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TISSUE DISTRIBUTION OF [^{3}H]ACTINOMYCIN D ADSORBED ON POLYBUTYLCYANOACRYLATE NANOPARTICLES

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SUMMARY

Suspensions of polybutylcyanoacrylate nanoparticles have been characterized morphologically. The influence of the adsorption of [^{3}H]actinomycin D on these particles upon the tissue distribution of the drug has been studied in rats. It has been demonstrated that 24 h after injection, adsorbed [^{3}H]actinomycin D is 5.6-, 44- and 64-fold more concentrated than the free drug in muscle, spleen and liver respectively. Furthermore, the urinary excretion of [^{3}H]actinomycin D is diminished when the drug is bound to polybutylcyanoacrylate nanoparticles.

INTRODUCTION

An obstacle to the effective use of pharmacologically active agents is their poor tissue specificity. The development of ultrafine formulations able to contain a variety of drugs would contribute to improving the selectivity of these agents. Thus liposomes (Tyrell et al., 1976; Fendler and Romero, 1977), macromolecular complexes (Trouet et al., 1974; Rowland, 1977) albumin microspheres (Kramer, 1974; Kramer and Burnstein, 1976) and nanocapsules (Kreuter and Speiser, 1976; Couvreur et al., 1977) can be useful as drug carriers owing to their size.

More recently, a new approach was realized by the development of polymethyl- and polyethylcyanoacrylate nanoparticles (Couvreur et al., 1979a and c). The main advantage of these particles is that they are more or less quickly degraded depending on the length of the alkyl chain (Couvreur et al., 1979b). These ultrafine particles of about 0.2 μm in diameter are able to adsorb with good efficiency a variety of drugs in a stable and repro-

ducible way. We have shown previously that the association of cytostatic drugs to nanoparticles of polymethyl- and polyethylcyanoacrylate can modify the distribution of these drugs in rat tissues (Couvreur et al., 1980).

However, because the methyl- and ethyl-homologs are locally highly histotoxic, possibly due to the relatively high concentration of degradation products, the less toxic higher homologs are presently receiving more attention. Indeed, the degradation products of the alkylcyanoacrylate polymers may include formaldehyde and an alkylcyanoacetate (Leonard et al., 1966). Furthermore, it has been demonstrated that the polymethyl- and polyethylcyanoacrylate polymers are dramatically cytotoxic (Rice et al., 1978). Owing to these considerations, we developed more recently polybutylcyanoacrylate nanoparticles (PBN). This paper describes the preparation of these particles loaded with [^{3}H]actinomycin D with emphasis on their distribution in various tissues of the rat.

MATERIALS AND METHODS

Materials

Monomers of butylcyanoacrylate were kindly obtained from Loctite (Dublin, Ireland). Polyoxyethylene–polyoxypropylene surfactant was purchased from Marles-Kuhlmann-Wyandotte (Paris, France).

[^{3}H]Actinomycin D (spec. act. 13.7 Ci/mmol., radioactive concentration 1.0 mCi/ml) was obtained from Amersham (M.B.L.E., Brussels, Belgium). This radiochemical was at least 95% pure, as specified in batch analysis. Non-radioactive actinomycin D was generously provided by Merck, Sharp and Dohme (Brussels, Belgium).

The chemicals for radiation counting (Soluène and Instagel) were purchased from Packard Instrument S.A. Benelux (Brussels, Belgium). Other chemical compounds were of reagent grade and used as purchased.

The tritiated molecules were measured by scintillation counting (Philips model PW 4510). The morphological appearance of the nanoparticles was observed with a transmission electron microscope (Philips EM 301).

Preparation of polybutylcyanoacrylate nanoparticles loading [^{3}H]actinomycin (PBN)

Nanoparticles of PBN were prepared by polymerization of the cyanoacrylic monomer. To an aqueous solution (10 ml) of 0.01 M HCl containing 0.2% of dextran 70 and 0.8% of a polyoxyethylene–polyoxypropylene surfactant (Pluronic L63), [^{3}H]actinomycin D (400 μl) was added. Then, under mechanical stirring, butylcyanoacrylate (150 μl) was dropped in the medium. After polymerization (at least 2 h), the milky suspension of nanoparticles was passed through a fritted glass filter (pore size: 9–15 μm).

Likewise, a solution of free [^{3}H]actinomycin D (F) containing all the reagents mentioned above except butylcyanoacrylate was prepared.

The PBN and F solutions were stored at 8°C during the rest of the experiments.

Before injection, each 'stock solution' (1.5 ml) was buffered to pH 7 by addition of 5.5 ml of a phosphate buffer solution (containing 25% (v/v) of a 0.2 M KH_2PO_4 solution and 15% (v/v) of 0.2 M NaOH).

Morphometrical characterization of polybutylcyanoacrylate nanoparticle suspensions

For preliminary size estimations, we used a nano-sizer (Coulter) based on the scatter-

ing of light arising from a laser source. The morphological properties of polybutylcyanoacrylate nanoparticle suspensions were established by stereological analysis of freeze-fracture replicas from spray-frozen samples. A detailed description of the method can be found elsewhere (Guiot et al., 1980).

Determination of [^{3}H]actinomycin D adsorbed on PBN

The PBN suspension was centrifuged at 20,000 r.p.m. After separation and dissolution of the sediment in dimethylformamide, [^{3}H]actinomycin D was measured in both sediment and supernatant by scintillation counting.

Animals

Male Wistar rats weighing between 180 and 220 g were used in all experiments. The animals were anaesthetized with ether.

Procedures of injection and preparation of rat tissues for ^{3}H analysis

Two groups of 23 rats were injected by puncture in the femoral vena. Each group received one of the two forms of [^{3}H]actinomycin D: F and PBN in a single injection of 0.80 ml. Thus the doses of radioactivity administered to each rat were about 4 and 6 μCi respectively for PBN- and F-injected rats. At 0.5, 3 and 24 h, respectively, 11, 6 and 6 rats were sacrificed in each of the two groups.

Triplicate samples of blood and fresh tissues including spleen, small intestine, muscle (pectoral muscles and muscular wall of the abdomen), kidneys, liver and lungs were taken for [^{3}H]analysis. The samples of organ were solubilized in 1 ml of 0.5 N quaternary ammonium, then they were decolorized using 0.2 ml of H_2O_2 (30%) followed by warming to 40°C during 30 min. Blood samples (200 μl) were solubilized in 1.5 ml of quaternary ammonium in toluene/isopropanol (1/1) and decolorized with 0.5 ml of H_2O_2. After cooling

TABLE 1

TISSUE CONTENT OF [^{3}H]ACTINOMYCIN D, 24 h AFTER INJECTION (% OF INJECTED DOSE PER GRAM OF WET TISSUE) [a]

Organs	Free [^{3}H]actinomycin D (F) [b]	[^{3}H]actinomycin D-loaded nanoparticles (PBN) [b]	Ratios PBN/F	Student's *t*-test
Blood	0.23 ± 0.4	0.32 ± 0.05	1.39	$0.02 < P \leq 0.05$
Spleen	1.10 ± 1.04	48.75 ± 20.90	44.31	$P \leq 0.002$
Small intest.	0.99 ± 0.25	1.90 ± 0.34	1.91	$P \leq 0.001$
Muscle	0.13 ± 0.03	0.73 ± 0.16	5.61	$P \leq 0.001$
Kidneys	4.22 ± 0.46	5.05 ± 2.38	1.19	$P > 0.1$
Liver	0.70 ± 0.21	44.85 ± 8.54	64.07	$P \leq 0.001$
Lungs	0.95 ± 0.31	4.46 ± 2.31	4.69	$P \leq 0.001$

[a] Drug concentration in μCi/g of wet organ weight and per injected mCi.
[b] Results are the mean ± S.E. from 6 animals.

at room temperature, scintillation fluid (15 ml) was added to each sample and counting was performed in a liquid scintillator analyzer.

The amount of radioactivity injected was calculated on basis of triplicate samples (0.2 ml) of F or PBN form, measured with the same syringe that was used for injection. These samples were treated in the same manner as the tissues.

Drug concentration in each tissue was expressed in μCi/g of wet organ weight and per injected mCi. This expression is represented in Fig. 3 by: 'Radioactivity/g weight (% of injected dose)', and in Table 1.

Determination of the urinary excretion of [^{3}H]actinomycin D

Two groups of 9 rats were injected as described above; each group received an F or PBN sample. The rats were then placed individually into metabolic cages. Urinary samples were taken at various time intervals. Dosage was performed by liquid scintillation counting as described previosuly for blood samples.

RESULTS AND DISCUSSION

Morphometrical characterization of polybutylcyanoacrylate nanoparticles

Fig. 1 shows the general appearance of fractured polybutylcyanoacrylate nanoparticles. The contour is often irregular but in most (98%) cases the general shape of the profiles can be approximated to a circle. If the few elongated profiles are considered as ellipses, the maximal ratio between the apparent major and minor axes is smaller than 1.5. On the electron micrographs, particles often appear as formed of a porous core, surrounded by a more homogeneous ring. Size distribution of the particles performed as described (Guiot et al., 1980) is presented in Fig. 2a. A total of 250 profiles, from 2 different preparations were measured. The distribution ranges in radius from 25 to 340 nm and the average radius ± S.D. is 80.6 ± 73.2 nm. The average external surface of particles is 148,800 nm^2 and the average volume per particles is 11×10^6 nm^3.

In some case, laser light scattering technique was used to measure the average size particles. An average value of 276 nm was found for the radius. This is much larger than the value arising from the stereological analysis reported above. However, assuming Rayleigh scattering, the latter measurement is a function of the square of the particle volume and should be very sensitive to a small proportion of large particles in a population. This is clear from Fig. 2b, which represents the volume distribution for the suspension analyzed. From the stereological analysis, the particles corresponding to the average volume or to the average squared volume have a radius of 262 or 291 nm respectively. The agreement with the values from light scattering and stereological analyses may thus be considered as satisfactory; the value deduced from the stereological analysis is, however, the one corresponding to the statistical definition of the mean. These calculations stress the possible influence of the measurement techniques for evaluating the mean size of rather disperse particle populations.

In principle, the stereological analysis yields also an evaluation of the number of particles per unit volume. As explained elsewhere (Guiot et al., 1980), this parameter is, however, overestimated when the method is applied to freeze-fracture faces, which are not true planes. In the case of lipid vesicles, it was found that the overestimation could be as

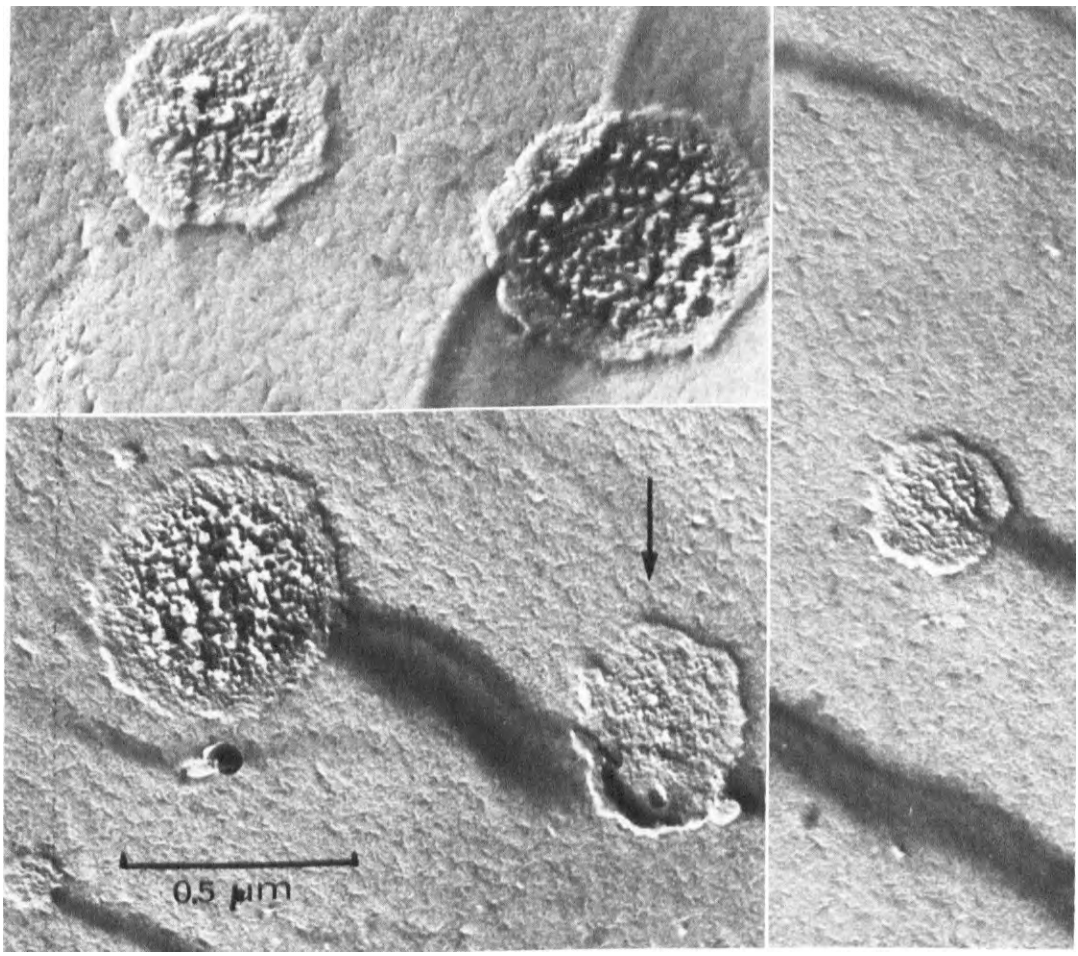

Fig. 1. General appearance of a freeze-fracture replica from spray-frozen sample of polybutylcyanoacrylate nanoparticle suspension. Contours of profiles are often irregular. Note also the porous core surrounded by a more homogeneous ring visible in several profiles. Arrow shows example of elongated profile, which form a minor component of the population.

large as 1.6 times. Hence, we can here only make an estimation of the maximum number of particles per unit volume in the suspension. This parameter can nevertheless be used to assess the efficiency of the actinomycin D adsorption to the nanoparticles. In our preparations, we had a maximum of 1.2×10^{12} particles/ml and actinomycin D was 2.4×10^{-7} M. Hence, a minimum of 120 actinomycin D molecules were on average adsorbed on a single nanoparticle. However, this assumes that actinomycin D is distributed evenly between all particles. It cannot be excluded that the distribution of the drug is a function of the surface or the volume of the particles, and in this case, the smallest one may contain much fewer molecules of actinomycin D or even be devoid of the drug.

Tissue distribution of free [^{3}H]actinomycin D and [^{3}H]actinomycin D bound to the nanoparticles

After centrifugation, 65% of the [^{3}H]actinomycin was bound to the PBN. Fig. 3

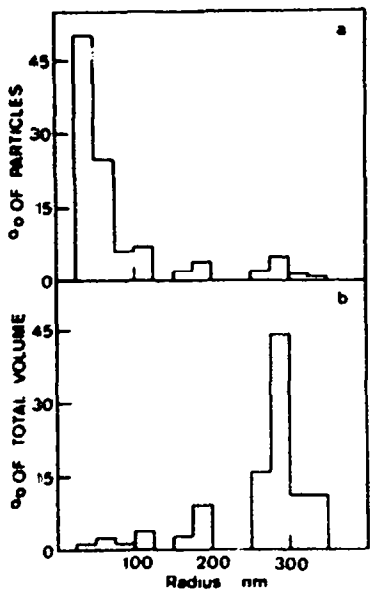

Fig. 2. Size distribution of polybutylcyanoacrylate nanoparticles. The upper graph gives the size distribution, on a number basis, as it is obtained from the profile size distribution in freeze-fracture replica. The method of Wicksell (1925) is used to transform the profile size distribution into particle size distribution. The histogram on the lower graph was obtained by calculating the total particle volume corresponding to each class of the upper histogram. The volume distribution was then normalized.

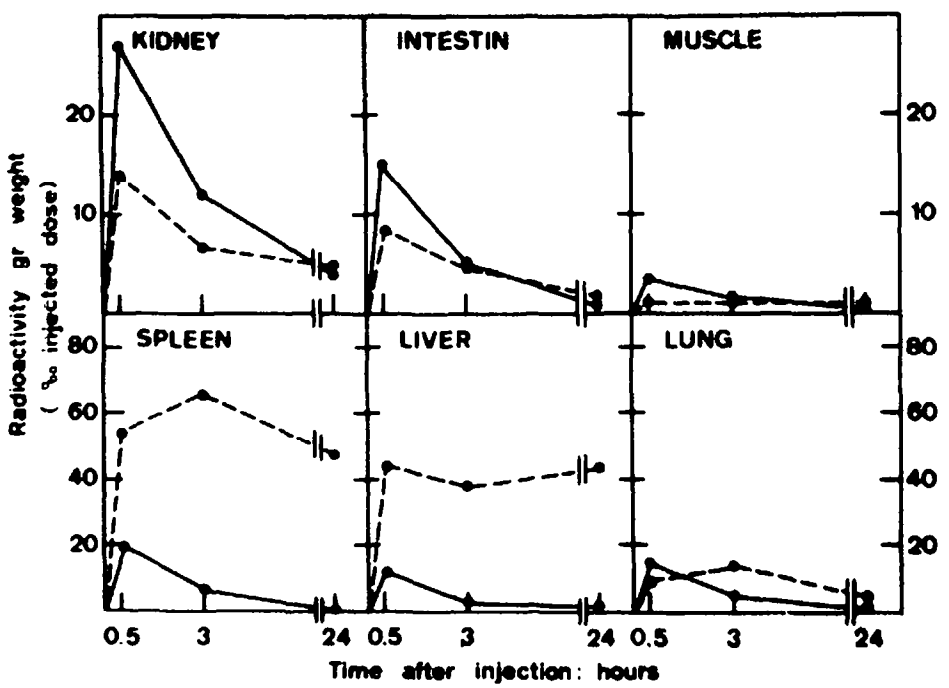

Fig. 3. Tissue concentrations of [^{3}H]actinomycin D after a single injection into rats. ———, free [^{3}H]actinomycin D; - - - - - -, [^{3}H]actinomycin D adsorbed on polybutylcyanoacrylate nanoparticles. Each point represents the mean result from minimum 6 animals. Radioactivity/g weight (% of injected dose) = drug concentration in μCi/g of wet organ weight per injected mCi.

shows that in PBN-treated rats the concentration of [^{3}H]actinomycin in liver and in spleen is strikingly increased from 1 to 24 h. Likewise the lungs' content of [^{3}H]actinomycin D is increased from 3 to 24 h. Inversely, compared to F-injected rats, PBN-treated rats showed low concentrations of [^{3}H]actinomycin D in small intestine and kidneys from 1 to 3 h after injection. Table 1 shows the concentrations of [^{3}H]actinomycin D found in blood, spleen, small intestine, kidneys, liver and lungs 24 h after administration of either F and PBN forms. The statistical analysis (Student's *t*-test) of these results indicates that PBN notably modifies the distribution pattern of the drug. Indeed, the tissue concentrations observed with PBN-injected rats are 64-fold higher for the liver, 44-fold higher for the spleen and 4.7-fold higher for the lungs compared to the F-injected rats. Likewise, after the same time, the muscle retains 5.6-fold more actinomycin D when the drug is bound to the nanoparticles.

Rats injected with the free form presented more important urinary excretion of [^{3}H]-actinomycin compared to the rats treated with PBN form (Fig. 4). The difference between the elimination levels of the two formulations is significant with a probability of 0.900 at 34 and 48 h (Table 2). These results provide evidence that nanoparticles of polybutylcyanoacrylate can be successfully used to modify the tissue distribution of actinomycin D. It is noteworthy that the carrier greatly increases the uptake of the drug by the tissues rich in reticulo-endothelial cells. Although the mechanism of this uptake is not yet completely elucidated, it could be possible that PBN gains access to the cells via endocytosis. More surprising is the increase in muscle content [^{3}H]actinomycin D when using the PBN form. Similar observations were previously made with [^{3}H]vinblastin-loaded nanoparticles of polyethylcyanoacrylate (Couvreur et al., 1980).

The lower levels of actinomycin D found in the kidneys of PBN-injected rats could be explained by the relative delay in urinary excretion.

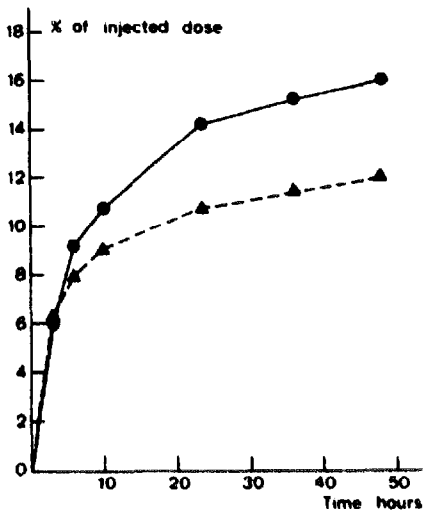

Fig. 4. Cumulative curves of urinary excretion of [^{3}H]actinomycin D after a single injection into rats. ———, free [^{3}H]actinomycin D; - - - - - -, [^{3}H]actinomycin D adsorbed on polybutylcyanoacrylate nanoparticles. Each point represents the mean from 9 animals.

TABLE 2

CUMULATIVE URINARY EXCRETION OF [^{3}H]ACTINOMYCIN (% OF INJECTED DOSE)

Time after injection (h)	Free [^{3}H]actinomycin D (F) [a]	[^{3}H]actinomycin D-loaded nanoparticles (PBN) [a]	Student's *t*-test
6	9.18 ± 1.93	8.04 ± 1.22	$P > 0.2$
10	10.97 ± 2.07	8.95 ± 1.22	$P > 0.2$
24	14.24 ± 2.46	10.89 ± 1.18	$0.1 < P < 0.2$
34	15.11 ± 3.27	11.38 ± 1.50	$0.05 < P < 0.1$
48	16.05 ± 3.26	12.15 ± 1.48	$0.05 < P < 0.1$

[a] Results are the mean ± S.E. from 9 animals.

Considering the observations previously made with polymethyl- and polyethylcyanoacrylate nanoparticles (Couvreur et al., 1980), it is interesting to note that the differential tissue distribution between free and bound drug is correlated to the polymeric nature of the carrier as well as to the nature of the transported drug. Similar observations were made with liposomes (Kimelberg, 1976; Caride et al., 1976; Juliano and Stamp, 1978).

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