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Polyether polyurethane delivery systems. I. Evaluation of monolithic systems for N^6-(Δ^2-isopentenyl)adenosine

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Summary

The nucleoside, N^6-(Δ^2-isopentenyl)adenosine (IPA) is reported to have inhibitory and cytotoxic properties against several types of cancer cells. The efficacy of this potent antileukemic agent is diminished by rapid enzymatic degradation in the body. In an effort to optimize the release properties of IPA, the drug was incorporated in polymeric delivery systems. Polyether–polyurethane was the polymer of choice because of its hydrophilic–hydrophobic character. Release studies from monolithic devices revealed linear relationships when the cumulative amount of IPA was plotted against the square root of time. IPA release from devices (1.12 mm thick) showed presence of lag time. In vitro L1210 cell culture studies showed that the IPA–polyether polyurethane devices maintained effective drug concentrations for ca. 100 h. Compared to conventional solutions, the polymeric system was more effective in controlling cell proliferation. Almost total cell death occurred when IPA was released at controlled rates due to increased contact time. Results of this study indicate that effective in-vitro antileukemic activity was obtained by the prepared polymeric system.

Introduction

The goal of effective cancer treatment is to inhibit the proliferation of neoplastic cells with minimal toxicity to the host. Non-specificity of most chemotherapeutic agents coupled with the inadequacy of the present modes of drug administration create a formidable problem in the treatment of cancer. In addition, many potent antineoplastic agents have been found to be unstable. Polymeric implants, containing a protected supply of the drug, can present an alternative mode of drug administration. Drugs released continually at or near the tumor sites may result in greater effectiveness and less toxicity. Use of polymers as drug carriers in cancer therapy has proved to be promising (Blackshear, 1979; Langer et al., 1981; Miyazaki et al., 1985).

The nucleoside N^6-(Δ^2-isopentenyl)adenosine (IPA) is known to have an inhibitory and cytotoxic action on human leukemic myeloblasts and sarcoma-180 cells (Grace et al., 1967). The ability of IPA to alter RNA synthesis and its immunosuppressive properties are attributed to the fact that it interferes with the transport of unmodified nucleosides through the cytoplasmic membrane (Hacker and Feldbush, 1971; Hare and Hacker,

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1972). The effective antineoplastic and cytotoxic effects of IPA against leukemic cells in humans are limited due to enzyme degradation. Adenosine deaminase has been reported to be the primary enzyme responsible for the conversion of IPA to inosine (Chassey and Suhadolnik, 1967). After i.v. administration the biological half life of IPA was found to be 4 h (Chedda and Mittelman, 1972). Various reactions, biosynthesis and metabolism of IPA have been extensively reviewed (Hall, 1970).

The polyether–polyurethane copolymer has been shown to be biocompatible and this makes it suitable for implantation studies (Marchant et al., 1984a and b; Takahara et al., 1985). This copolymer is composed of hydrophilic polyethylene glycol blocks and hydrophobic urethane segments thereby imparting a hydrophilic–hydrophobic character to the membrane. As a result, it has been used to fabricate devices for steroids (Sharma and Kim, 1984) and also for ionizable compounds (Hwang and Chang, 1984). Superior biocompatibility, availability, as well as its hydrophilic–hydrophobic character makes this copolymer ideally suited for use in implantation experiments. In this study, monolithic delivery systems made of polyether–polyurethane were evaluated as a potential carrier for IPA.

Materials and methods

Materials

Segmented polyether–polyurethane (Biomer) was supplied as a 30% solution of the polymer in *N*,*N*-dimethyl acetamide (Ethicon, Inc.). IPA was purchased from Sigma Chemicals Co. and used without further purification. Parent strains of L1210 cells were provided by the Fredrick Cancer Repository, through the National Cancer Institute. Materials used in cell culture medium were obtained commercially (Gibco Laboratories). All other chemicals were of analytical grade.

Analysis of IPA

Quantitation of IPA was done either by UV spectroscopy or by using a HPLC method. UV spectroscopy was used to quantitate IPA released, in in vitro studies, from monolithic devices. In all other cases IPA was quantitated by the HPLC method. In the first method a UV-vis spectrophotometer (Model 139, Perkin Elmer) was adjusted to monitor IPA at the wavelength of 267 nm. The conditions of the HPLC method were as follows: column, MicroPak MCH-10; flow rate, 1 ml/min; mobile phase, methanol : water (75 : 25). Samples of 30 μl were injected into a Varian 5000LC equipped with a Varian UV 50 variable wavelength detector.

Preparation of devices

The monolithic devices were prepared by a solvent casting method. Accurately weighed quantities of IPA were added to the copolymer solution. After thorough mixing, the drug–copolymer mixture was poured on a glass plate in the form of a thin sheet. The solvent was subsequently evaporated in a vacuum oven monitored at 30 psi and 50 °C for a period of 24 h. Monolithic devices with 4 different loading doses of IPA (1.04, 4.78, 8.89, 9.17% w/w) were prepared. Calculation of the loading dose was based on the weight-ratio of the drug and the polymer used.

Release studies from monolithic devices

Release studies of IPA from the monolithic devices were performed by the Rotating Basket Method using a USP XX Dissolution Apparatus. The dissolution medium consisted of pH 7.4 phosphate-buffered saline solution previously equilibrated at 37 ± 0.05 °C. The devices used in this study had thicknesses of 0.35 and 1.12 mm and surface areas of 8 cm^2 in both cases. The baskets containing the devices were rotated at 100 ± 1 rpm and aliquots were drawn at predetermined time intervals for quantitation. A constant volume was maintained in the vessel by replacing with fresh buffer solution.

Cell culture conditions

The L1210 cells used in the in vitro culture experiments were maintained as stationary suspension cultures in growth medium comprising of RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). The cell cultures were incubated

in 5.0 ml of growth medium per tissue culture flask in a carbon dioxide incubator (Forma Scientific) maintained at 37°C with 98% relative humidity and 5% CO_2. Stock cell suspensions were maintained by asceptically transferring 0.1 and 0.5 ml aliquots to fresh growth medium twice every week. The cultures were visually monitored daily to assess general growth characteristics and to ensure the absence of contaminants using an inverted microscope connected by a Panasonic minicamera to a television screen as described by Martinez et al. (1978).

Evaluation of devices against L1210 cells

Release of IPA from the devices was tested using two different inoculum sizes of L1210 cells (2×10^5 and 11×10^5 cells/ml). The IPA–polyether–polyurethane monolithic systems were sterilized in a gas sterilizer and placed in the cell culture flasks containing a definite number of cells. The devices (containing 4.78% w/w of IPA) were 1.12 mm thick with an effective surface area of 8 cm^2. All procedures were carried out under aseptic conditions. The cell numbers were counted before and after introduction of the polymeric devices. These were compared with sterile solutions containing the same number of cells and the free drug. The free drug solutions were sterile filtered before introduction into the culture flasks. Blank polymeric devices (devoid of IPA) were also tested, for the requisite period of time, to see if they had any untoward effect on cell growth. Cell viability counts were done by using the Trypan blue exclusion technique of Phillip and Terryberry (1957). All experiments were performed in quadruplicate. Aliquots were removed at periodic intervals for determination of IPA concentration and cell numbers in quadruplicate. Samples were centrifuged and subsequently filtered in order to get cell-free samples for HPLC determination of IPA.

Results and Discussion

Experimental data obtained from the release studies of the monolithic devices were analysed

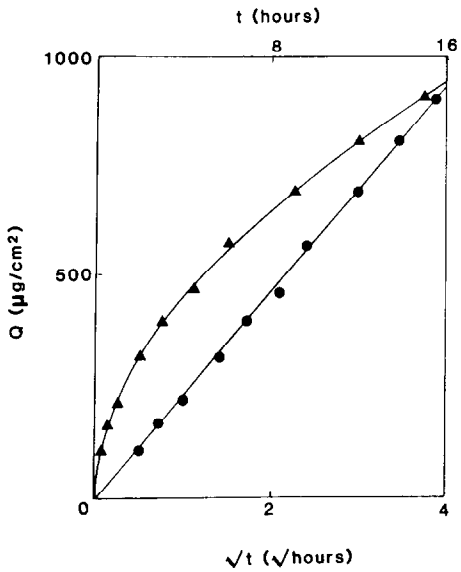

Fig. 1. Release of IPA from monolithic devices (4.78% w/w). ▲, Q vs t; ●, Q vs $\sqrt{t}$.

according to Eqn. 1 (Higuchi, 1961) based on diffusion controlled transport in a polymeric matrix:

$$Q = [D(2A - C_s)C_s t]^{1/2} \quad (1)$$

where Q is the cumulative amount of drug released per unit area of the device at time t, D is the diffusivity of the drug in the matrix, A is the loading dose or amount of drug dispersed in a unit volume of the device, and C_s is the solubility of the drug in the matrix. Fig. 1 shows the plot of the cumulative amount released, Q, versus time, t, and $\sqrt{t}$. The curvilinear profile obtained by plotting Q against t was found to be similar to observations reported previously for drug release from other polymeric systems (Samuelov et al., 1979). Linear Q–$\sqrt{t}$ profiles obtained were predicted by Eqn. 1. Q was found to increase linearly as a function of $\sqrt{t}$ for all concentrations used. The slopes of the Q–$\sqrt{t}$ profile are given by

$$Q/\sqrt{t} = [D(2A - C_s)C_s]^{1/2} \quad (2)$$

which reduces to :

$$Q/\sqrt{t} = [2D \cdot C_s]^{1/2} \cdot A^{1/2} \quad (3)$$

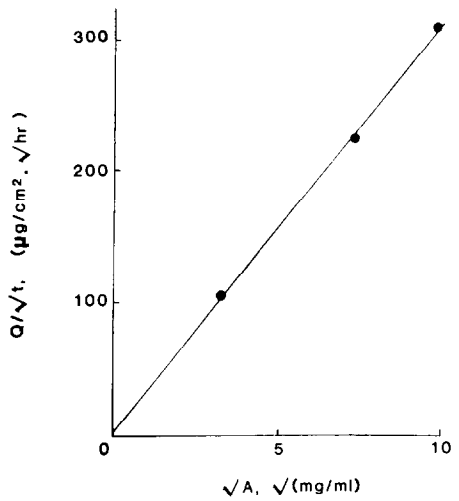

Fig. 2. Relationship between the release rate of IPA and the square root of the loading dose.

when $2A \gg C_s$. The slopes of the $Q-\sqrt{t}$ plots increased with an increase in the loading dose. Fig. 2 shows the linear relationship obtained by plotting $Q/\sqrt{t}$ against $\sqrt{A}$. The slope of the line $\sqrt{(2DC_s)}$ was 31.19 $\mu g/cm^2 \cdot h^{1/2}$ and the intercept was 2.64 $\mu g/cm^2 \cdot h^{1/2}$. These values can be used to predict optimum loading doses for specific conditions. Similar linear relationships were obtained for IPA release (Chang and Chaudhuri, 1983) and prostaglandin release (Roseman et al., 1981) from monolithic silicone device.

The slopes and the intercepts obtained from the $Q-\sqrt{t}$ plots for different loading doses are listed in Table 1. It is evident that for the thin monolithic devices (0.35 ± 0.05 mm), the $Q-\sqrt{t}$ relationships almost passed through the origin even at high loading doses. Similarly, when IPA was released from silastic devices a "burst effect" was observed at high loading doses (Chang and Hacker, 1982; Chang and Chaudhuri, 1983). On the other hand, the $Q-\sqrt{t}$ profiles for the thick monolithic devices (1.12 ± 0.05 mm) had intercepts on the time axis. This indicated the presence of a lag time for all loading doses. Similar lag times were also reported in release studies of hydroxylated progesterone derivatives from silicone matrix (Chien et al., 1979). The devices of both thicknesses were subjected to the same temperature and the same period of drying in the solvent casting process. It

TABLE 1

Slopes and intercepts of Q vs $\sqrt{t}$ plots obtained from monolithic devices of different thicknesses

Loading dose, % (w/w)	Monolithic devices thicknesses		Significance level [c]
	0.35 ± 0.05 mm	1.12 ± 0.05 mm	
1.04	105.3 ± 2.08 [a]	105.7 ± 6.11	n.s.
	0.0009 ± 0.00003 [b]	0.069 ± 0.0043	s.
4.78	225.3 ± 1.53	224.0 ± 4.58	n.s.
	0.0023 ± 0.00050	0.075 ± 0.0075	s.
9.17	311.8 ± 5.25	309.7 ± 4.73	n.s.
	0.0021 ± 0.00048	0.061 ± 0.0024	s.

[a] Mean of slope $\pm$S.D.

[b] Mean of x-axis intercept $\pm$S.D.

[c] Probability of differences in slopes and x-axis intercepts due to variation in thickness. Significance (s) and non-significance (n.s.) based on a two-tailed t-test ($P < 0.05$).

was speculated that during the drying process a portion of IPA, dissolved in the solvent, may have migrated to the inner core of the matrix while the solvent evaporated from the outer surfaces. In such cases a lag time is usually observed due to the release of the drug from the inner core since the outer surfaces had little or no drug.

In vitro cell culture studies were performed to evaluate differences in L1210 cell kill patterns between IPA in solution form and that released from devices. L1210 mouse leukemic cells are known to possess the necessary enzyme systems to phosphorylate IPA to the nucleotide level (Hacker, 1960). The cell proliferation patterns after IPA was introduced either in the device or in the solution form are shown in Fig. 3. The studies showed that IPA released from the fabricated device proved to be much more effective in controlling L1210 cell growth as compared to the solution. Although IPA in the solution form was quick to elicit antileukemic properties, this action was short-lived as the cells began to multiply after 50 h. The devices, on the other hand, had a prolonged effect with almost complete cell kill. The few cells remaining were deformed and nonviable. Table 2 shows the concentration of IPA at different time periods. The concentration of IPA released from devices at the end of 24 h was determined previously. The IPA solution had a concentration equal to that of IPA released at the end of 24 h. The decrease in IPA concentration

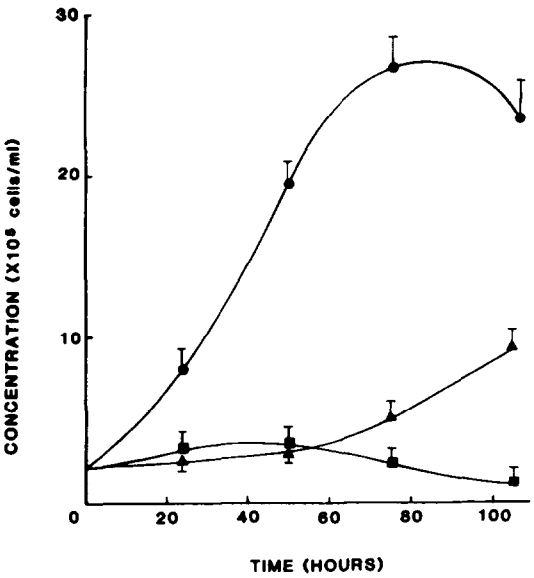

Fig. 3. Effects of IPA on L1210 cell proliferation with initial inoculum size of 2×10^5 cells/ml. ●, Control; ▲, IPA solution; ■, IPA polymeric device.

observed after 50 h is probably due to enzyme deactivation. Although IPA released from the polymer sheet was also subjected to degradation, the release rate was fast enough to maintain its concentration. Table 3 shows percent inhibition of cell growth as a function of the loading dose. The increased inhibition rate observed at the high loading doses was expected as higher amounts of the drug were released. The antileukemic activity persisted even when initial cell concentrations were as high as 11×10^5 cells/ml.

It appears that polymeric devices were more effective in controlling cell growth since they allow a greater contact time between the cell and the drug. Release of the drug at a controlled rate

TABLE 2

Changes in IPA concentration (μg/ml) as a function of time in RPMI-1640 culture medium containing L1210 cells

Treatment	Time (h)		
	24	50	106
IPA solution	4.40	4.30	2.94
IPA polymeric device	4.33	6.20	5.02

TABLE 3

Percent inhibition of cell growth by IPA released from monolithic devices of three different loading doses

Loading dose % (w/w)	Time (h)		
	24	48	84
1.04%	23.9	57.2	62.6
4.78%	54.8	79.7	89.0
9.17%	59.9	88.7	92.9

Initial concentration of cells was 11×10^5 cells/ml.

for a prolonged period from the protected supply enhanced the therapeutic effectiveness to a marked extent. Choice of polymer is also an important factor. Compared to previous studies with silicone polymer (Chang and Hacker, 1982; Chang and Chaudhuri, 1983) IPA was released at a faster rate from the less hydrophobic polyether–polyurethane. However, it should be pointed out that the release rate may change with a change in the polymer structure. As mentioned before, the polyether–polyurethane copolymer has hydrophilic as well as hydrophobic properties. This dual character of the membrane is expected to change with an alteration in the ratio of the two segments. Proper choice of polymer and optimization of the device can result in controlled release of the therapeutic agent such that drug levels are maintained in excess of its degradation rate to provide the necessary therapeutic action. This type of device holds promise as implants in cancer therapy. Evaluation of devices of different designs as well as implantation studies in mice are presently being performed in our laboratory.

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