A comparative study of antitumour and toxicologic properties of related polyanions*

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Poly(maleic anhydride) (PMA) homopolymer, DIVEMA copolymer (PCP) and poly(acrylic acid–maleic anhydride) (PAAMA) copolymer were synthesised and separated into discrete molecular weight *(MW)* fractions. These fractions were tested for antitumour (Lewis lung carcinoma LLC), anti-Friend leukemia (FLV), immunoadjuvant activity, phagocytic activity, sensitization to bacterial lipopolysaccharide (LPS) and inhibition of microsomal mixed functional oxidase enzymes (MMFO). Molecular weight preparations of the three polymers, 10 000 and above, possess pronounced activity against LLC (increased life-span > 35%), FLV (ED_{50} between 1.6–4.0 mg/kg) and enhanced B lymphocyte activity as measured by increased number of hemolytic plaque forming cells. Molecular weight preparations below 1000 were devoid of these activities. PCP was the most potent inhibitor of MMFO as measured by prolongation of hexabarbital sleeping times. All polymers induced a sensitization to LPS. PCP was the most potent sensitizer followed by PAAMA and PMA. PCP at *MW* 30 000 and above markedly suppressed phagocytosis as measured by the vascular clearance rate of colloid carbon in mice while *MW* preparations <5000 >2000 stimulated phagocytosis. PMA inhibited phagocytosis to a much lesser degree than PCP. In contrast to PCP, PMA did not stimulate phagocytosis until 7 days after drug administration. Peritoneal exudate cells (PEC) and serum from PCP and PMA-treated mice conferred complete protection against FLV but not LLC in recipient mice. Supernatants from the PEC also transferred protection but not to the same degree as whole cells. These studies begin to delineate the structures and polymer size which possess therapeutic and toxicologic potential.

Anionic and cationic polyelectrolytes of both natural and synthetic origin have been found to exhibit an inhibitory effect on viruses, bacteria, tumours, and enzymes. Polyanions in particular have a broad range of biological activity and have received considerable interest in the areas of oncology and virology. The prolonged protective action of synthetic polyanions when given prior to virus inoculation has significant clinical potential. Consequently, an impetus was established for assaying the fundamental role of polyanions in controlling host resistance to a variety of pathophysiology.

The action of polyanions as mitotic inhibitors and their functional role in neoplastic processes have been widely studied[1–3] as has the role of polynucleotides in immunology[4] and virus resistance[5]. A possible mechanism for the activity of polyanions on tumour growth may be related to coupling of the polyanion to tumour antigen. However, the action of polyanions on a wide range of enzymes, such as alteration of the isoelectric point of proteins, displacement of nucleohistone and antiviral action all indicate possible alternative concepts of antitumour action. For example, immunopotentiators, or 'host-resistance inducing agents', have been used for decades in an attempt to treat tumour-bearing animals and man. These attempts have been largely empirical until recently, when the interactions of the basic immune response to tumours have been partly delineated. Cytotoxic lymphocytes, cytotoxic antibody and 'activated' macrophages have all been shown capable of inhibiting or destroying tumour cells[6–8]. However, more emphasis has been placed on the thymus-derived cytotoxic lymphocyte as the effector cell in both transplantation and tumour immunity.

Considerable evidence has emerged that implicated the macrophage as a major effector of tumour cytotoxicity and/or cytostasis. Both synthetic reticuloendothelial stimulants such as poly(acrylic acid–maleic anhydride), DIVEMA (pyran) and poly(riboinoinic–cytidylic acid) as well as biologic reticuloendothelial stimulants are known to enhance macrophage function as well as to induce resistance to tumour growth[6–9]. Moreover, macrophage from animals treated with polyanionic stimulants have been demonstrated to be cytostatic and/or cytotoxic for tumour cells while demonstrating quantatively less cytotoxicity for normal cells[10].

PHARMACOLOGIC STUDIES OF RELATED POLYANIONS

One of the most interesting polyanions is DIVEMA copolymer prepared from divinyl ether and maleic anhydride through copolymerization. This material shows a variety of biological activities and has elicited a considerable amount of interest by several researchers in different areas[1].

Our interest is to investigate methods of improving DIVEMA's therapeutic efficacy by preparing discrete molecular weight fractions with narrow polydispersity and by preparing and evaluating other polyanions with similar structural features.

Consequently, pharmacologic studies were conducted on five different anionic polymers of similar structure with

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Table 1 Pharmocologic studies of some polyanion polymers

Properties	Control	DIVEMA XA 124–177	PAAMA	MA	FMA	$(Asp–Glu)_n$
Intrinsic viscosity	–	0.21	0.29	0.04	0.07	0.025
Toxicologic properties:						
LD_{50}[1]	–	74 (69–79)	110	110	130	200
Liver Weight[2]	5.4	7.8*	6.9*	6.2	5.2	6.1
(% Body weight)	±0.4	±0.2	±0.3	±0.4	±0.1	±0.2
Spleen weight[2]	0.36	1.08	0.98	0.80	0.50	0.64
(% Body weight)	±0.02	±0.04	±0.05	±0.04	±0.04	±0.02
LD_{50} (Endotoxin) (mg/kg)[3]	25	0.12*	1.*	15	15	15
Hexobarbital sleeping time (min)[4]	50.7	106*	63.7	58.1	47	53.1
	±2.6	±9.7	±5.2	±2.7	±3.2	±4.1
Antitumour and antiviral properties:						
Antitumour activity Lewis lung (% inhibition)[5]	0	76*	74*	75*	15	10
Increase survival time	0	39%	33%	15%	0	0
Antiviral (EMC) (% protection)[6]	0	89%	90%	30%	0	0

Poly(acrylic acid–maleic anhydride) copolymer (PAAMA), maleic anhydride homopolymer (MA), furan–maleic anhydride homopolymer (FMA), aspartic acid–glutamic acid polymer $(Asp–Glu)_n$

[1] LD_{50} (95% confidence limits)calculated by the method of Litchfield and Wilcoxon. Polymers administered intravenously. Mortality recorded after 24 h. [2] Polymers administered in a dose of 25 mg/kg intravenously except Asp–Glu – given in a dose of 100 mg/kg. Organ weights determined 7 days after drug injection and expressed as percent of total body weight (Munson *et al. J. Reticuloendothelial. Soc.* 1970, **7**, 375. [3] LD_{50} of *S. typhosa* 0904 lipopolysaccharide 24 h after a single administration of 25 mg/kg of polymer (Munson *et al. Proc. Soc. Biol. Med.* 1971, **137**, 553). [4] Group of NYLAR–A mice were inoculated with 25 mg/kg i.v. of the polymer – (except (Asp–Glu); 100 mg/kg). 24 h later an anesthetic dose (80 mg/kg) of sodium hexobarbital was administered i.v. and duration of anesthesia recorded. (Munson *et al. Proc. Soc. Exp. Biol. Med.* 1971, **137**, 553). [5] BDF mice were inoculated with 10^6 Lewis' lung cells into right-hand gluteus muscle. Polymers were administered daily by the intraperitoneal route for 10 consecutive days following tumour implantation. Primary tumour size was determined on day 14 (% inhibition) and increased life span (% ILS) calculated from mean time to death. [6] Mice were inoculated intravenously with 10 LD_{50} of encephalomyocarditis virus 24 h after administration (i.v.) of 25 mg/kg of polymer. Percent protection based on 20 mice/group calculated from mean time to death.
* $P < 0.05$ mean ± s.e. derived from 8 mice per group except for the LD_{50} studies which employed at least 100 animals.

DIVEMA XA 124-177 as a comparison. These polymers included: poly(acrylic acid–maleic anhydride) (PAAMA) copolymer[11], maleic anhydride homopolymer (MA)[12], furan–maleic anhydride copolymer (FMA)[13] and aspartic acid–glutamic acid copolymer (Asp–Glu)[14].

The results of these tests are listed on *Table 1*. DIVEMA exhibits a LD_{50} of 74, causes a hepatosplenomegaly, sensitizes to gram negative bacterial endotoxins, possesses antitumour activity against the Lewis lung adrenocarcinoma, shows broad spectrum of antiviral activity and inhibits macrosomal enzyme activity as manifested in greater than 100% prolongation in hexobarbital sleeping time[15,16]. Poly (acrylic acid–maleic anhydride) with a viscosity slightly higher than DIVEMA also shows a hepatosplenomegaly but not to the extent of DIVEMA. PAAMA sensitizes to endotoxin, shows equal antitumour activity to DIVEMA, possesses antiviral activity against encephalomyocarditis (EMC) virus and did not produce a significant effect on microsomal enzymes as indicated by the prolongation of hexobarbital sleeping time. Although maleic anhydride polymer slowed the growth of the primary Lewis lung tumour, it did not cause a significant increase in life span. However, it did provide a moderate degree of protection from EMC virus. Maleic anhydride polymer caused a slight hepatosplenomegaly but did not alter the hexobarbital sleeping time. In these latter polymers, the molecular weight may have been too low to provide either tumour or antiviral activity and are being further investigated.

DECREASED TUMOUR GROWTH IN POLYANION-TREATED MICE

The primary tumour systems used for testing of antineoplastic activity are Lewis lung carcinoma, Friend leukemia virus, and leukemia L1210. Although the latter tumour has many advantages, it is primarily affected by drugs active against rapidly proliferating tumours. Little if any antitumour activity against the L1210 has been reported using polyanion polymers[17]. Lewis lung carcinoma is transplantable as subcutaneous implants. A 25 mg tumour transplant has a 100% take and the time of death ranges between 11 and 45 days with metastases occurring as early as 48 h post implant. Most organs are involved but the lung is most susceptible to metastases and death is usually due to respiratory inadequacy. The Lewis lung adenocarcinoma is a low growth fraction solid metastasing neoplasia which very closely mimics host human solid tumour that are poorly responsive to presently available chemotherapeutic drugs.

To evaluate DIVEMA as an antitumour agent, Lewis lung carcinoma was transplanted subcutaneously in two groups of mice[10]. One group was treated with 0.15 M sodium chloride and the other with DIVEMA intraperitoneal injection (i.p.). After implantation, tumours in the saline treated animals grew through from 0.1 to 1.0 cm^3 in 14 days. However, from 14 to 28 days the tumour volume increased to 9.5 cm^3 and average survival was 31.0 ±1.2 days. The group given a daily i.p. injection of 50 mg DIVEMA/kg on days 1–8 after tumour transplantation only showed an increase of 0.1 cm^3 from day 1–33. Further, DIVEMA treated mice survived an average of 50.4 ±0.9 days with several surviving to 60 days. Autopsies indicated that pulmonary metastasis was the cause of death. Further metastatic tumours were observed in tissue sections of lung of saline-treated mice at day 15, but of DIVEMA-treated mice at day 28. These data exhibit DIVEMA's potential as an effective agent in depressing tumour growth as well as prolonging the life span of Lewis lung infected mice. Intraperitoneal administration of DIVEMA in doses from 0.1 to 75 mg/kg to mice infected with Lewis lung carcinoma re-

Table 2 Comparison of antitumour activity of polyanionic polyanionic polymers

	Dose (mg/kg)	Days after tumour implant[a]		
		15	24	Mean survival time in days
Control		1304 ± 104	4627 ± 317	29.4 ± 1.0
Maleic anhydride homopolymer	25	1074 ± 184 (18%)	2078 ± 724	
	50	774 ± 200 (41%)	2387 ± 492 (49%)	30.2 ± 1.5
	75	390 ± 99 (70%)	1505 ± 234 (78%)	33.8 ± 2.6
Acrylic acid–maleic anhydride copolymer	25	856 ± 130 (34%)	2309 ± 446 (50%)	39.4 ± 1.3
	50	322 ± 69 (75%)	1576 ± 271 (66%)	37.0 ± 2.5
	75	337 ± 141 (74%)	1053 ± 359 (77%)	39.1 ± 2.8
DIVEMA copolymer	25	546 ± 130 (58%)	1889 ± 353 (59%)	36.4 ± 3.5
	50	473 ± 106 (76%)	2159 ± 468 (54%)	37.2 ± 1.1
	75	319 ± 109 (76%)	1542 ± 284 (77%)	41.0 ± 2.3

[a] Tumour weight derived from tumour measurements, mean ± s.e. derived from 15 control mice and 8 treated mice per group, polymers administered i.p. on days 1–10 after tumour transplantations

sulted in a biphasic curve[18]. Although the dose-response curve of cytotoxicity for normal cells paralleled that of the tumour cells, it was much lower, suggesting the selectivity of DIVEMA to activate the immune response to tumour cells. To evaluate the antitumour activity of other polyanions, we conducted a comparative study of maleic anhydride homopolymer and acrylic acid–maleic anhydride copolymer with DIVEMA copolymer. The results are listed in *Table 2*. Acrylic acid–maleic anhydride copolymer was as effective as DIVEMA in both suppressing tumour growth (50–77%) as compared to DIVEMA (59–77%) as well as exhibiting an increase in mean survival time by 33%. Although maleic anhydride homopolymer was effective in suppressing tumour size 49–78% it did not change the mean survival time to any significant extent. We have since found that maleic anhydride homopolymer is more effective against the primary tumour than the metastatic cancer.

EFFECT OF MOLECULAR WEIGHT OF POLYANIONS ON BIOLOGICAL ACTIVITY

A variety of fractionations, degradations, and synthetic techniques have been used to produce active antitumour DIVEMA samples with different molecular weights. Degradation and large scale fractionations of DIVEMA on sand columns both failed to give consistent and/or enough material for evaluation[19]. Breslow subsequently prepared DIVEMA copolymer at low temperature in acetone with tetrahydrofuran as a chain transfer agent using a free radical initiator. This method provided controlled molecular weight as well as narrow polydispersity copolymers. These copolymers were then evaluated for biological activity by Regelson *et al.*[20]. It was found that low molecular weight samples of DIVEMA copolymer with narrow molecular weight distribution are not only lower in toxicity but retain the antitumour activity exhibited by the higher molecular weight samples against both Ehrlich adrenocarcinoma and Lewis lung carcinoma in mice. The serum glutamic pyruvate transaminase (SGPT) levels and inhibition of drug metabolism increased with increasing polymer size, as did sensitization to endotoxin. Phagocytosis was stimulated by low molecular weight polymers and depressed by high molecular weight polymers.

In our laboratory we have isolated from Hercules XA 124-177 DIVEMA copolymer, a broad molecular weight range polymer, two fractions with lower molecular weight and narrow polydispersity. These fractions were obtained by using membrane ultrafiltration techniques. *Table 3* compares the biological activities of the original XA 124-177 DIVEMA copolymer and two lower molecular materials which were obtained from the PM-10 and PM-30 membrane filters. It was observed that the parent DIVEMA XA 124-177 caused the expected hepatosplenomegaly, sensitization to endotoxin and inhibition of the microsomal enzymes as manifested by the increased hexobarbital sleeping time. However, the polymer fractions that were obtained by passing the polymer through the PM-10 and PM-30 filters exhibited lower intrinsic viscosities and showed much higher LD_{50}'s than the parent polymer, caused no hepatosplenomegaly, did not sensitivze to bacterial endotoxin, maintained their antitumour activity but were devoid of the antiviral activity. They did not inhibit the microsomal enzyme as measured by hexobarbital sleeping time. Both fractions were active against Lewis lung carcinoma but only the larger molecular weight fraction retained its activity against encephalomyocarditis. In addition, similar to the parent DIVEMA, both PM-10 and PM-30 fractions included peritoneal macrophages activated to destroy tumour cells non-specifically while not affecting normal cells. Also, both of these fractions increased the liver and spleen weight only slightly compared to parent DIVEMA and increased the LD_{50} (endotoxin) from 0.12 mg/kg for parent polymer to 15 mg/kg for each of these fractions. Consequently the use of discrete molecular weight fractions does have a decided effect on the toxicities of these polymers without much loss on their antitumour activity.

The fractionation of poly(acrylic acid–maleic anhydride) copolymer (PAAMA) was carried out by the same technique. *Table 4* compares the antitumour activity for the whole polymer PAAMA with the polymer which would pass through the PM-50 membrane filter. The antitumour activity of these two polymer preparations were both effective as measured by primary tumour size and increased life span. Although the large whole polymer appeared to suppress tumour size more effectively, the lower molecular weight fraction produced higher increased life spans. Whole

Table 3 Pharmocologic studies of DIVEMA fractions

Properties	Control	DIVEMA XA 124–177 whole polymer	PM-10	PM-30
Intrinsic viscosity	–	0.21	0.05	0.06
Toxicologic properties:				
LD_{50}		74 (69–79)	120 (105–135)	115 (108–122)
Liver weight[2] (% body weight)	5.4 ± 0.4	7.8* ± 0.2	5.1 ± 0.2	5.9 ± 0.8
Spleen weight[2] (% body weight)	0.36	1.08* ± 0.04	0.40 ± 0.04	0.44 ± 0.03
LD_{50}[3] (Endotoxin) (mg/kg)	25	0.12	15	15
Hexobarbital Sleeping time (min)[4]	36.8 ± 2.6	97.6* ± 4.1	42.8 ± 3.8	48.6 ± 5.3
Antitumour and antiviral properties:				
Antitumour[5] activity Lewis lung (% inhibition)	0	76*	69*	64*
Antiviral (EMC) (% protection)	0	89*	0	30

PM-10 Filtrate of XA124–177 passed through Amicon PM-10 filter.
PM-30 Filtrate of XA124–177 passed through Amicon PM-30 filter.
[1] LD_{50} (95% confidence limits) calculated by the method of Litchfield and Wilcoxon. Polymers administered intravenously. Mortality recorded after 24 h. [2] Polymers administered in a dose of 25 mg/kg intravenously. Organ weights determined 7 days after drug injection and expressed as percent of total weight (Munson, *et al. J. Reticuloendothelial. Soc.* 1970, **7**, 375). [3] LD_{50} of *S. typhosa* 0904 lipopolysaccharide 24 h after a single administration of 24 mg/kg of polymer. (Munson, *et al. Proc. Soc. Exp. Biol. Med.* 1971, **137**, 553. [4] Group of NYLAR-A mice were inoculated with 25 mg/kg i.v. of the polymer (except Asp–Glu; 100 mg/kg). 24 h later an anesthetic dose (80 mg/kg) of sodium hexobarbital was administered i.v. and duration of anesthesia recorded. (Munson *et al. Proc. Soc. Exp. Biol. Med* 1971, **137**, 553. [5] BDF mice were inoculated with 10^6 Lewis' lung cells into right-hand gluteus muscle. Polymers were administered daily by the intraperitoneal route for 10 consecutive days following tumour implantation. Primary tumour size was determined on day 14 (% inhibition) and increased life span (% ILS) calculated from mean time to death.
* $P < 0.05$ mean ± s.e. derived from 8 mice per group. LD_{50} studies employed at least 100 animals

Table 4 Comparison of antitumour activity for PAAMA-whole polymer with PAAMA-PM-50 Polymer[a]

Treatment	Dose (mg/kg)	Body weights[b] change (g)	Tumour weights Days post tumour inoculation[c] 14	21	28	Mean survival time in days	Increased life span (%)
Physiologic saline		+0.4	2287 + 266	5457 + 366	12300 + 636	29.8 + 1.4	
Whole polymer PAAMA	25	–0.4	948* + 110	2851* + 382	ND	40.7* + 2.4	36.5
	50	–0.8	980* + 107	2938* + 366	ND	38.1 + 2.4	27.9
	100	–1.2	601* + 140	1410* + 310	ND	43.9* + 1.9	44
PM-50	25	+0.2	1318* + 404	3663 + 2008	7409 + 2115	43.9* + 1.9	47
	25	+1.5	1058* + 122	3475* + 315	8052 + 1368	49.2* + 2.1	65
	50	+1.3	1163* + 198	3737* + 246	7245 + 1257	44.1* + 1.8	48

[a] Groups of BDF_1 male mice were inoculated with 10^6 Lewis lung cells into the right hind gluteus muscle. Polymers were administered daily i.p route for 10 consecutive days following tumour implantation. Primary tumour size was determined on day 14, 21, and 28 after the inoculation of the tumour cells and increased life span (% ILS) calculated from the mean time to death. [b] Mean survival days ± standard error was calculated from *N*. [c] Inreased life span. [d] Poly(acrylic acid–maleic anhydride) – polymer before filtration. [e] Poly(acrylic acid–maleic anhydride)– passed through a PM-50 filer. [f] Change in whole body weight after 10 days of drug treatment. * $P < 0.05$ mean ± s.e. derived from 8 mice per group.
ND, Not determined

body weight loss caused by the lower molecular weight polymer was markedly less than that of the parent polymer. Further fractionation and biological studies of this polymer are in progress.

We also prepared high molecular weight poly(maleic anhydride) homopolymer (PMA) and separated this material into six fractions (A–E) ranging from 1000 to 100 000 daltons. The phagocytic index and organ weights from PMA treated mice were determined in *Table 5*. One day after injection all fractions except E depressed the phagocytic index 11–28%. On day 7 all fractions produced some stimulation of phagocytic activity with fraction B showing as much as 243% as determined by the vascular clearance of colloidal carbon[21]. The higher molecular weight fractions A and B produced the greatest hepatomegaly with an increase of 23 and 21% over control liver weights. Lung and thymus weights were not effected by any PMA fractions. The lowest PMA fraction F produced no significant change in the phagocytic index or organ weight.

PMA also was evaluated for anti-Friend leukemia activity by measuring inhibition of splenomegaly on day 10. All fractions showed anti-FLV activity but with less potency than DIVEMA. ED_{50}'s against Friend leukemia disease were determined for all PMA fractions and DIVEMA copolymer (*Table 6*). The unfractionated DIVEMA polymer proved to be the most potent against FLV possessing the lowest ED_{50} with PMA fractions, D, B, C, E, A and F in order of decreasing potency.

In order to determine the cell type(s) responsible for protection against FLV, passive transfer studies were performed using serum peritoneal exudate cells (PEC), bone marrow cells and spleen cells from PMA-D treated mice (*Table 7*). PEC (2×10^7) produced a 40% inhibition of splenomegaly and spleen cells (1.8×10^8) or 1 ml of serum produced no signi-

Table 5 Effect of poly(maleic anhydride) on vascular clearance rate of colloidal carbon and organ weights

	Molecular weight fraction	Phagocytic index day		Organ weights expressed as % body weight			
		1	7	Liver	Spleen	Lungs	Thymus
	Control	.037 ±.002 (15)	.042 ±.002 (10)	5.96 ±.31 (10)	0.55 ±0.03 (10)	0.98 ±0.05 (10)	0.17 ±0.02 (10)
A	100 000	.027 ±.003 (5)	.087 ±.012 (5)	7.35 ±.11 (6)	0.93 ±0.06 (6)	1.03 ±0.04 (6)	0.16 ±0.01 (6)
B	100 000 50 000	.027 ±.002 (5)	.101 ±.009 (5)	7.26 ±0.39 (6)	1.04 ±0.04 (6)	0.84 ±0.04 (6)	0.17 ±0.01 (6)
C	50 000 30 000	.030 ±.003 (5)	.088 ±.009 (5)	6.78 ±0.24 (6)	0.96 ±0.07 (6)	0.76 ±0.18 (6)	0.15 ±0.02 (6)
D	30 000 10 000	.027 ±.003 (5)	.095 ±.008 (5)	6.85 ±0.42 (6)	1.25 ±0.14 (6)	0.97 ±0.05 (6)	0.19 ±0.01 (6)
E	10 000 1000	.037 ±.006 (5)	.144 .033 (5)	7.14 ±0.43 (6)	0.90 ±0.14 (6)	0.97 ±0.10 (6)	0.15 ±0.01 (6)
F	1000	.027 ±.003 (5)	.0521 ±.005 (5)	6.74 ±0.24 (6)	0.60 ±0.05 (6)	1.10 ±0.04 (6)	0.18 ±0.01 (6)

Groups of Balb/c mice were injected i.v. with various fractions of poly(maleic anhydride) at a dosage level of 25 mg/kg. Vascular clearance of colloidal carbon was performed on days 1 and 7 after treatment. Organ weights (%BW) were determined 7 days after treatment. Mean ± s.e. was derived from the number of mice indicated in parentheses.

ficant inhibition of FLV disease. The leucocyte counts obtained decreased correlatively with decreased spleen weights. Further peritoneal exudate cells, serum and supernatants from PEC, harvested from mice treated with similar molecular weight fractions of PMA and DIVEMA were compared to PEC and serum from DIVEMA-treated mice (*Table 8*). FLV produced a 7-fold increase in spleen weight as compared with the naive control. PMA-C peritoneal exudate cells (2×10^7) inhibited splenomegaly by 74%. Both DIVEMA-C and DIVEMA PEC inhibited FLV splenomegaly similarly.

Table 6 Comparison of molecular weight of PMA to ED_{50} for Friend leukemia disease

Groups of Balb/c mice were treated i.p. with polymers (100 mg/kg–1.25 mg/kg) for 5 consecutive days prior to i.p. inoculation with FLV. Splenomegaly was the index of efficacy and was determined 10 days after virus inoculation

Fraction	Approximate *mw*	ED_{50} (mg/kg)
PMA–A	>100 000	3.4
PMA–B	100 000/50 000	2.1
PMA–C	50 000/30 000	2.5
PMA–D	30 000/10 000	1.6
PMA–E	10 000/1000	3.3
PMA–F	<1000	25.0
DIVEMA	Average *MW* 30 000	0.8

CONCLUSIONS

Polyanionic polymers possess many biologic activities. Two of the more important effects are antitumour and antiviral. Studies of these polymers as possible chemotherapeutic agents utilizing these effects may be summarized as follows. (a) Many polyanions such as DIVEMA and PAAMA activate the immune system to inhibit tumour cells. (b) The antitumour activity appears to be related to certain structural requirements since DIVEMA and PAAMA, copolymers of maleic anhydride, increase the life span of mice implanted with Lewis lung carcinoma significantly, while maleic anhydride copolymer has been much less effective; however, all three of these polymers are active against Friend leukemia virus. (c) The toxicities of these polymers appear to be related to both structure and molecular weight. Toxicities of DIVEMA polymer decreased with decreasing molecular weight while antitumour activity is retained with molecular weights as low as 5000. However, maleic anhydride homopolymer showed no significant change in toxicity with molecular weight. Antiviral activity (i.e. EMC) requires a higher molecular weight polymer (>30 000) than for antitumour activity for all these polymers. The toxicities of similar molecular weight polymer fractions are greatest with DIVEMA followed by PAAMA and PMA. The degree change in toxicity with change in molecular weight is also in the same order.

The future of synthetic polyanions in clinical and veterinary medicine resides on the attention given to structure of the polymer, control of molecular weight and biodegrada-

Table 7 Passive transfer of serum, spleen cells, peritoneal exudate cells and bone marrow cells from PMA-D treated Balb/c male mice Poly(maleic anhydride)-fraction D (30 000 > *MW* > 10 000) was administered i.p. in a dose of 25 mg/kg on days −6 through −2. Harvested serum and cells were injected i.p. into recipient mice on day −1. FLV was injected i.p. on day 0 and splenomegaly and leucocytes were determined on day 10. Mean ± s.e. was derived from 8 mice per group

Treatment	FLV infection	Splenomegaly (% body wt)	Inhibition (%)	Leucocytes (count/mm)	Inhibition (%)
Intact Mice:					
Normal	−	0.6 ± 0.1		9900 ± 900	
Normal	+	4.8 ± 0.6		76 400 ± 8900	
PMA–D	−	0.7 ± 0.1		10 500 ± 700	
PMA–D	+	0.9 ± 0.1[a]	95	10 200 ± 600[a]	100
Recipient Mice:					
Serum 1 ml	+	4.0 ± 0.6		57 000 ± 12 200	
Spleen cells 1.8×10^8	+	5.3 ± 0.8		91 600 ± 17 800	
PEC 2.7×10^7	+	3.1 ± 0.4[a]	40	36 400 ± 9700[a]	60
Bone marrow cells 4.5×10^7	+	5.1 ± 1.1		80 100 ± 22 900	

[a] $P < 0.05$ as compared to control group

Table 8 Effect of FLV disease of passive transfer of serum and peritoneal exudate cells from PCP-C, PMA-C, and PCP treated mice. DIVEMA copolymer fraction C (50 000 > *MW* > 30 000), poly (maleic anhydride) fraction C (50 000 > *MW* > 30 000) and unfractionated DIVEMA copolymer were administered i.p. in a dose of 25 mg/kg on days −6 through −2. PEC supernatant fluids, HMEB[b] harvested PEC and serum were injected into naive mice on day −1. FLV was injected i.p. on day 0 and splenomegaly was determined on day 15. Mean ± s.e. was derived from 8 mice per group.

Treatment	Dose	FLV Infection	Splenomegaly (% body wt)	Inhibition (%)
Intact Mice:				
Normal		−	0.5 ± 0.1	
Normal		+	3.7 ± 0.3	
DIVEMA-C		−	0.6 ± 0.1	
PMA-C		−	0.5 ± 0.1	
DIVEMA		−	1.0 ± 0.1	
DIVEMA-C		+	1.8 ± 0.2[a]	62
PMA-C		+	0.9 ± 0.1[a]	89
DIVEMA		+	1.6 ± 0.1[a]	81
Recipient Mice:				
DIVEMA-C-serum	1 ml	+	1.8 ± 0.2[a]	61
PMA-C serum	1 ml	+	1.7 ± 0.1[a]	62
DIVEMA serum	1 ml	+	1.6 ± 0.3[a]	67
DIVEMA-C PEC	2×10^7	+	1.2 ± 0.1[a]	77
PMA-C PEC	2×10^7	+	1.3 ± 0.3[a]	74
DIVEMA PEC	2×10^7	+	1.4 ± 0.4[a]	72
DIVEMA-C PEC	1×10^7	+	1.8 ± 0.2[a]	61
PMA-C PEC	1×10^7	+	2.0 ± 0.4[a]	52
DIVEMA PEC	1×10^7	+	2.1 ± 0.3[a]	49
DIVEMA-C Supernatant[b]	1 ml	+	1.0 ± 0.1[a]	84
PMA-C Supernatant[b]	1 ml	+	1.5 ± 0.2[a]	70
Hank's minimum[b] essential medium	1 ml	+	2.9 ± 0.4	

[a] $P < 0.05$ as compared to control group. [b] Supernatants were derived from the second HMEM wash of PE cells collected from DIVEMA-C and PMA-C treated mice. Supernatants and HMEM were injected 24 h prior to FLV

bility. The most immediate clinical situation of polyanions is as immunoadjuvants to enhance the biological effectiveness of relatively inactive vaccines. This application has been exhibited to be useful in the treatment of tumours and viruses[22]. Clinically, the concomitant adjuvant use of vaccines with synthetic polyanions will be feasible when the quantity of polymer necessary is below the toxic threshold.

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