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Polyether polyurethane delivery systems. II: Effect of coadministration of pentostatin with N^6-(Δ^2-isopentenyl)adenosine

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Summary

Pentostatin, a tight binding inhibitor of adenosine deaminase, was used together with N^6-(Δ^2-isopentenyl)adenosine (IPA) to increase the efficacy of the latter. IPA, although cytotoxic to several types of tumor cells, is unstable due to deamination to inosine by adenosine deaminase. Monolithic polymeric devices were made using segmented polyether polyurethane with both drugs, either individually or together. Drug release data for both drugs gave linear relationships when the amount released per unit area was plotted against the square root of time. Release rates of IPA from the devices were higher than that of pentostatin. The devices were also evaluated for cytotoxic activity of the drugs against cultured L1210 murine leukemic cells. Compared to the intact drugs alone, the polymeric devices enabled a sustained and controlled release of the agents, for a longer period of time. Cell replication was decreased to a marked extent although the initial action was slow. Release of both drugs, when incorporated in the same device, resulted in almost total cell death (> 98%) of an initial population of 11×10^5. Even though the drugs were partially effective individually, the presence of pentostatin together with IPA increased cytotoxic action. The results indicated that sustained release of the enzyme inhibitor together with IPA gave rise to a better delivery system.

Introduction

Maximum success in chemotherapy has been attained by using an approach called the 'combined modality treatment' (Sartorelli, 1976). This method of treatment uses a combination of a chemical agent with irradiation and/or surgery. Use of two different chemical entities to complement each other in the treatment of cancer has also been successful. The present study uses an extension of the latter concept.

Many potent antineoplastic agents have been found to be unstable. It has also been reported that the efficacy of these compounds can be increased if they are released at controlled rates from polymeric implants that constitute protected

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supplies (Chang and Chaudhuri, 1983; Chaudhuri et al., 1988). Although the effectiveness of the polymeric implants for such unstable compounds has been proved, the problem of degradation has not yet been addressed.

The nucleoside, N^6-(Δ^2-isopentenyl)adenosine (IPA), was chosen as a model compound. IPA has been proven to have inhibitory and cytotoxic properties (Grace et al., 1967). Although it is potent, the cytotoxic and antineoplastic effects are curtailed due to enzymatic degradation. IPA undergoes rapid deamination to inosine primarily due to the action of adenosine deaminase (Chassey and Suhadolnik, 1967). The present study was aimed at decreasing the in-vivo degradation rate of the drug. It was hypothesized that administration of an adenosine deaminase inhibitor could probably decrease the deamination rate. Pentostatin [(*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo(4,5-*d*)(1,3)diazepin-8-ol] is a potent inhibitor of the enzyme adenosine deaminase. By competitively binding with adenosine deaminase, pentostatin was found to potentiate the activity of adenosine analogs (Gray et al., 1982; Woo, 1982). Pentostatin markedly potentiated the action of IPA against L1210 cells (Hacker and Chang, 1983). This adenosine deaminase inhibitor has additional attributes. Pentostatin possesses both antilymphocytic (Cass et al., 1976) and immunosuppressive properties (Chassin et al., 1977) and was also found to cause a successful level of clinical remission in patients with acute or chronic lymphocytic leukemia (Gray et al., 1982).

The present study extends previous work using polyether polyurethane copolymers (Chaudhuri et al., 1988). Both compounds, IPA and pentostatin, were incorporated into monolithic devices which were then evaluated for their release properties as well as their effectiveness against L1210 leukemic cells. The premise was that when released simultaneously from the monolithic devices, pentostatin would potentiate the action of IPA.

Materials and Methods

Materials

N^6-(Δ^2-Isopentenyl)adenosine (IPA) was obtained from Sigma. Pentostatin was supplied by Flow Labs through the National Cancer Institute. Parent L1210 cell strains were provided by the Fredrick Cancer Repository. Materials used in cell culture studies were obtained commercially (Gibco). All chemicals were used without further purification. Segmented polyether polyurethane (Biomer) was obtained commercially from Ethicon as a 30% solution in *N*,*N*-dimethylacetamide.

Quantitation of drugs

An HPLC method was used for quantitation of IPA and pentostatin. 30 μl of sample were injected into a high-pressure liquid chromatograph (Varian 5000LC). The separation was performed by using a Micropak MCH-10 (Varian) C-18 reverse-phase column with a mobile phase comprising 0.02 M phosphate buffer (25%) and methanol (75%) at a flow rate of 1 ml/min. The column effluent was monitored by a fixed-wavelength detector (Beckman Model 153) set at 254 nm at ambient temperature.

Preparation and evaluation of devices

The monolithic devices were prepared according to procedures outlined previously (Chaudhuri et al., 1988). The loading doses for the monolithic devices for IPA were 1.04, 4.78, 8.89 and 9.17% w/w. In the case of pentostatin, the loading doses used were 1.53, 2.99 and 4.47% w/w. When both drugs were coadministered from the same device, the loading doses were 8.9 and 4.5% w/w for IPA and pentostatin, respectively.

All devices made were evaluated for release of the drugs by the rotating basket method using a USP XX Dissolution Apparatus. Details of the drug release experiments have been described earlier (Chaudhuri et al., 1988) and these analyses performed in quadruplicate.

Determination of antineoplastic activity

The antineoplastic activity of IPA and pentostatin released from the devices were tested in vitro using murine L1210 leukemic cells. The cells were routinely cultured in supplemented growth medium, using two different inoculum sizes (2×10^5 and 11×10^5 cells/ml), and monitored daily for growth characteristics. Cell culture conditions were as mentioned previously (Chaudhuri et al.,

1988). The devices containing the drugs (either singly or in combination) were introduced into the cell culture flasks containing 5 ml of the media maintained at 37°C at time zero or 24 h after incubation. In all cases, the devices had an effective surface area of 8 cm^2. The effectiveness of the drugs released from the devices was assessed by counting the cell numbers before and after introduction of the devices. Sterile-filtered solutions of the free drugs were also used as comparisons. The control consisted of sterile-filtered saline solutions being added to the media. Aliquots were withdrawn, at periodic intervals, for determination of cell number as well as quantitation of the concentration of the drugs.

Results and Discussion

Release patterns of IPA and pentostatin from the polyether polyurethane monolithic devices were analyzed using Eqn 1 based on diffusion-controlled transport in a polymeric matrix:

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

where Q is the cumulative amount of drug released per unit area of the device at time t, D is the diffusivity of 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. In the case of IPA, linear profiles were obtained when Q, the amount released, was plotted against the square root of time. A linear relationship was also obtained when the slopes of $Q/\sqrt{t}$ were plotted vs $\sqrt{A}$, the square root of the loading dose. As mentioned before (Chaudhuri et al., 1988), this line had a slope 31.19 $\mu g\ cm^{-2}\ h^{-1/2}$. Drug release data in the case of pentostatin also showed linear Q-$\sqrt{t}$ relationships. However, for all the loading doses tested, intercepts were found on the y-axis indicating a 'burst effect'. The slopes of the $Q/\sqrt{t}$ relationships yielded a straight line when plotted vs the square root of the loading dose, $\sqrt{A}$. The latter linear plot had a slope of 2.90 $\mu g\ cm^{-2}\ h^{-1/2}$. Unlike IPA, pentostatin does not dissolve in the polymer-solvent mixture during preparation. As a result, pentostatin mostly remained dispersed throughout the matrix as fine suspended particles. The burst effect observed may be attributed to the dispersed pentostatin particles residing on the surface of the matrix. Similar results were observed for IPA release from silastic devices (Chang and Hacker, 1982). Values of the slopes of the straight lines obtained by plotting $Q/\sqrt{t}$ profiles against $\sqrt{A}$ were higher for IPA as compared to that of pentostatin. It has been pointed out that reductions in $Q/\sqrt{t}$ vs $\sqrt{A}$ plots could reflect changes in matrix diffusivity and/or polymer solubility of different molecules (Chien et al., 1979). The observation made in this study can be explained on the basis of the poor solubility of pentostatin in the polymer.

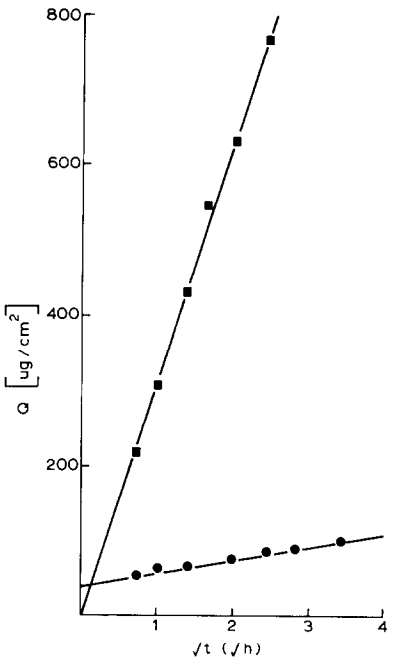

Fig. 1. Release of IPA and pentostatin from the same monolithic device. (■) IPA, (●) pentostatin.

Fig. 1 shows the profiles of IPA and pentostatin when released from the same device. The slope for IPA was 319.5 (±6.1) compared to 18.7 (±0.59) $\mu g\ cm^{-2}\ h^{-1/2}$ for pentostatin. No significant difference was observed, statistically, when these values were compared to the release rates of the drugs from the Biomer sheets when present

independently. This indicated that the presence of one does not affect the release rate of the other in any way.

The effectiveness of the devices was tested in vitro with murine L1210 leukemic cells. The sensitivities of L1210 to IPA and pentostatin have been reported earlier (Hacker and Chang, 1983) and it has also been demonstrated that IPA released from polymeric devices has been successful in controlling L1210 cell growth (Chang and Hacker, 1982; Chaudhuri et al., 1988). Fig. 2 shows the cell proliferation patterns of L1210 cells after the addition of various devices onto a culture inoculated with 2×10^5 cells. The cells were found to be sensitive to both drugs, IPA and pentostatin when given in concentrations of 4.5 and 0.35 μg/ml, respectively, in solution form either separately or together. These concentrations indicate the respective amount of drug released from the devices at the end of a 24 h period. Drugs in the solution form had the fastest inhibition rate. However, this inhibition lasted for approx. 2 days. The cytotoxic

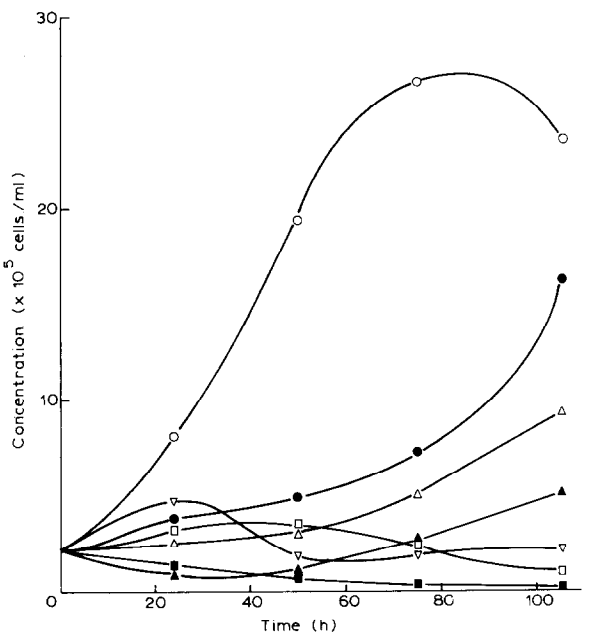

Fig. 2. Effects of IPA and pentostatin on L1210 cell proliferation with initial inoculum size of 2×10^5 cell/ml. (○) Control, (△) solution with IPA, (●) solution with pentostatin, (▲) solution with IPA and pentostatin, (□) device with IPA, (▽) device with pentostatin, (■) device with IPA and pentostatin.

TABLE 1

Percent inhibition of L1210 cell growth by IPA and pentostatin released from monolithic devices and solutions at initial concentrations of 2×10^5 cells/ml

Treatment	Time (h)			
	24	50	75	106
Solution of IPA	69.6	84.8	83.1	59.8
Solution of pentostatin	52.0	75.0	72.5	31.2
Solution of IPA and pentostatin	87.6	95.2	90.4	78.7
Device with IPA	60.6	84.3	91.0	94.9
Device with pentostatin	41.7	90.1	92.3	90.6
Device with IPA and pentostatin	84.1	96.5	98.1	98.7

action of all the solutions was more prompt when compared to the drugs in the devices. Although the monolithic devices, with one or both drugs, were initially slow in eliciting cytotoxic response, their action was seen to be more sustained and prolonged. As seen from Table 1, almost complete inhibition was obtained with the polyether polyurethane devices containing both drugs within 2 days. The few cells remaining, at the end of the study period, were found to be deformed or nonviable. The effectiveness of the monolithic sheets indicated that contact time was important in inhibition of L1210 cell growth. It was also observed that IPA appeared to be more effective compared to pentostatin. However, in contrast to previous reports (Hacker and Chang, 1983), pentostatin was found to have substantial inhibitory properties. An increase in cell kill ability was observed in all cases when both drugs were used together as compared to when they were administered separately.

The difference between different drugs and their dosage form was much more pronounced when the inoculum size was 11×10^5 cells/ml as seen in Fig. 3. The reason for this increased cytotoxicity was not clear as the devices used had the same loading dose. It is known that a given dose of any drug is responsible for killing a constant fraction of cells, not a constant number, regardless of the

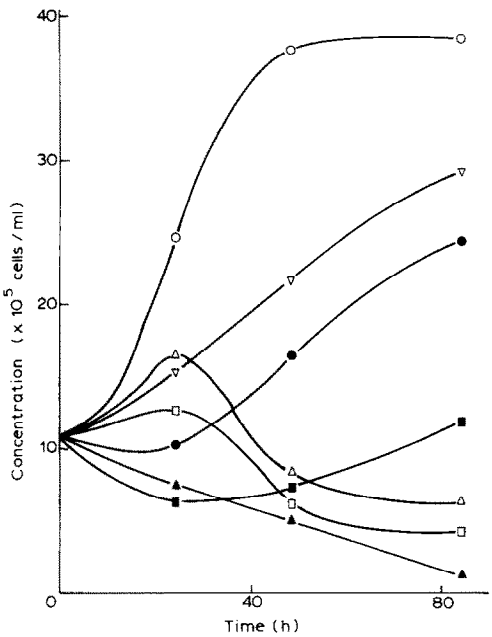

Fig. 3. Effects of IPA and pentostatin on L1210 proliferation with initial innoculum size of 11×10^5 cells/ml. (○) Control, (●) solution with IPA, (▽) solution with pentostatin, (■) solution with IPA and pentostatin, (□) device with IPA, (△) device with pentostatin, (▲) device with IPA and pentostatin.

cell number present (Carter, et al., 1977). This observation agreed with the results obtained.

Potency of any antileukemic agent depends on the number of cells present. The stage of the disease is thus an important factor in the determination of efficacy. In order to determine the effect of time on the inhibitory capacity, devices were placed in the culture media 24 h after the start of the cell cycle. Table 2 shows the same pattern of cell inhibition observed earlier. In all cases a slight decrease in inhibition was observed pointing to the fact that a greater efficacy is achieved if the devices are administered at the start of the cell cycle. The results of the present study indicated that pentostatin itself had good cytostatic action. Thus, the tendency in increase in cytotoxic action may have been due to the combined therapy concept in addition to the protective action against deamination. The actual mechanism of action will be further pursued.

TABLE 2

Percent inhibition of L1210 cell growth by IPA and pentostatin released from monolithic devices when incorporated 24 h after the start of the cell cycle (initial cell concentration 2×10^5 cells/ml)

Treatment	Time (h)		
	50	75	106
Device with IPA	78.8	90.4	90.8
Device with pentostatin	74.1	88.0	87.8
Device with IPA and pentostatin	86.9	93.3	95.8

The in vitro studies with the L1210 leukemic cells clearly show that use of IPA and pentostatin together increases the cytotoxic effect. Cell kill was complete when both agents were used in the device as they prevented any further cell propagation. The results of this study add more credence to the use of polymeric drug delivery systems, especially in the treatment of chronic conditions.

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