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Note

Effect of polybutylcyano acrylate nanoparticles on primary immune response of mice to sheep erythrocytes

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Summary

The effect of polybutylcyanoacrylate nanoparticles (PBCN) on the primary immune response of mice to sheep erythrocytes (SRBC) was studied by estimating antibody production (hemagglutinins) and rosette-forming cells (E-RFC). The data obtained indicated that the ability of treated mice to establish a specific immune response was markedly impaired when PBCN were administered at high doses prior to antigenic stimulation. The degree of depression was dose-dependent. When PBCN were injected after immunization or on the day of immunization with SRBC, there were no significant differences with the control. Small doses of PBCN given before immunization or shortly thereafter stimulated the primary immune response to SRBC. These data revealed that the inductive phase of primary immune response had been affected preferably by PBCN. The conclusion might be drawn that the specific immune response induced in animals before treatment with PBCN would not be affected by nanoparticles and that they could be used as a drug carrier in chemotherapy.

Polyalkylcyanoacrylate (PACA) nanoparticles have been developed as a colloidal lysosomotropic carrier, designed to achieve site-specific drug delivery and, hence, to increase the therapeutic index by reducing drug distribution in non-target tissue (Couvreur et al., 1979; Couvreur et al., 1980a,b, 1982). These nanoparticles are well tolerated in vivo because of their biocompatibility, biodegradability (Grislain et al., 1983; Lenaerts et al., 1984; Leyh et al., 1984), bioelimination and low toxicity (Kante et al., 1982).

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The uptake of nanoparticles by marcophages of the reticuloendothelial system (RES), mainly the liver and spleen macrophages and to a lesser degree the bone marrow macrophages (Kreuter et al., 1979; Sjöholm and Edman, 1979; Couvreur et al., 1980a,b; Illum and Davis, 1982; Illum et al., 1982, 1984; Grislain et al., 1983; Leu et al., 1984; Gipps et al., 1986, 1988), defined the relevance for an investigation of the action of PACA nanoparticles on some immunological indices in mice.

The present study was undertaken to evaluate the effect of polybutylcyanoacrylate nanoparticles (PBCN) on the primary immune response of mice to sheep erythrocytes (SRBC) – antibody produc-

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tion (hemagglutinins) and rosette-forming cells (E-RFC).

0.2 ml of butyl-2-cyanoacrylate monomer (Scientific Research Centre for Speciality Polymers, Bulgaria) were dispersed at room temperature in 10 ml of distilled water containing 0.2% (w/v) citric acid (POCH, Poland) and 0.8% (w/v) dextran 40 (Pharmachim, Bulgaria) under magnetic stirring. After polymerization was complete (3 h), the resultant suspension was adjusted to pH 7.0 with 1 N NaOH. All solutions used, with the exception of the monomer, were sterile and aseptic techniques were used throughout the preparation of the polymer suspension.

The diameter of nanoparticles, measured by photon correlation spectroscopy (PCS) (Malvern 4700 C, Malvern Instruments, U.K.) was 154 ± 5 nm.

Male inbred BALB/c mice, 8–10 weeks old and weighing 20–30 g, were used throughout the experiments. Mice had free access to standard rodent chow and water.

Healthy mice were injected intraperitoneally (i.p.) with non-toxic doses of PBCN, namely, 400.0, 200.0, 100.0, 50.0, 10.0, 5.0, and 2.5 mg/kg. PBCN were administered before immunization on day -5 and -1, on the day of immunization designated 0 and after immunization on day $+2$. The control animals were only immunized.

Sheep red blood cells (SRBC) preserved in Alsevers solution, were washed three times with sterile saline to remove serum proteins and adjusted to a concentration of 10^9 cells/ml. Groups of mice ($n = 5$–8) were immunized by i.p. injection of 2×10^8 SRBC/mouse.

The primary immune response (antibody production and rosette-forming cells) was tested individually for each mouse on day 5 after immunization. The mice were bled via the retro-orbital plexus. The titre of antibodies (hemagglutinins) was read by recording the $\log_2$ of the highest dilution of serum which produced complete hemagglutination. Rosette-forming cells were measured within the splenocytes and were expressed as a percentage. Preparation of single-cell suspensions from the spleens and formation of rosettes with SRBC were as described previously (Kostadinov et al., 1979). Briefly, the spleens were teased and erythrocytes were removed using Tris-buffered NH_4Cl solution. Splenocytes were then washed three times in Hanks' balanced salt solution and adjusted to 10^7 cells/ml in tissue culture medium (RPMI 1640, Sigma) supplemented with 5% fetal calf serum (FCS, Sigma). The cell viability was estimated by the trypan blue exclusion test. Equal volumes (250 μl) of splenocytes and SRBC (1%) were mixed and centrifuged (5 min, $150 \times g$). E-RFC were measured after overnight incubation at 4°C.

Student's t-test was used for statistical analysis. The results were expressed as mean $\pm$ S.D.

The results in the present paper have shown that the treatment of mice with different doses of PBCN (50.0, 100.0, 200.0 and 400.0 mg/kg) before immunization, on day -5 and -1, significantly reduced levels of the serum antibody titers (Figs 1A and 2A) and the percentage of E-RFC (Figs 1B and 2B). The degree of inhibition was dose- and time-dependent. The greatest suppression was induced with the highest doses of 400.0 mg/kg of nanoparticles, given on day -5 as shown in Fig. 1A,B. In contrast, the same doses of PBCN, administered shortly after immunization, on day 0 or on day $+2$, did not induce significant differences in serum antibody titers (Figs 1A and 2A) and percentage of E-RFC (Figs 1B and 2B) in comparison with the control. Small doses of 2.5 and 5.0 mg/kg of nanoparticles, given before immunization or shortly thereafter, stimulated the primary immune response against SRBC (Fig. 3A,B).

These results indicated that the effect of PBCN was dependent on the phase of the immune response, both inductive and productive, and that the inductive phase was affected preferentially. The time dependence of the effect of nanoparticles might be explained to some extent by their tissue distribution and especially their uptake by the macrophages of RES, as mentioned above. A possible explanation is that the PBCN themselves as well as the products of their biodegradation block or stimulate the antigen-presenting function of the macrophages and in this way the induction of the immune response. Further studies should evaluate the role of the degradation products (n-butanol and polycyanoacrylic acid).

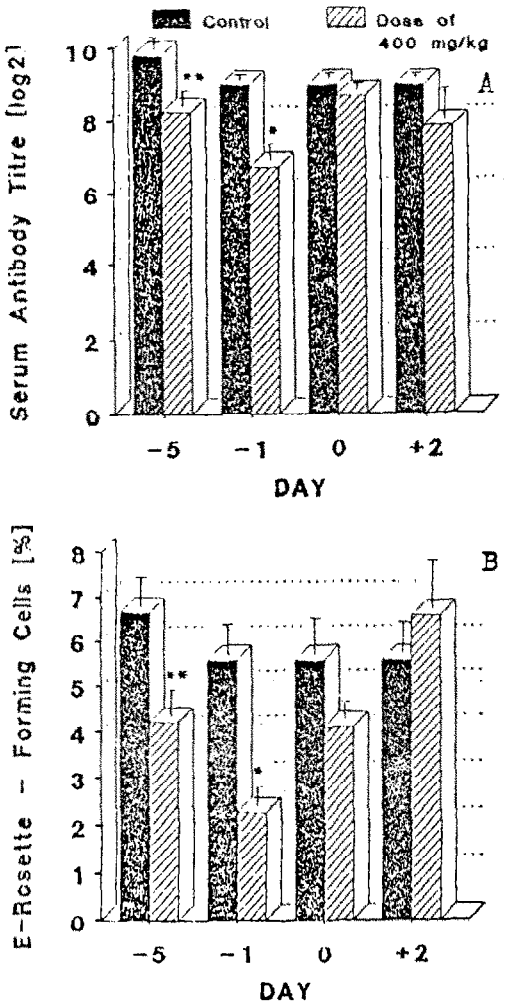

Fig. 1. Time-dependent effect of a single i.p. dose of 400 mg/kg of PBCN on primary immune response of mice to SRBC: (A) serum antibody titres (hemagglutinins), expressed as $\log_2$ of the highest serum dilution capable of the complete agglutination of a 1% suspension of SRBC; (B) E-RFC within splenocytes, expressed as a percentage. Immune response was measured on day 5 after the immunization with SRBC (2×10^8 SRBC/mouse). Each bar represents the mean of 5–8 mice ± S.D. P values: * < 0.001; ** < 0.02.

On the other hand, the PBCN did not affect the proliferation of committed cells, their differentiation to antibody formers and the synthesis and release of antibodies.

Therefore, in conclusion, we might suggest that a specific immune response induced before the treatment with PBCN would not be affected by

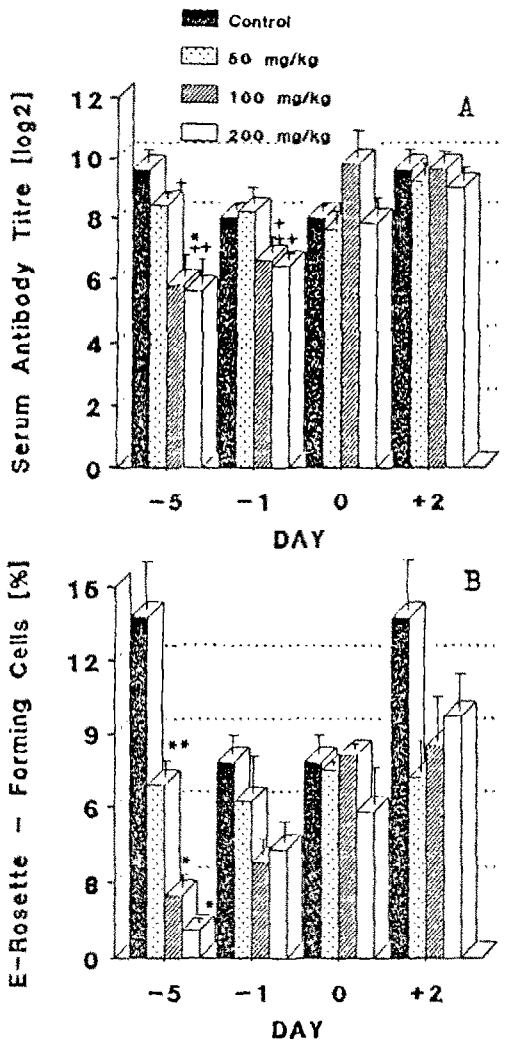

Fig. 2. Time-dependent effect of single i.p. doses of 50, 100, and 200 mg/kg of PBCN on primary immune response of mice to SRBC: (A) serum antibody titres (hemagglutinins), expressed as $\log_2$ of the highest serum dilution capable of the complete agglutination of a 1% suspension of SRBC; (B) E-RFC within splenocytes, expressed as a percentage. Immune response was measured on day 5 after the immunization with SRBC (2×10^8 SRBC/mouse). Each bar represents the mean of 5–8 mice ± S.D. P values: * < 0.001; ** < 0.02; $^{++}$ < 0.01 and $^{+}$ < 0.05.

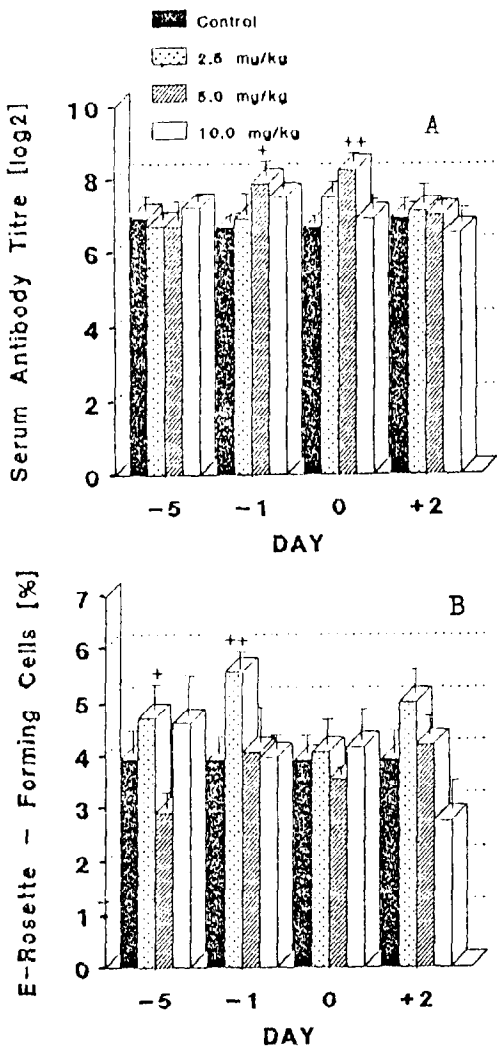

Fig. 3. Time-dependent effect of single i.p. doses of 2.5, 5.0, and 10.0 mg/kg of PBCN on primary immune response of mice to SRBC: (A) serum antibody titres (hemagglutinins), expressed as $\log_2$ of the highest serum dilution capable of the complete agglutination of a 1% suspension of SRBC; (B) E-RFC within splenocytes, expressed as a percentage. Immune response was measured on day 5 after the immunization with SRBC (2×10^8 SRBC/mouse). Each bar represents the mean of 5–8 mice ± S.D. P values: $^{++} < 0.01$; $^{+} < 0.05$.

them. These data might be useful when the nanoparticles are used as a drug carrier in therapeutic protocols.

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