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New magnetic drug carrier

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We have previously demonstrated the use of polyalkylcyanoacrylate nanoparticles as a drug carrier (Couvreur et al 1979a,b). They are able to adsorb a wide variety of drugs and degrade at a rate depending on the length of their alkyl chain (Couvreur et al 1979c). Their use with adsorbed dactinomycin enhanced its activity against an experimental subcutaneous sarcoma(Brasseur et al 1980). The possibility of significantly reducing the toxicity of an anticancer drug such as doxorubicin by adsorbing it on polyisobutylcyanoacrylate nanoparticles has been demonstrated (Couvreur et al 1982). Such a carrier could profoundly modify the pattern of tissue distribution of various drugs (Couvreur et al 1980; Kante et al 1980), however, excessive accumulation by the reticuloendothelial system proved difficult to avoid. Therefore biodegradable drug-carriers with magnetic properties have been developed since, magnetic guidance of intravascular particles by an externally placed electromagnet has proved feasible (Widder et al 1978, 1980). Until now such particles were generally not smaller than 1 μm. We describe preparation of polyalkylcyanoacrylate nanoparticles of about 0·22 μm which respond to a magnetic field. Some toxicological data are also given.

Materials and methods

Materials. Radioactive monomer ([^{14}C]isobutylcyanoacrylate: spec. act. 1·4 mCi ml^{-1}) was purchased from Institut des Radioéléments (Fleurus, Belgium). The monomer was prepared according to Mc Keever (1959). [^{14}C]Formaldehyde was used in the synthesis, which resulted in the monomer being tagged on C3. Cold monomer was obtained from Loctite (Dublin, Ireland). [^{3}H]Dactinomycin (spec. act. 13·7 mmol^{-1} corresponding to a concentration of 1·0 mCi ml^{-1}) was obtained from M.B.L.E. (Brussels, Belgium). This radiochemical was at least 95% pure, as analytically specified in batch analysis. Soluène and Instagel were purchased from Parckard Instruments S. A. Benelux (Brussels, Belgium). Magnetite particles were very kindly supplied by Professor Puisieux of Pharmacie Centrale des Hôpitaux (Paris, France). Other chemicals were of reagent grade and used as purchased.

The magnets (Samarium sizing 1, 0·5, 0·2 cm, with a magnetic field of 8500 Oe) were obtained from Van Battel (Brussels, Belgium). The nanoparticle size was measured with a Coulter-Nano-Sizer (Coulter Electronics, Harpenden, U.K.) based on laser light scattering. Nanoparticles were dispersed ultrasonically (Ultrasonic Ltd, Yorks, England). Centrifugation was with a Beckman J-21C (Beckman Ltd, California, U.S.A.). Living tissues were treated with a tissue sample oxidizer (Oxymat, In-Intertechnique, Plaisir, France) before the radioactivity was measured by scintillation counting (Philips model PW 4510, Brussels, Belgium). This method permitted the separation of ^{3}H and ^{14}C (Peterson et al 1969).

Preparation of magnetized nanoparticles. Magnetically responsive polyalkylcyanoacrylate nanoparticles were prepared by anionic polymerization of the monomer in the presence of ultrafine magnetic particles of between 0·01 to 0·05 μm.

After 1 g of glucose and 1 g of citric acid had been dissolved in 100 ml of distilled water, 0·7 g of magnetite particles were dispersed by ultrasonic treatment over 15 min. The suspension was passed through a fritted glass filter (pore size 9–15 μm) to avoid magnetite agglomerates. [^{3}H]Dactinomycin (2 ml) and [^{14}C]isobutylcyanoacrylate monomer (1·5 ml) were added and stirred ultrasonically (400 W). After 3 h, nanoparticles were formed and filtered through a fritted glass filter (suspension A).

To separate magnetized nanoparticles, the suspension was allowed to flow through a magnetic field at a rate of 1 ml per 3 min, using a pumping circulation tube system. Four permanent magnets were attached to the external surface of the circulation tubes (Fig. 1). After removal of the magnets, the nanoparticles attached to the internal surface of the tubes were washed with 100 ml of an aqueous solution containing NaCl (0·7%) and $CaCl_2.2H_2O$ (0·2%). This magnetically responsive particle suspension was finely resuspended by ultrasonic treatment for 15 min, at 400 W and filtered through fritted glass (suspension B).

Polyalkylcyanoacrylate nanoparticles were prepared by the same procedure as suspension A, but without magnetite (suspension C).

The determination of the [^{3}H]dactinomycin content in the nanoparticles was performed by centrifugation of suspensions A, B and C for 1 h at 20 000 rev min^{-1}. The measurement of the ^{3}H in sediment and supernatant was made by a liquid scintillation counting technique (Couvreur et al 1979c). ^{14}C in suspensions A and B was measured and compared to determine the percentage of magnetized nanoparticles.

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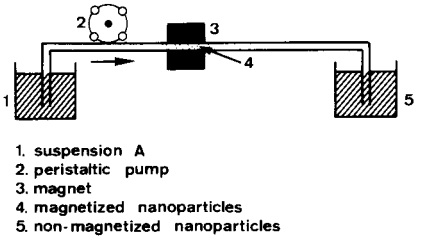

FIG. 1. Scheme of constant flow apparatus for the separation of magnetized nanoparticles.

Acute toxicity. Six groups of 10 male NMRI mice (20 g) were treated by intravenous injection of nanoparticle suspensions B and C in the tail vein to determine the LD50 of magnetized and non-magnetized nanoparticles. Each group received respectively one of the following doses in a single injection: 150, 225, 262, 300, 375 and 450 mg kg^{-1} of polyisobutylcyanoacrylate nanoparticles. The mortality was recorded 24 h after administration. The toxicity of pure magnetite suspended in aqueous solution of glucose 5% was determined using the same procedure.

Magnetic guidance testing of nanoparticles in-vivo. Mice kidneys were chosen for targeting the magnetized drug-carrier behaviour. This is because of their accessibility and of ease of comparing the drug concentration in a magnet-bearing kidney with the paired kidney.

A magnet was placed on the left kidney of each of ten mice; the right kidney was used as reference. The mice were then intravenously injected with 0·3 ml of nanoparticle suspension B. After 10 min, they were killed. Each kidney was isolated and separately homogenized with 0·5 ml of distilled water. The homogenized tissue suspension (100 µl) was treated with tissue oxidizer and its radioactivity determined.

To test the possibility of avoiding excessive accumulation of the carrier in the liver, the same experiment was made on 8 mice with a magnet on each kidney. Kidneys and liver radioactivity was determined by tissue counting as described above.

Results and discussion

Both, magnetized and non-magnetized nanoparticles had a diameter ca 0·220 µm which was reproducible; they were the smallest magnetic spheres reported in the literature (Widder et al 1978; Kato et al 1979; Morimoto et al 1980).

Comparison between ^{14}C-radioactivity obtained with nanoparticle suspensions A and B showed that 93% of the prepared carrier was magnetically responsive. Furthermore, these magnetized particles adsorbed 80% of [^{3}H]dactinomycin (suspension B) whilst non-magnetized nanoparticles adsorbed 88% of the drug (suspension C). These results showed that the binding of magnetite to nanoparticles did not significantly change the drug adsorption at the carrier.

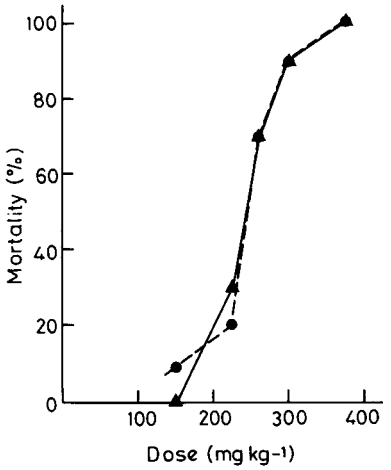

FIG. 2. Cumulative mortality of mice after i.v. administration of various doses of magnetized (– – –) and non-magnetized (——) polyisobutylcyanoacrylate nanoparticles.

With constant-flow conditions to test the magnetic susceptibility of the nanoparticles, we found that 28% w/w magnetite was necessary to obtain a high percentage of responsive magnetic nanoparticles.

Fig. 2 shows the cumulative mortality of mice after intravenous administration of different nanoparticle doses. Based on dry polymer kg^{-1}, the LD50 was determined and found to be 242 mg kg^{-1} for polyisobutylcyanoacrylate nanoparticles and 245 mg kg^{-1} for polyisobutylcyanoacrylate nanoparticles loaded with magnetite, showing that the addition of ultrafine magnetite particles to the formulation did not affect the acute toxicity of the carrier preparation. This was confirmed by the lack of mortality after administration of free magnetite particles (range of doses: 250 to 1500 mg kg^{-1}) suspended in nanoparticles polymerization medium.

Ten min after intravenous administration of magnetized nanoparticles loaded with [^{3}H]dactinomycin, an average three times higher radioactive concentration was found in the kidney bearing the magnet compared with the control. The mean radioactive concentration g^{-1} tissue was equivalent to 440 245 d min^{-1} for the left kidney and to 155 581 d min^{-1} for the right kidney. This difference was significant at P of 0·005 (Student's $t = 2{\cdot}88$).

In another experiment, nanoparticles loaded with [^{3}H]dactinomycin were injected into mice with a magnet on each kidney; the mean radioactivity found in these organs was three times higher than in the kidneys of control mice without magnet (Fig. 3). At the same time, a one third reduction of radioactivity was found in the liver of mice with magnets on their kidneys compared with the controls.

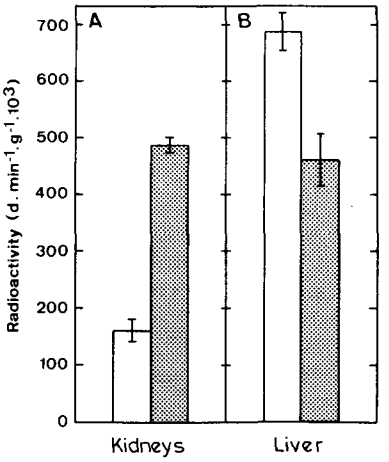

FIG. 3. Kidneys (A) and liver (B) distribution of radioactivity at 10 min after intravenous injection of magnetic polyisobutylcyanoacrylate nanoparticles. Open columns, control (no magnet); shaded columns, treatment with magnets attached to each kidney. Each column represents mean radioactive concentration (d min^{-1} g^{-1}) in the liver or in the kidney of 8 animals. Total radioactivity injected 6 840 000 d min^{-1} g^{-1}.

Conclusions

These results relate to magnetized submicroscopic biodegradable nanoparticles obtained from a polymerized isobutylcyanoacrylate in the presence of a finely dispersed magnetite. These particles with a diameter smaller than 0·3 μm are able to adsorb dactinomycin with high efficiency. Furthermore, the high magnetic susceptibility of this ultrafine drug-carrier is shown.

The addition of magnetite to the formulation does not influence the acute toxicity of the carrier. The possibility of localizing a high concentration of drug in a desired target by using nanoparticles as a magnetized drug-carrier is apparent. Furthermore, the possibility of reducing accumulation in the reticuloendothelial system was demonstrated.

The fixation of magnetite on a colloidal drug carrier could allow therapeutic levels of drugs to be attained at a desired target with smaller doses allowing side effects due to accumulation of the drug in other tissues to be reduced.

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REFERENCES

Brasseur, F., Couvreur, P., Kante, B., Deckers-Passau, L., Roland, M., Deckers, C., Speiser, P. (1980) Eur. J. Cancer 16: 1441–1445

Couvreur, P., Kante, B., Roland, M., Guiot, P., Baudhuin, P., Speiser, P. (1979a) J. Pharm. Pharmacol. 31: 331–332

Couvreur, P., Roland, M., Speiser, P. (1979b) Brevet Belge d'Invention, n° 869.107

Couvreur, P., Kante, B., Roland, M., Speiser, P. (1979c) J. Pharm. Sci. 68: 1521–1524

Couvreur, P., Kante, B., Lenaerts, V., Scailteur, V., Roland, M., Speiser, P. (1980) Ibid. 69: 199–202

Couvreur, P., Kante, B., Grislain, L., Roland, M., Speiser, P. (1982) Ibid. 71: 790–792

Kante, B., Couvreur, P., Lenaerts, V., Guiot, P., Roland, M., Baudhuin, P., Speiser, P. (1980) Int. J. Pharmaceut. 7: 45–53

Kato, T., Nemoto, R., Mori, H., Sato, S., Unno, K., Honma, M., Okada, M., Minowa, T. (1979) Pharmacology 7: 621

McKeever, C. H. (1959) US Patent, 2,912,454

Morimoto, Y., Sugibayashik, Okumura, M., Kato, Y. (1980) J. Pharm. Dyn. 3: 264–267

Peterson, J., Wagner, F., Siegel, S., Nixon, W. (1969) Anal. Biochem., 31: 189–203

Widder, K. J., Senyei, A. E., Scarpelli, D. G. (1978) Proc. Soc. Exp. Biol. Med. 58: 141–146

Widder, K. J., Senyei, A. E., Ranney, D. F. (1980) Cancer Res. 40: 3512–3517