DEVELOPMENT OF MACROMOLECULAR PRODRUGS OF THE ANTITUMOR ANTIBIOTIC ADRIAMYCIN

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INTRODUCTION

The anthracycline antibiotics daunomycin and 14-hydroxydaunomycin (adriamycin, ADR) have been shown to be very effective in the treatment of a number of malignancies (1,2). Besides the non-specific side effects of cytostatic agents, adriamycin exerts a specific toxic activity on the heart which may eventually lead to a life-threatening congestive heart failure when a cumulative dose of 550 mg/m^2 has been exceeded (1,2). To effect a decrease in cardiotoxicity we are developing adriamycin-polymer conjugates which are not taken up by heart muscle cells but are readily incorporated in tumor cells. After internalization of a macromolecular substance by endocytosis a phagosome is formed which is then fused with a lysozome (Fig. 1). The substance is then exposed to many digestive enzymes at an acidic pH (4-5) (3). We have concentrated on the use of poly(α-L-glutamic acid)(PGA) as a carrier, because this polymer can readily be degraded by lysozomal enzymes and is rather plasma-stable. After partial derivatization the conjugate will be water-soluble owing to residual carboxylic acid groups.

RESULTS AND DISCUSSION

The adriamycin moiety was covalently bound to PGA with various labile linkages including acylhydrazone (Fig. 2) and amide either without or with various peptide spacers (Fig. 3). Details about the syntheses of these conjugates will be described elsewhere (4,5). The cytotoxic activity of polymeric conjugates with either complex-bound or covalently-bound adriamycin was evaluated using *in vitro* L1210 clonogenic and B16 melanoma liquid assays (Table 1). These data indicate that adriamycin when complexed or linked to the carrier with an acylhydrazone bond at C-13 retains a significant cytotoxicity. The latter type of covalent bond appears promising for adriamycin prodrugs, although the polymeric conjugate as well as the acylhydrazide tend to become insoluble on standing for reasons still under study. Amide-bound prodrugs of adriamycin are found to be stable in plasma for at least 48 hours in accordance with their possible applicability as an endocellular release system. The cytotoxicity of these conjugates can be improved by the use of peptide spacers but remains relatively low. The data suggest that

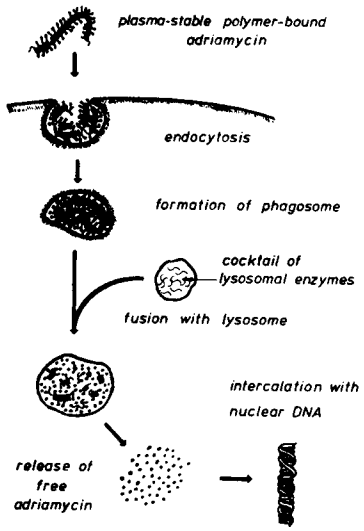

Fig. 1 Endocytosis and lysozomal digestion of adriamycin--polymer conjugates

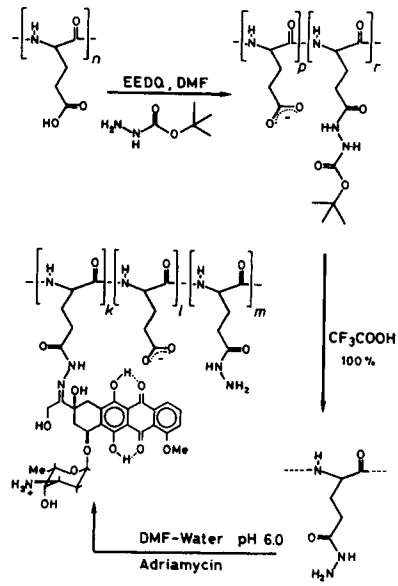

Fig. 2 Conjugation of adriamycin onto PGA using an acyl-hydrazone linkage

Monomeric unit in protease-activated macromolecular prodrug of adriamycin

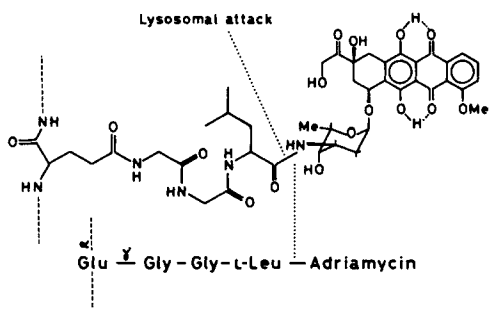

Fig. 3 Example of a prodrug of adriamycin bound as the C-terminal amide of a PGA-peptide graft

Table 1. Cytotoxic activities, expressed as dose with 50 % inhibition of cell growth (ID_{50}) of adriamycin prodrugs

TYPE	CODE*	LOAD (mole-%)	ID_{50} L1210 assay (ng ADR/ml)	ID_{50} B16 assay (ng ADR/ml)
Free drug	A	-	21/24	4-5
Acylhydrazone	P_3-H-A	13/0.4	53-71	17
Conjugate	P_2-A	5	1100-4100	-
Complex	P-A/A	6/3.3	24-39	-
	P_3-GGL-A/A	76/4/3	-	2-4
Spacer	P_3-GGL-A	96/4.3	200	70
	P_2-G-GGL-A	95;67/10	1100/3300	270
	P_3-GT-GGL-A	100;53/9	$\geq$2200	180
	P_7-GGL-A	89/8	3000	220
	P_7-GGGL-A	84/8	560	200
	P_8-[GGL-A]	4.4/4.4	>65000	>>650
	P_8-[GGGL-A]	4.5/4.5	>65000	>>650

* indices indicate batch number; A = adriamycine; H = acylhydrazone; P = poly(α-L-glutamic acid); G = Gly; L = Leu; T = Tyr

in the conjugates prepared thus far and having peptide spacers with up to 5 amino acid residues, the velocity of the enzymatic release of adriamycin by lysozomal enzymes inside tumor cells is still too low for efficient drug action. Using the same batch of PGA, the increase in spacer length from three to four amino acid residues improves the cytotoxicity fivefold (P_7-GGL-A and P_7-GGGL-A), consistent with an increased accessibility of the leucyl-adriamycin bond to enzymes. On the other hand, on decreasing the load of peptide spacer from 84-89 mole-% to 4.5 mole-% the cytotoxic activity decreases to very low values (P_8-[GGL-A] and P_8-[GGGl-A]) which contradicts the expected decrease in steric hindrance to enzymatic hydrolysis. On the basis of these results a more detailed study on the enzymatic degradation of macromolecular prodrugs using PGA and derivatives as the carrier including the effects of pH (6) seems warranted. Such studies, including the use of *p*-nitroaniline as a model drug (3), are currently in progress.

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