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Effect of opsonins on the macrophage uptake of polyacrylstarch microparticles

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Summary

The macrophage uptake of polyacrylstarch and polyacrylamide microparticles was investigated in vitro. Polyacrylstarch microparticles were rapidly phagocytosed by macrophage monolayers. The uptake was dependent on opsonization and could be partly inhibited with a monoclonal antibody against a macrophage-specific receptor for complement. Moreover, the polyacrylstarch microparticles activated complement of the alternative pathway. The uptake of polyacrylamide microparticles by the macrophages was 50–100 times lower and not dependent on opsonins. The polyacrylamide microparticles did not activate complement of the alternative pathway and the uptake was not inhibited by monoclonal antibodies. These results may explain the different vascular half-lives of the polyacrylstarch microparticles ($t_{1/2} < 5$ min) and polyacrylamide microparticles ($t_{1/2} = 60$ min).

Introduction

When a small particulate material is introduced into the circulation, it will normally be opsonized and rapidly cleared from the blood by the macrophages of the reticuloendothelial system (RES). Therefore, small particles or colloids have been utilized as drug carriers for the selective delivery of drugs to the RES (Davis et al., 1984). The rapid clearance from the circulation of foreign particles such as microorganisms or microparticles is an important part in the host defence system against various infectious agents (Stålenheim, 1981). Since the microparticles localize to the same compartment as many infectious agents, the microparticulate drug delivery system has been used to treat severe infections in animals (Alving, 1983; Lopez-Berestein et al., 1984) and more recently in humans (Lopez-Berestein et al., 1985).

However, the efficient delivery of microparticles to the RES has been frustrating to investigators who wish to target the microparticles to other sites in the body (Poste, 1983). Therefore, attempts have been made to circumvent the RES uptake of the particles. Artursson et al. (1983) showed that the half-life of microparticles could be increased by coating the microparticles with monomethoxy-polyethyleneglycol, but no change in the distribution pattern was found. Illum and Davis (1983, 1984) found that colloidal particles

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coated with poloxamer 338 were partly diverted from the RES and they surmised that this property was due to the minimal adhesive properties of this surfactant. This is supported by results obtained in vitro, which showed that poloxamer-coated emulsions were taken up at a very slow rate by macrophage monolayers (Davis and Hansrani, 1985).

Although the opsonization process is considered one of the most important mechanisms in host defence and most probably is of importance in the clearance of any particulate material from the circulation, the opsonization of microparticulate drug carriers has not been extensively studied (Kondo, 1984). Thus no attempts have been made to analyze the importance of complement for the blood clearance and macrophage uptake of microparticles.

In this study, the vascular half-life, macrophage uptake, and complement-activating properties of polyacrylamide and polyacrylstarch microparticles were investigated. Moreover, a macrophage receptor of importance for the uptake of the microspheres was identified. Knowledge of these factors may be of importance for the understanding of the mechanisms underlying the rapid elimination of various particulate drug carriers from the circulation.

Materials and Methods

Materials

Maltodextrin, a soluble hydrolysate from potato starch (M.W.: 5000), was a gift from Dr. Lars Svensson, Stadex AB, Malmö, Sweden. [^{14}C]Starch (M.W. 7000–8000; spec. radioact. 1.9 mCi/mg) and 2,3-[^{14}C]acrylamide (spec. radioact. 75.3 mCi/mmol) were obtained from New England Nuclear, Boston, MA. Complement fixation test diluent tablets were obtained from Oxoid, Basingstoke, Hants, U.K. Rabbit blood in Alsevers solution was purchased from Statens Veterinärmedicinska Anstalt, Uppsala, Sweden. Male NMRI mice weighing 18–22 g were used for in vivo studies and were obtained from A-lab, Solna, Sweden and Balb/C mice, 6–12 weeks old, used as sources for macrophages and mouse serum, were obtained from Bomholtsgård, Ry, Denmark. The tissue culture media were purchased from Gibco-Biocult, Paisley, Scotland. Monoclonal rat IgG antibody against mouse type three complement receptor (MI/70; Beller et al., 1982) was purchased from Sera-lab Ltd., U.K.

Preparation of microparticles

Microparticles were prepared essentially according to a reported method (Artursson et al., 1984). A solution of acrylamide (6% w/v) and N,N′-methylenebisacrylamide (2% w/v) or acryloylstarch (10% w/v) in sodium phosphate buffer (0.1 M, pH 7.4) containing 1×10^{-3} M EDTA were used to produce polyacrylamide microparticles and polyacrylstarch microparticles, respectively. Ammonium peroxydisulphate (0.43 M, pH 7.4; 200 μl) was added to the deoxygenated monomer solution (5 ml) and the water-phase was homogenized in an organic phase consisting of toluene/chloroform (4:1, v/v; 300 ml) containing poloxamer 188 as detergent. Upon addition of N,N,N′,N′-tetramethylethylenediamine (100 μl) the monomer in the water droplets polymerized to microparticles. The microparticles were freed from the organic phase by extensive washes with distilled water and physiological saline. To produce radioactive microparticles [^{14}C]acrylamide or [^{14}C]acryloyl starch was included in the monomer solution. The polyacrylamide microparticles used for i.v. injection had a mean diameter of 0.9 μm and a specific radioactivity of 2.02×10^6 dpm/mg dry weight, while the corresponding microparticles used for the in vitro experiments had a mean diameter of 1.4 μm and a specific radioactivity of 3.94×10^6 dpm/mg dry weight. The polyacrylstarch microparticles had a mean diameter of 1.5 μm and a specific radioactivity of 1.96×10^6 dpm/mg dry weight. The mean size of the microparticles was estimated by scanning electron microscopy as reported previously (Artursson et al., 1984). About 90% of the polyacrylstarch microparticles had a diameter of 0.5–2.2 μm. The polyacrylamide microparticles were characterized according to the nomenclature suggested by Hjertén (1962) and the polyacrylstarch microparticles according to the same nomenclature as modified by Edman et al. (1980). Thus, the poly-

acrylamide microparticles had a T-C-value of 8–25 while the polyacrylstarch microparticles were characterized by the D-T-C-value of 10–0.5–0. The microparticle suspensions were autoclaved and stored in sealed injection vials at 4°C until further use.

Blood clearance of microparticles

0.5 mg of radioactive microparticles were injected into the tail vein of the mice and blood samples were collected with heparinized capillary tubes from the orbital plexus at different times after the injection (Artursson et al., 1983).

Opsonization of microparticles

10 mg of packed microparticles were suspended in 0.5 ml of fresh mouse serum and the mixture was incubated at 37°C for 30 min. The opsonized microparticles were washed 3 times in tissue culture medium lacking fetal calf serum and immediately added to the macrophages.

Macrophage uptake

Macrophages were collected from the peritoneal cavity of untreated mice and seeded on cell culture dishes (diameter 5.0 cm) at a concentration of 1.0×10^6 cells/ml. After incubation for 45 min, non-adherent cells were removed by 8 washes with phosphate-buffered saline. By this procedure, > 99% of the adherent cells were macrophages as characterized by morphology and uptake of latex particles. The cells were incubated in RPMI 1640 supplemented with 10% fetal calf serum, benzylpenicillin (100 U/ml), and streptomycin (10 μg/ml) at 5% CO_2–air and 95% humidity for 24 h, before addition of microparticles. All uptake experiments were performed in the same medium without fetal calf serum.

Opsonized radioactive microparticles were added to the monolayers at a concentration of 0.25 mg/ml and the cells were incubated for 15, 30, 60, 120, 180, 240 and 360 min. At these times, the macrophage uptake was stopped by 8 careful washes of the monolayers. The macrophages were then treated with sodium dodecyl sulphate (1% in distilled water for 60 min) and the radioactivity of the solubilized cells was determined in a liquid scintillation counter.

Incubation with monoclonal antibodies

A monoclonal antibody against the type three complement receptor was added to the macrophages in saturating amounts 15 min prior to incubation with the microparticles (Ezekowitz et al., 1984). The antibodies were present throughout the incubation period.

Activation of the alternative complement pathway

The capacity of the microparticles to activate the alternative complement pathway was measured according to Richer and Stanworth (1980). An optimal dilution of fresh human serum supplemented with 1×10^{-3} M EGTA to block the classical complement pathway caused 50% hemolysis of unsensitized rabbit erythrocytes when incubated for 30 min at 37°C, as measured in a spectrophotometer at 413 nm. If the serum was preincubated with microparticles prior to the treatment with rabbit erythrocytes, a possible complement consumption of the microparticles could be detected as a decreased lysis of the rabbit erythrocytes. The complement activation capacity of the microparticles was expressed as % of inhibition of the lysis of the rabbit erythrocytes, after subtraction of appropriate blanks.

Results

Blood clearance of microparticles

The circulatory half-life of the polyacrylamide microparticles was estimated as reported previously (Artursson et al., 1983), and was found to be 60 min. The polyacrylstarch microparticles were more rapidly eliminated from the circulation and after 15 min, only 2.4 ± 1.4% of the total dose per ml blood remained in the circulation (Fig. 1). This corresponds to 4% of the injected dose as estimated from a total blood volume of 1.7 ml. The half-life of the polacrylstarch microparticles was estimated to be less than 5 min.

Uptake of microparticles by macrophages

Opsonized microparticles were added to serum-free macrophage cultures and the uptake of the ^{14}C-labelled spheres was followed for 6 h (Fig 2). The polyacrylstarch microparticles were rapidly

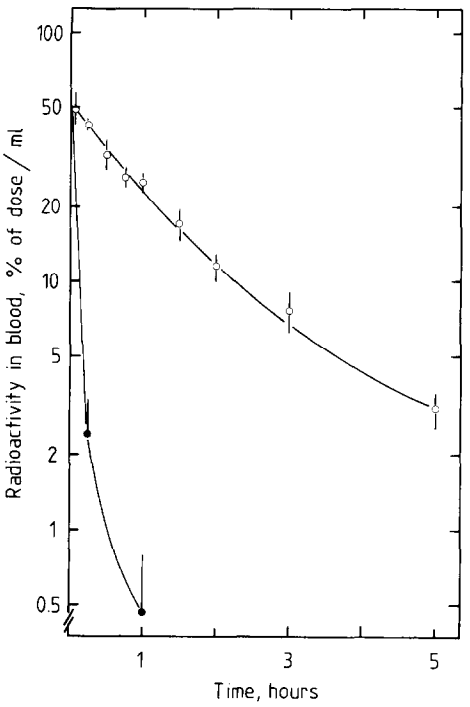

Fig. 1. Disappearance of radioactive polyacryl starch (●) and polyacrylamide microparticles (○) from blood.

taken up by the macrophages and after 1 h 10.7 ± 1.3 $\mu g/10^6$ cells were phagocytosed by the macrophages. After this time the rate of uptake levelled off and after 4 h in culture 10.3 ± 2.6 $\mu g/10^6$ cells of polyacrylstarch microparticles were taken up by the macrophages. The uptake of polyacrylamide microparticles was much lower and showed no plateau. After 1 h, 0.1 ± 0.05 $\mu g/10^6$ cells were internalized and the corresponding value at 6 h was 0.5 ± 0.1 $\mu g/10^6$ cells. Thus, after 1 h, the uptake of polyacrylstarch microparticles was about 50 times higher than the uptake of polyacrylamide microparticles.

The uptake of unopsonized microparticles by the macrophages was also investigated. One hour after addition of 0.25 mg/ml microparticles to the monolayers, 2.2 ± 0.2 $\mu g/10^6$ cells polyacrylstarch microparticles and 0.1 ± 0.05 $\mu g/10^6$ cells polyacrylamide microparticles were phagocytosed by the macrophages (data not shown). Thus, the uptake of polyacrylstarch microparticles increased about 5 times when the spheres were opsonized while the uptake of polyacrylamide microparticles

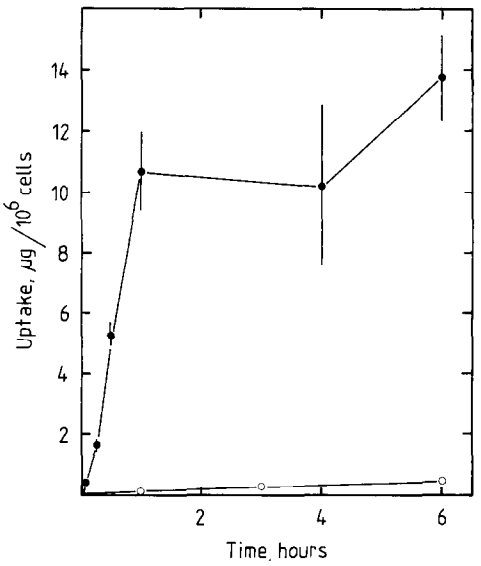

Fig. 2. Uptake of opsonized polyacrylstarch (●) and polyacrylamide (○) microparticles by macrophages.

was essentially unaffected by the opsonization procedure.

Activation of the alternative pathway of complement by microparticles

Microparticles were incubated with fresh human serum and the microparticle-depleated serum was tested for the capacity of lyze unsensitized rabbit erythrocytes. By this procedure, activation of the alternative pathway of complement by the microparticles can be detected as a decreased capacity of the serum to lyze the erythrocytes (Richer and Stanworth, 1980). Incubation of polyacrylstarch microparticles with fresh serum resulted in a dose-dependent activation of the alternative complement pathway (Fig. 3). At the highest concentration of microparticles, complement consumption was maximal and 98% of the complement activity was adsorbed on the microparticles. The polyacrylamide microparticles did not consume significant amounts of complement.

Inhibition of macrophage uptake of microparticles

The relative contribution of the type three complement receptor to the uptake of opsonized microparticles was investigated by means of a monoclonal antibody directed against this receptor.

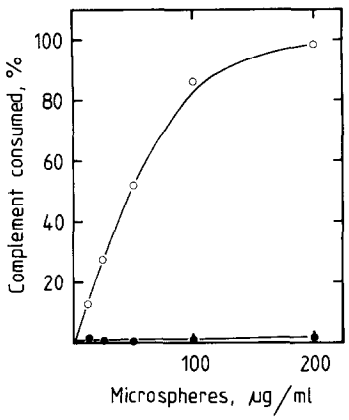

Fig. 3. Dose-dependent activation of the alternative complement pathway by polyacrylstarch microparticles (○). The corresponding amounts of polyacrylamide microparticles did not activate complement (●).

When macrophages were preincubated with anti-complement receptor antibody, the uptake of polyacrylstarch microspheres decreased from 9.8 $\pm$ 0.7 $\mu g/10^6$ cells to 3.6 $\pm$ 1.7 $\mu g/10^6$ cells ($P < 0.05$). When polyacrylamide microparticles were incubated with the anti-complement receptor antibody no significant difference in uptake was found as compared with controls.

Discussion

Since complement-dependent opsonization can be activated even before antibodies have been produced against an invading microorganism, this system is considered as one of the most important mechanisms against infections (Stålenheim, 1981). For instance, the rate of phagocytosis of a number of *Salmonella* strains was reported to be dependent on the ability of the bacteria to activate complement of the alternative pathway and, more importantly, was inversely proportional to the virulence of these strains (Liang-Takasaki et al., 1982).

To investigate if complement activation was of importance for the clearance of microspheres from the vascular compartment, two types of microparticles with different circulatory half-lives, i.e. polyacrylamide and polyacrylstarch microparticles, were investigated for their ability to activate complement of the alternative pathway. It was found that the polyacrylstarch microparticles, having a half-life of less than 5 min, effectively activated complement, while no complement activation was found for the polyacrylamide microparticles having a half-life of about 1 h. The importance of opsonization for the uptake of polyacrylstarch microparticles was demonstrated in cell cultures. When these microparticles were opsonized, they were more rapidly taken up by the macrophages than the corresponding untreated microparticles. The opsonization procedure did not significantly increase the uptake of polyacrylamide microparticles. In addition, polyacrylamide microparticles were taken up to a lower extent. This was not due to the reported smaller size of the polyacrylamide spheres (Sjöholm and Edman, 1979) since the polyacrylamide microparticles used here were specially prepared to have the same size as the polyacrylstarch microparticles.

During the emulsion polymerization of the microparticles, poloxamer 188 was added as a surfactant. It may therefore be surmised that the hydrophobic central part of this surfactant is adsorbed to the surface of the more hydrophobic polyacrylamide microspheres in a similar way as suggested for polystyrene microspheres (Illum and Davis, 1984), whereas the hydrophilic polyacrylstarch microparticles would not adsorb the surfactant. Since poloxamers increase the half-life of microparticles (Illum and Davis, 1983) and decrease the macrophage uptake of emulsions (Davis and Hansrani, 1985), the possible absorption of poloxamer 188 to the surface of polyacrylamide microparticles may explain the longer half-life and lower macrophage uptake obtained with these microspheres.

The relative importance of the complement type three receptor for the uptake of microparticles by the macrophages was also investigated.

When the type three complement receptor was inhibited with a specific monoclonal antibody, a 63% reduction of the uptake of microparticles was noted after 1 h. This may be compared with the 50–90% inhibition of zymosan uptake by different macrophage populations obtained with the same antibody (Ezekowitz et al., 1984). These results indicate that the opsonized polyacrylstarch micro-

particles were partly taken up through the complement type three receptor on macrophages. However, since the uptake was only partly inhibited with the anti-complement receptor antibody, it is probable that in addition to complement other serum proteins such as albumin, immunoglobulins or fibrinectin also were present on the particle surface. Thus, a monoclonal antibody directed against the Fc-receptor for immunoglobulins on macrophages partly inhibited the uptake of the opsonized polyacrylstarch microparticle (unpublished results). These undefined serum components may also contribute to decrease the circulatory half-life of the microspheres since polyacrylamide microparticles coated with human serum albumin showed a shorter half-life and were more rapidly phagocytosed by macrophages than the corresponding uncoated microparticles (Artursson et al., 1983).

The macrophage specific mannose-fucose receptor did not interact with the uptake of polyacrylstarch microspheres (Artursson et al., unpublished results 1985).

In summary, these results show that complement adsorption as well as more unspecific coating of microparticles with other serum proteins seem to be of importance for the macrophage uptake of polyacrylstarch microparticles. The same factors may also be of importance for the clearance of these as well as other microparticulate carrier systems from the circulation.

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