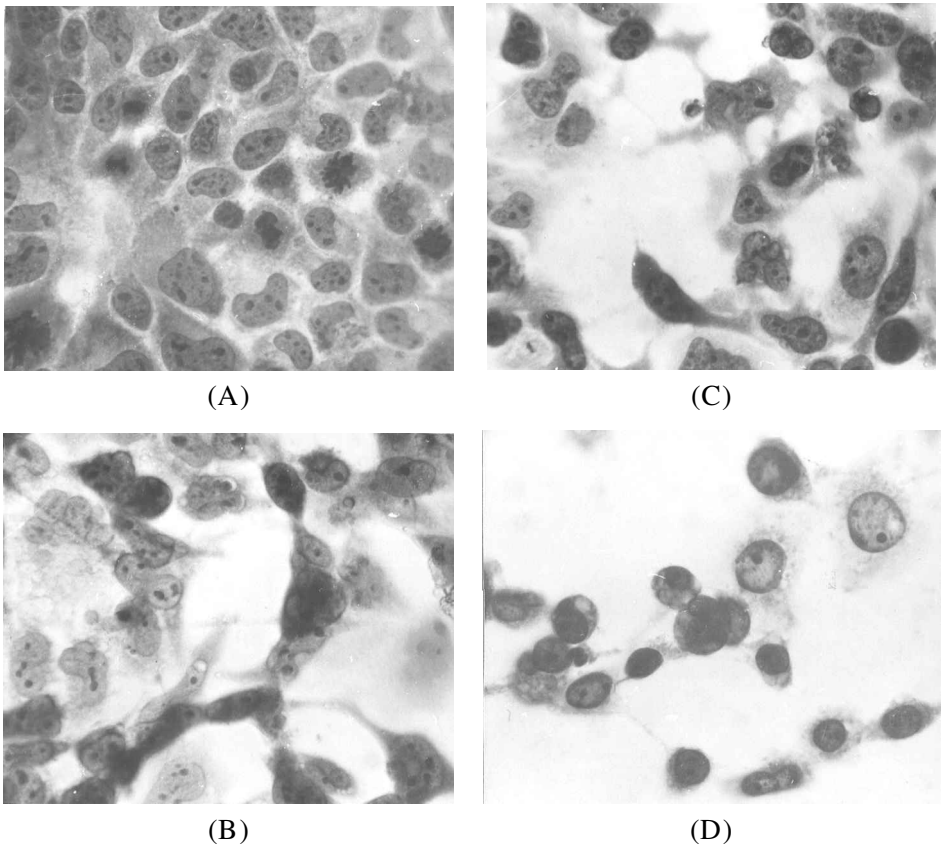

Figure 6. UMR106 cells cultured in: (A) DMEM alone (basal); (B) 0.1 mM free VOAspi; (C) 10% (w/w) VOAspi–PβPL film; and (D) 30% (w/w) VOAspi–PβPL film, at 37 °C after 24 h. At the end of the incubation, cells were fixed and stained with Giemsa (450×).

film with a higher load of VOAspi (30% w/w) induced a strong cytotoxic effect on the UMR106 cells (Fig. 6D). A few cells survived under these conditions and showed a reduction of cytoplasm and a pronounced condensation of the nuclei. A concentration of 2.5 mM VOAspi in the media was observed after overnight incubations with 30% w/w drug-loaded film.

In the control experiments, the effect of a solution of 0.1 mM aspirin or the unloaded PβPL film (without vanadium) was also evaluated for possible cytotoxicity. In both cases, the cells grew well, with no morphological alterations in comparison with control cell cultures without drug or polymer film. These observations suggest lack of toxicity either of aspirin that could have been released from the VOAspi complex, or the monomer from the PβPL film.

UMR106 growth was also evaluated by adding different amounts of the 10% w/w VOAspi–PβPL film in the culture media. As can be seen in Fig. 7, VOAspi inhibited cell proliferation in a dose-dependent manner, with an $ED_{50} = 0.45$ mM (50% surviving cells). In contrast, the ED_{50} value for free VOAspi (without

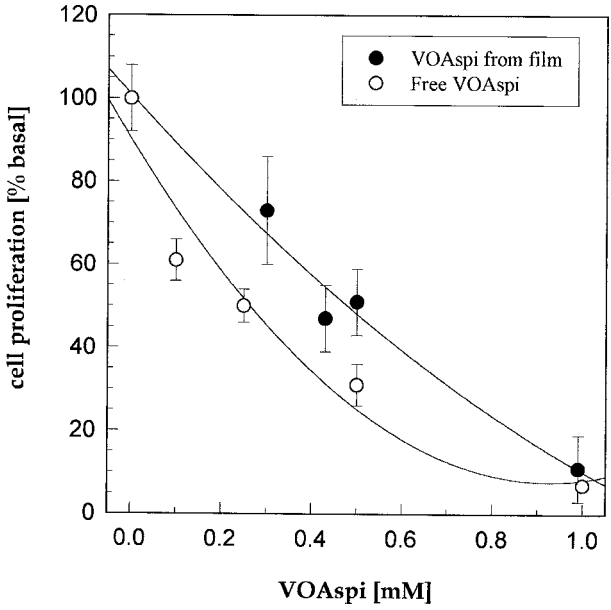

Figure 7. Dose-response curve of VOAspi on cell proliferation. UMR106 osteoblasts were incubated in DMEM alone (basal) or with the addition of various amounts of 10% w/w VOAspi–PβPL film or free VOAspi, at 37°C for 24 h. At the end of this period, cells were fixed, stained with Giemsa and the nuclei counted in 10 fields per well. The results are presented as the mean ± SEM.

polymer) was only 0.25 mM. Thus, VOAspi released from the PβPL film induced an antiproliferative effect on UMR106 cells but with less cytotoxicity than the free VOAspi. On the other hand, a 30% w/w VOAspi–PβPL film strongly inhibited cell proliferation, with a 10% cell survival after overnight incubation (data not shown). The mitotic index (MI) was assessed in the control as well as in the VOAspi-treated cultures. Under basal conditions, a MI $= 0.018 \pm 4 \times 10^{-3}$ was found for the UMR106 cells. The MI was zero for the cells cultured either with a 0.1 mM solution of VOAspi or with 0.3 mM VOAspi released from the PβPL films, indicating an arrest of the proliferative process under these conditions.

These results suggest that VOAspi embedded in the polymer retained the antiproliferative effects previously reported [8] in osteosarcoma cells with lower cytotoxicity than the free drug.

Oxidative stress studies

We have previously shown that vanadium compounds generate reactive oxygen species in osteoblast-like cells, and produce an oxidative stress [5]. Figure 8 shows that vanadium-induced TBARS formation is increased in a concentration-dependent manner. As can be seen, while 0.25 mM of free VOAspi was highly toxic (i.e. an increase of 370% TBARS over basal value), an equivalent concentration of VOAspi released from the controlled delivery system induced approximately half as much

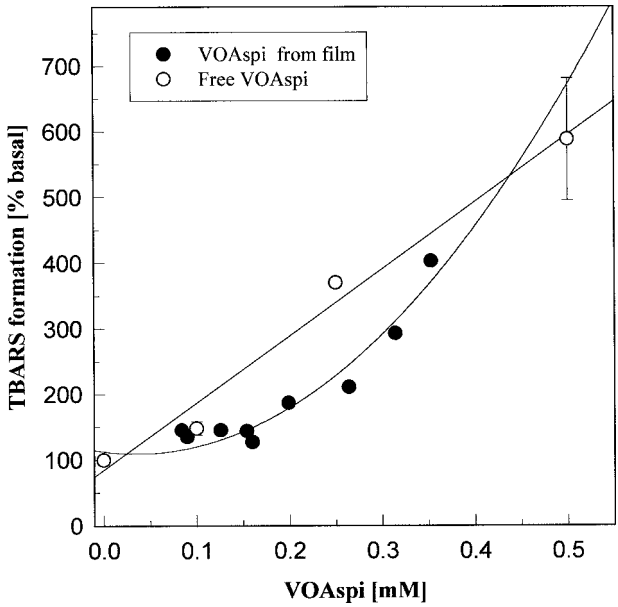

Figure 8. Effect of VOAspi on lipid peroxidation. UMR106 cells were incubated in DMEM alone (basal) or with the addition of different concentrations of free VOAspi in solution or with various amounts of 10% w/w VOAspi–PβPL film at 37 °C for 4 h. The lipid peroxidation was evaluated by assaying the TBARS production as described in Materials and Methods.

of TBARS formation. However, at concentrations of VOAspi greater than 0.45 mM, the extent of lipoperoxidation was the same whether the drug was free released from the PβPL films. In control experiments, 0.1 mM aspirin or the unloaded PβPL film did not generate lipid peroxidation in the UMR106 osteosarcoma cells (data not shown).

CONCLUSIONS

Based on the data presented in this communication, PβPL film appears to be a promising polymer-based delivery approach for drugs requiring sustained and controlled delivery. The release at the initial stage is dominated by a diffusion-based mechanism. Swelling experiments demonstrated a rapid diffusion of water into the film; thus allowing for controlled diffusion of the drug out of the film. The two mathematical models used to analyze the data support the experimental results up to 5 hours and their corresponding diffusion coefficient do not display significative differences from each other. Experimental results obtained over longer time periods could be described by erosion effects due to degradation of the polymer, which is more relevant after 2 days, as shown by our size exclusion chromatography experiments.

The PβPL film does not cause toxic effects on cells in culture suggesting a good biocompatibility.

The results obtained with VOAspi–PβPL film and UMR106 cells point towards a promising application of this system in antitumor chemotherapy. The morphological and biochemical experimental evidence presented in this study supports the concept that sustained released VOAspi maintains its antiproliferative action on osteosarcoma cells, with substantially lower cytotoxicity than that previously reported for this drug in solution.

The polymeric drug delivery system described in this communication provides a sustained release of a vanadium–aspirin complex for up to 7 days. The system is potentially useful for a topical application of selected drugs for cancer chemotherapy. Further experiments in appropriated animal models are needed to determine its full utility.

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REFERENCES

1. B. D. Ratner, in: *Comprehensive Polymer Science,* G. Allen (Ed.), Vol. 7, p. 201. Pergamon Press, Oxford (1989).
2. N. A. Peppas and A. R. Khare, *Adv. Drug Deliv. Rev.* **11**, 1 (1993).
3. A. K. Dash and G. C. Cudworth II, *J. Pharmacol. Toxicol. Meth.* **40**, 1 (1998).
4. J. L. Domingo, *Mol. Cell. Biochem.* **203**, 185 (2000).
5. A. M. Cortizo, L. Bruzzone, S. Molinuevo and S. B. Etcheverry, *Toxicology* **147**, 89 (2000).
6. A. M. Cortizo, M. Caporossi, G. Lettieri and S. B. Etcheverry, *Eur. J. Pharmacol.* **400**, 279 (2000).
7. J. H. McNeill, V. G. Youen, H. R. Hoveyda and C. Orvig, *J. Med. Chem.* **35**, 1489 (1992).
8. S. B. Etcheverry, P. A. M. Williams, D. A. Barrio, V. C. Sálice, E. G. Ferrer and A. M. Cortizo, *J. Inorg. Biochem.* **80**, 169 (2000).
9. S. B. Etcheverry and A. M. Cortizo, in: *Vanadium in the Environment. Advances in Environmental Science and Technology*, J. O. Nriagu (Ed.), p. 359. Wiley, New York (1998).
10. A. M. Cortizo and S. B. Etcheverry, *Mol. Cell. Biochem.* **145**, 97 (1995).
11. S. B. Etcheverry, D. C. Crans, A. D. Keramidas and A. M. Cortizo, *Arch. Biochem. Biophys.* **338**, 7 (1997).
12. D. A. Barrio, M. D. Braziunas, S. B. Etcheverry and A. M. Cortizo, *J. Trace Elem. Med. Biol.* **11**, 110 (1997).
13. V. C. Sálice, A. M. Cortizo, C. L. Gómez Dumm and S. B. Etcheverry, *Mol. Cell. Biochem.* **198**, 119 (1999).
14. J. K. Jackson, W. Min, T. F. Cruz, S. Cindric, L. Arsenault, D. D. Von Hoff, D. Degan, W. L. Hunter and H. M. Burt, *Br. J. Cancer.* **75**, 1014 (1997).

15. A. Bishajee, S. Roy and M. Chatterjee, *Oncol. Res.* **11**, 41 (1999).
16. J. Aubrecht, R. K. Narla, P. Gosh, J. Stanek and F. M. Uckun, *Toxicol. Appl. Pharmacol.* **154**, 228 (1999).
17. A. Sagrario Casas, I. H. Sorokin, H. A. Andreetta and R. V. Figini, in: *Proceedings of the 4th International Meeting of Science and Technology of Polymer*, La Plata, Argentina, p. 21 (1985).
18. D. Rosenvasser, A. Sagrario Casas and R. V. Figini, *Makromol. Chem.* **183**, 3067 (1982).
19. M. Miyajima, A. Koshika, J. Okada, A. Kusai and M. Ikeda, *J. Control. Rel.* **56**, 85 (1998).
20. J. Crank, *The Mathematics of Diffusion.* Clarendon Press, Oxford (1975).
21. H. Ohkawa, N. Ohishi and K. Yagi, *Anal. Biochem.* **95**, 351 (1979).
22. M. Bradford, *Anal. Biochem.* **72**, 248 (1976).
23. N. Wang and X. S. Wu, *J. Biomater. Sci. Polymer Edn 9* (1), 75 (1997).
24. T. G. Park, *J. Control. Rel.* **30**, 161 (1994).
25. M. Miyajima, A. Koshika, J. Okada and M. Ikeda, *J. Control. Rel.* **60**, 199 (1999).
26. A. Apicella and H. B. Hopfenberg, *J. Appl. Polym. Sci.* **27**, 1139 (1982).
27. N. M. Franson and N. A. Peppas, *J. Appl. Polym. Sci.* **28**, 1299 (1983).