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Actinomycin D Adsorbed on Polymethylcyanoacrylate Nanoparticles: Increased Efficiency Against an Experimental Tumor*

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Abstract—*This paper describes preliminary results of experimental chemotherapy using actinomycin D loaded polymethylcyanoacrylate nanoparticles on the growth of a transplantable soft tissue sarcoma of the rat. These results indicate that the use of polymethylcyanoacrylate nanoparticles as a drug carrier enhances the activity of the drug towards the subcutaneous sarcoma.*

INTRODUCTION

IT HAS been demonstrated that the encapsulation of cytotoxic drugs inside endocytosable carriers can help to improve the specific uptake of these drugs by the cancer cells and thereby reduce their toxicity towards healthy cells.

Works in this field have resulted in the development of liposomes [1, 2], macromolecular complexes [3, 4] and albumin microspheres [5]. More recently, nanocapsules of polyacrylamide have been used [6, 7]. Although their lysosomotropic character has been demonstrated [8, 9], such polyacrylamide nanocapsules are probably not degradable in biological fluids.

We have, therefore, developed a new hydrolysable drug carrier: nanoparticles made of various polyalkylcyanoacrylates [10, 11]. The advantage of these particles is that they are more or less quickly degraded depending on the length of the alkyl chain [12]. These ultrafine particles of about 0.2 μm are able to efficiently adsorb a variety of drugs, including cytotoxic agents, in a stable and reproducible manner. Furthermore, they can greatly modify the pattern of tissue distribution of these agents in the rat [13].

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This paper describes preliminary results of experimental chemotherapy using actinomycin D loaded polymethylcyanoacrylate nanoparticles against a transplantable soft tissue sarcoma of the rat. Actinomycin D was chosen as a representative cytotoxic drug because of its high adsorption to nanoparticles [12] and its activity against the soft tissue sarcoma.

MATERIALS AND METHODS

Preparation of carrier and drug

Actinomycin D loaded nanoparticles (ND) and free nanoparticles (N). Methylcyanoacrylate (100 μl) was added to an aqueous solution (25 ml) of 0.01 M HCl containing 0.4% polysorbate 20 while undergoing continuous mechanical stirring. After polymerization (30 min), the nanoparticles obtained formed a milky suspension displaying a Tyndall effect. This suspension was passed through a fritted glass filter (9–15 μm pore size) and 2.0 mg of actinomycin D was added while being continuously stirred. After 30 min the preparation was buffered to pH 7 with 0.25 ml of a 1.0 M NaOH solution and 4.75 ml of a phosphate buffer [containing 25% (v/v) of a 0.2 M KH_2PO_4 solution and 15% (v/v) of a 0.2 M NaOH solution]. Previous studies have demonstrated that, under similar conditions, 66% of the actinomycin D was bound to the nanoparticles [13]. One gram of polymer captures

approximately 13 mg of actinomycin D. The unbound drug was not removed prior to administration. Suspensions of polymethylcyanoacrylate nanoparticles without actinomycin D were similarly prepared (N).

The morphology and the size of the nanoparticles were regularly controlled using a scanning electron microscope (super Mini SM, I.S.I., Mountain View, U.S.A.) which showed that they were spherical, with a diameter of about 0.2 μm. Because nanoparticles are degraded at neutral pH, the ND and N samples were immediately injected after buffering into the rats.

Free actinomycin D (D). A solution of actinomycin D containing all of the above mentioned reagents except methylcyanoacrylate monomer was also prepared.

Animals and tumor

Adult male rats, weighing 150–250 g, of the pure LOU/dec strain [14] were used.

Among the possible tumors transplanted serially in this strain, the soft tissue sarcoma S 250 was chosen because of its high sensitivity to actinomycin D. The primary tumor appeared in 1973 in the inguinal region of a male rat following fortnightly per os administration of 16 doses of 15 mg of procarbazine.

This tumor line has subsequently been maintained in the LOU/dec strain in the ascites form.

For all experiments, 10^6 isolated viable tumor cells, suspended in Hanks' balanced salt solution, were grafted by s.c. injection in the flank of each animal. Cell viability had been determined by trypan blue exclusion. Under these conditions almost 100% of the rats developed a palpable tumor within about 10 days. Further growth of the tumor was followed by regular estimates of the surface (product of two perpendicular diameters measured with calipers), recorded in the following tables and figures.

In all the experiments, six rats were injected for each sample tested and six untreated rats were considered as a control group (C). Each treated rat received two successive injections of ND, D or N in the femoral vein under ether anaesthesia.

Detailed injection schedules (doses, injection days) are provided in Tables 1 and 2. The doses of ND, D and N are expressed in ml/kg body weight. One millilitre of ND or D contained 66 μg of actinomycin D.

Chemicals

Monomer of methylcyanoacrylate were generously provided by Loctite (Dublin, Ireland) as was the Actinomycin D by Merck Sharp and Dohme (Brussels, Belgium). The Polysorbate 20 was obtained from I.C.I. (Atlas Company, Everberg, Belgium) and the procarbazine (Natulan) from Roche Laboratories (Brussels, Belgium).

RESULTS

Figure 1 shows the evolution of the tumor size for ND, D and C groups in two different experiments (Nos. 1 and 2a). Compared to the control group, a reduction of the tumor size was noted both in D and ND injected rats.

This reduction is, however, considerably larger for the ND groups, especially after the second injection. A statistical analysis (Student's *t*-test) revealed a significant difference ($P=0.975$) in tumor size between ND and D groups (Table 1, exp. No. 1 and 2a). These observations were fully confirmed by further experiments carried out with somewhat different injection schedules (Table 1, exp. No. 2b, 2c, 3, 4a). In one case (exp. No. 3) the ratio of tumor sizes between ND and D groups was reduced to zero.

Despite the interest of these results, when the mortality is considered in each experiment there was an appreciable toxic effect with ND samples (Table 1). This toxicity, however, depended on the dose of ND injected.

Among rats injected with the half dose (1.65 ml of ND/kg body weight), no deaths occurred (exp. No. 4a). In addition, an appreciable antitumor effect was only observed for the ND group at this dosage (Fig. 2).

In order to establish whether the nanoparticles themselves may contribute to the toxicity and to the antitumor activity of ND, rats were injected with N samples. No deaths occurred during these trials and no significant difference in the evolution of the tumor size was observed with N treated rats when compared to the control group (Table 2 and Fig. 3).

DISCUSSION

The present results provide evidence that actinomycin D loaded polymethylcyanoacrylate nanoparticles have a greater inhibitory action than the free drug on the growth of the S250 sarcoma and that nanoparticles alone do

Table 1. *Compared antitumor activities of free actinomycin D (D) and nanoparticles loading actinomycin D (ND) towards the S*250 *sarcoma*

Exp. No.	Experiment schedule: Dose*	Days of injection†	Day of tumor measure†	Tumor size (mm^2)‡: C	ND	D	Tumor sizes ratio ND/D	*t*§	Mortality‖: ND	D
1	3.30	10, 15	14	926	313	672	0.47	6.536	0	0
			17	1451	467	658	0.71	2.206	1	0
			21	(s)	254	1037	0.25	8.451	3	0
2a	3.30	11, 13	17	1764	357	955	0.37	8.951	1	0
2b	3.30	12, 14	17	1764	541	930	0.58	1.776	2	0
2c	3.30	13, 14	17	1764	471	1854	0.25	16.611	4	2
3	3.30	12, 14	19	1043	0	235	0.00	2.370	2	0
			24	(s)	32	684	0.05	2.739	4	0
4a	1.65	17, 18	20	722	403	651	0.62	3.099	0	0
			24	1239	633	1012	0.63	2.858	0	0

*Volume (ml) of either ND or D per kg b.w. and per injection; actinomycin D concentration in both ND and D: 222 μg per 3.30 ml.
†The day of tumor graft was considered as day 0.
‡Means from surviving rats.
§Results of Student's *t*-test: underlined value indicates that the difference in tumor size between ND and D is significant ($P=0.975$).
‖Total numbers of deaths recorded at the day of measure (initial groups contained six rats). No death was observed among controls.
(s)Sacrificed.

Table 2. *Activity of nanoparticles (N) on the S*250 *sarcoma*

Exp. No.	Experiment schedule: Dose*	Days of injection†	Day of tumor measure†	Tumor size (mm^2)‡: C	N	Tumor sizes ratio N/C	*t*§	Mortality‖: N
4b	1.65	17, 18	20	722	611	0.85	0.737	0
			24	1239	1085	0.88	0.701	0
5	3.30	14, 15	17	1178	1154	0.98	0.185	0
		14, 16	17	1178	1101	0.94	0.715	0

*Volume (ml) of N per kg b.w. and per injection.
†See Table 1.
‡Means from six rats.
§Results of Student's *t*-test, indicating no significant difference in tumor size between C and N.
‖See Table 1.

not demonstrate any significant antitumor effect. Thus, the increased efficiency of the nanoparticle–actinomycin complex might reasonably be explained by an improved delivery of the bound drug to the malignant cells. This could result from the carrier function of nanoparticles. The exact mechanism by which nanoparticles enhance the anticancer drug activity is, however, not yet known and should be investigated.

The ND complex induced a higher mortality than the free drug. This can be explained by the tendency of the carrier to accumulate actinomycin D in several vital organs [13]. This hypothesis is supported by the fact that nanoparticles alone do not influence the mortality rate. It should be noted that the ND injected rats which died during these trials were those in which a very marked antitumor effect (regression or even disappearance of a palpable tumor) was also observed.

Data have been presented showing that the problem of toxicity could be overcome by the

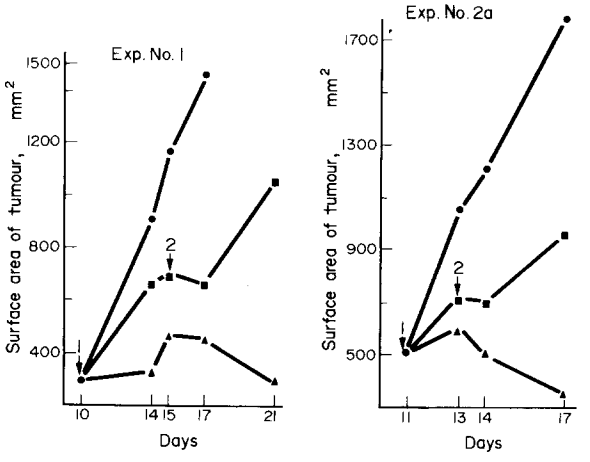

Fig. 1. Tumor size of S250 treated with free actinomycin D (D), (■); or with actinomycin D adsorbed on nanoparticles (ND) (▲); dosage: two injections (↓) of 222 μg of actinomycin D (free or sorbed) per kg b.w. *Control (C), (●).*

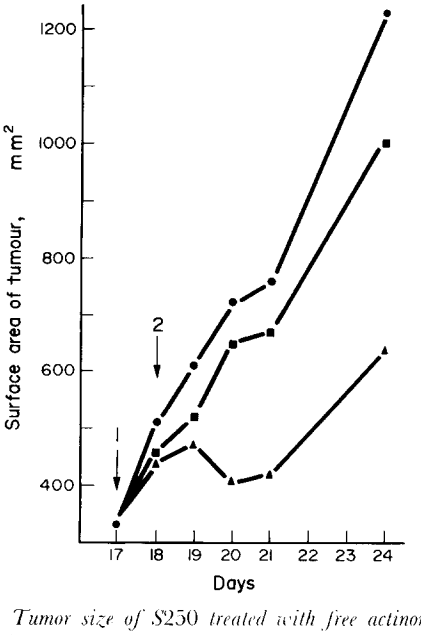

Fig. 2. Tumor size of S250 treated with free actinomycin D (D), (■); or with actinomycin D adsorbed on nanoparticles (ND), (▲); dosage: two injections (↓) of 111 μg of actinomycin D (free or sorbed) per kg b.w. *Control (C), (●).*

reduction of the dose of ND administered. A complete screening of doses and dose ratios of both polymethylcyanoacrylate nanoparticles and adsorbed actinomycin D is necessary in order to discover the optimal ND formulation.

Similar trials will subsequently be carried out with other polyalkylcyanoacrylates such as polybutyl, polyisobutyl and poly-carbalkoxyalkylcyanoacrylate which are known to be less toxic [15, 16]. Preliminary unpublished results

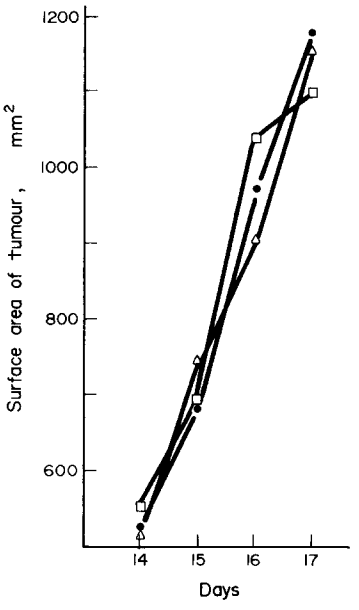

Fig. 3. Tumor size of S250 after free nanoparticles (N) injected at days 14 *and* 15 (△), *or* days 14 *and* 16 (□). *Control, (●).*

already indicate that polybutylcyanoacrylate nanoparticles present a rather different distribution pattern in rat tissues after i.v. administration when compared to the polymethyl nanoparticles. The former accumulates the drug mainly in the liver and the spleen while the latter concentrates actinomycin in the small intestine and the lungs [13].

The results reported here indicate that the use of nanoparticles may be interesting in experimental cancer chemotherapy. It is hoped that further investigations concerning the preparation, the size, the composition, the route and schedule of administration of nanoparticles as well as the choice of other drugs and tumor models may yet improve the results already obtained.

The future study of healthy and tumoral tissue disposition of nanoparticles may also permit an understanding of the mechanism leading to the enhanced effectiveness of the nanoparticle–drug complex.

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