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Tissue distribution of polybutylcyanoacrylate nanoparticles carrying spin-labelled nitrosourea

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Summary

The opportunities and advantages of EPR spectroscopy for studying tissue distribution of polyalkylcyanoacrylate nanoparticles are discussed. Tissue distribution of polybutyl-2-cyanoacrylate nanoparticles (PBCN) having diameters of about 100 nm, charged by 1-(2-chloroethyl)-3-(1-oxyl-2,2,6,6-tetra-methylpiperidinyl)-1-nitrosourea (spin-labelled nitrosourea, SLCNU) is investigated in C_{57} Black mice, both healthy and tumor-bearing (subcutaneously implanted Melanom B16). PBCN modifies the tissue distribution profile of SLCNU; the kidney excretion rate is reduced to one third of normal; drug accumulation in the liver and in the brain is also reduced, and drug concentrations are lowered. An increased accumulation of SLCNU loaded PBCN in the tumor tissue is estimated.

The interest in the polyalkylcyanoacrylate nanoparticles as colloidal drug transport carriers, continuously increases. This is reasonable taking into account their advantages: good biocompatibility, biodegradation (Leyh et al., 1984; Lenaerts et al., 1984a), an ability for stable and reproducible sorption of many drugs, an ability to lower drug toxicity (Couvreur et al., 1982) and improve therapeutic effect (Brasseur et al., 1980) of some anticancer preparations. Tissue distribution investigations in animals show a strong and fast entrapment by organs enriched with reticuloendothelial cells (Kante et al., 1980; Couvreur et al., 1980) and they can change tissue distribution profiles of the cytostatic drugs which they are charged with (Couvreur et al., 1980). Studies on the fate of this carrier in the body and its excretion ways are based on the radiolabelled nanoparticles (Couvreur et al., 1980; Lenaerts et al., 1984b; Grislain et al., 1983; Illum et al., 1984; Kreuter et al., 1979; Kreuter et al., 1983). EPR spectroscopy is successfully used in studying the pharmacokinetics of nanoparticles.

In the present work we demonstrate the opportunities and advantages of this method for tissue distribution investigations. This goal was attained

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by using polybutylcyanoacrylate nanoparticles (PBCN) having a diameter of about 100 nm, which are associated with 1-(2-chloroethyl)-3-(1-oxyl-2,2,6,6-tetramethylpiperidinyl)-1-nitrosourea (spin-labelled nitrosourea, SLCNU). Apart from its use as a spin-label for the nanoparticles, it is also an extremely interesting and effective cytostatic (Raikov et al., 1985; Sosnovski and Li, 1985).

Preparation of PBCN containing SLCNU. Heamodex 0.8% (w/v) (Dextran of mol. wt. 40,000, Pharmachim, Bulgaria) and 0.2% (w/v) citric acid (reagent grade, POCH, Poland) were added to 10 cm^3 distilled water and dissolved by stirring with a magnetic stirrer; 0.2 cm^3 butyl-2-cyanoacrylate (Scientific Industrial Centre for Special Polymers, Bulgaria) in which 20 mg SLCNU (prepared according to Raikov et al., 1982) was dissolved was carefully added. A polymer suspension of pH 7 was obtained for 30 min using 1 N NaOH. Stirring was continued for 2.5 h. The resulting suspension was filtered through a glass filter G3 (pore size 9-15 μm) and was injected i.p. into animals. An electron transmission microscope Philips EM 301, The Netherlands, was used in the nanoparticles' size estimation.

Procedures with animals. Two groups of C57 Black mice of 20 g b. wt. were used. The first group consisted of healthy animals and the second of animals subcutaneously implanted with tumor Melanom B16. Tumor-bearing animals were tested during the 11th–14th day after the implantation; 1 ml of nanoparticle suspension was administered i.p. to all animals in that group. Animals from both groups were killed by decapitation at intervals of 0.5, 1, 3, and 24 h. Blood samples, internal organ and peritoneal washes by DMSO (reagent grade, Fluka, Switzerland) were taken from the healthy animals. From tumor-bearing animals only tumor tissue was taken.

Fresh organs (lung, liver, spleen, brain, kidneys) and tumor tissue were weighed and homogenized with redistilled water. Blood samples were weighed and homogenized by DMSO. These tissue homogeneities were centrifuged (3000 rpm for 15 min, Centrifuge Janetzky K 70 D, Poland). Water supernatants were evaluated by an EPR-spectroscope (BRUKER EPR-spectrometer, F.R.G.). DMSO was added to every tissue sample (not to blood samples), homogenized and placed in an EPR-spectroscope.

Immediately before the spectroscope measurements 0.05 ml 1 N NaOH were added to every sample to reoxidize the reduced radical (Raikov et al., 1985).

Ultracentrifuging (20,000 rpm for 2 h, ultracentrifuge Sorval ARC-1, Du Pont, U.S.A.) of samples of the nanoparticle suspension and subsequent EPR-spectroscopy of the supernatant and precipitate (quantitatively dissolved in DMSO) revealed that 74% of SLCNU was bound to the nanoparticles.

Table 1 demonstrates the SLCNU-PBCN distribution in mice organs, calculated as a part of the total SLCNU content. Blood clears the nanoparticles away very quickly. Maximum concentration is reached 30 min after i.p. injection of the suspension and after 3 h the nanoparticles concentration is zero.

The lung distribution profile coincides with those established by other authors for nanoparticles of a different nature (Kreuter et al., 1979). Fig. 1a shows that the accumulation in the lung of the SLCNU non-entrapped in the nanoparticles is higher than the entrapped SLCNU. The highest concentration of SLCNU entrapped within nanoparticles is observed in the 30th min post-injection (a result from the reaction between the nanoparticles and blood serum elements). This concentration decreases nearly 4 times after 60 min (deagglomeration and redistribution). The secondary accumulation of the SLCNU-carrying nanoparticles in the lung (Fig. 1a) could be used for treating the lung tumors or its metastatic complication.

Surprisingly low was the concentration of the nanoparticles, carrying SLCNU in the liver (Table 1). Kreuter et al. (1983), described a similar low entrapment of PBCN carrying (75-Se)-norholestinol by the liver. As Fig. 1b shows, only SLCNU associated with the nanoparticles is entrapped by the liver. Besides, it is well known that the free SLCNU achieves its maximum concentration in the liver 10 min after i.p. injection in mice (Raikov et al., 1985).

Excluding the initial 30 min, the highest con-

TABLE 1

Tissue distribution of SLCNU-loaded nanoparticles (0, 100 μm) in the blood, liver, spleen, lung, kidney, brain of healthy mice and in the tumor-bearing mice after i.p. administration

Organs	30 min		60 min		3 h		24 h	
	mg of inj. dose/g $\times 10^{-4}$	mg of inj. dose in whole organ $\times 10^{-4}$	mg of inj. dose/g $\times 10^{-4}$	mg of inj. dose in whole organ $\times 10^{-4}$	mg of inj. dose/g $\times 10^{-4}$	mg of inj. dose in whole organ $\times 10^{-4}$	mg of inj. dose/g $\times 10^{-4}$	mg of inj. dose in whole organ $\times 10^{-4}$
Blood	13.00± 3.00	1.30±0.30	5.90±1.50	1.65±0.36	0.00	0.00	0.00± 0.00	0.00±0.00
Liver	0.00	0.00	5.80± 1.09	4.52±0.84	1.20± 0.48	1.06±0.10	0.00	0.00±0.00
Spleen	66.00±14.90	6.60±1.49	78.00±17.72	3.90±0.70	170.00±38.62	8.50±1.40	110.00±37	3.30±1.20
Lung	120.00±5.11	12.00±1.50	43.00± 6.82	5.16±1.3	43.00± 6.23	4.73±0.20	6.90± 1.70	1.66±0.40
Kidney	9.30± 2.42	3.26±0.12	41.00± 5.80	12.30±3.70	8.90± 1.70	2.49±0.60	0.00	0.00
Brain	10.00± 1.90	3.00±0.38	2.20± 0.23	0.55±0.10	1.70± 0.41	0.49±0.10	0.00	0.00
Tumor	11.00± 2.20	5.50±1.10	4.30± 1.20	2.75±0.60	21.00± 2.70	9.66±0.70	13.00±2.40	3.38±0.30

The table shows arithmetic means±S.D. obtained from 4 mice in each group.

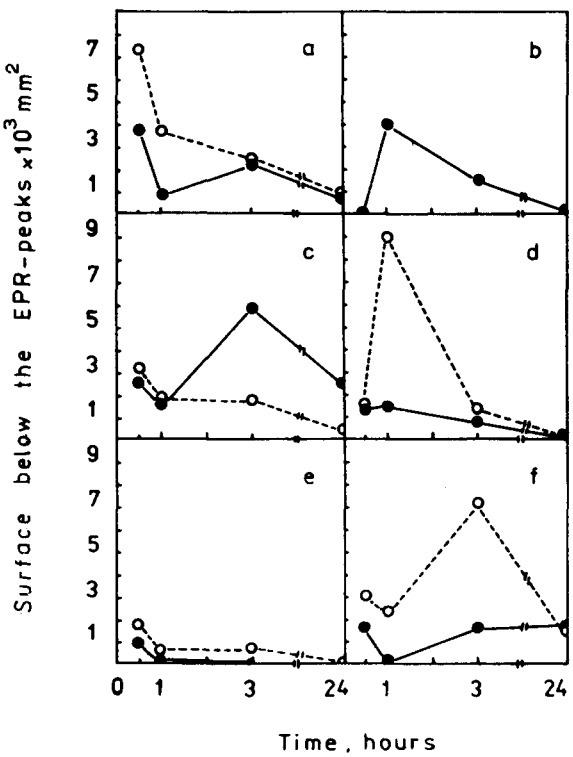

Fig. 1. Tissue distribution profile of the SLCNU entrapped into PBCN (●———●) and non-entrapped (○------○) in mice organs: (a) lungs; (b) liver; (c) spleen; (d) kidneys; (e) brain; (f) tumor (Melanom B16); determined by EPR-spectroscopy. The EPR spectra were measured at 9.44 GHz with 1 Gpp modulation and at 10 dB microwave power.

centration of the nanoparticles carrying SLCNU was measured in the spleen during the whole experimental time (Table 1). This phenomenon may be explained by the high concentration of macrophage cells in the spleen. Fig. 1c shows the entrapped and non-entrapped SLCNU distribution in the spleen. Accumulation of the nanoparticles in the spleen from the 60th min to the 3rd h is in good agreement with the possible particle redistribution between the organs and with the general spleen susceptibility to entrap them via phagocytosis.

Fig. 1d demonstrates a kidney distribution profile of the SLCNU entrapped and non-entrapped into the nanoparticles. From the 60th min up to the 3rd h postadministration the unbound SLCNU leaves the kidneys 3 times faster than the bound form.

It was established that the SLCNU association with PBCN does not readily enter the brain, where the lowest concentration of associated SLCNU was found (Fig. 1e). Studying the free SLCNU body distribution, Raikov et al. (1985) measured the highest SLCNU concentration in the spleen and the next highest in the brain.

The resultant tumor distribution profile confirms the opinion about the increased endocytose activity of the tumor cells. A higher accumulation in the spleen of healthy animals than in tumor tissue was found 24 h after administration of the suspension (Table 1). Fig. 1f shows the drug distribution in the tumor tissue, comparing the associated drug with nanoparticles and the non-associated in the suspension. The quantity of the SLCNU associated with nanoparticles smoothly increases up to the 24th hour. EPR-study on the peritoneal washings revealed that the applied dose decreases nearly 27 times up to the 24th h but still is present in a sufficient quantity as an unresorbed suspension.

In conclusion it can be said that the introduction of SLCNU bound to PBCN into the body results in an effective change of the tissue distribution profile.

The EPR-spectroscopy possibilities for studying body distribution of the nanoparticle suspension and the fate of the drugs associated with them is shown as well. The advantage of the method is its ability to give data about the fate of the unbound drug within the nanoparticles present in the suspension.

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