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Circulation time and body distribution of protein A-coated amino modified polystyrene nanoparticles in mice

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Abstract

Amino modified polystyrene nanoparticles of size 240 nm were covalently coated with protein A (cell wall protein of *Staphylococcus aureus*, Cowan strain) using glutaraldehyde as a spacer. The protein A was then radiolabeled using [^{14}C]formaldehyde and sodium cyanoborohydride by the reductive alkylation method. Blood circulation time and body distribution of ^{14}C-labeled protein A-coated particles were studied in mice. After rapid intravenous injection through the tail vein, the coated particles showed higher blood concentrations for the first 3 min compared with that of the uncoated particles. The increased circulation time of protein A-coated particles appears to be the result of reduced uptake by the liver.

Keywords: Circulation time; Body distribution; Protein A; Polystyrene nanoparticle

The circulation time and body distribution of particulate drug carriers, such as liposomes and nanoparticles, have been a subject of considerable interest due to the potential application of such systems in targeting of drugs to specific sites in the body. Surface characteristics (Troster et al., 1992; Dunn et al., 1994) and particle size (Kanke et al., 1980; Illum et al., 1982; Moghimi et al., 1991) are among the parameters of particulate drug carriers that have been varied to influence their circulation time or body distribution. The intent of most of the strategies is to prolong the blood circulation time by reducing the accumulation in the organs of the mononuclear phagocyte system (MPS) which is also called the reticuloendothelial system (RES), such as the liver. In general, increasing surface hydrophilicity of nanoparticles has been shown to decrease phagocytic uptake (Van Oss, 1978; Arturson et al., 1983).

Efforts to prolong the blood circulation time or change the body/tissue distribution of particulate drug carriers have also been made by suppressing the phagocytic function. This was achieved by saturating the MPS using dextran sulfate (Souhami et al., 1981; Patel et al., 1983), colloidal carbon (Freise et al., 1981), several rare earth metals (Lazar, 1973), polystyrene particles (Kao and Juliano, 1981), empty liposomes (Dave

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and Patel, 1986), liposomes containing toxic agent (Van Rooijen and Van Nieuwmegen, 1984), and toxic agents (Roerdinck et al., 1981). However, suppression of this important host defense mechanism leads to increased susceptibility to infection and shock (Loegering, 1983) and, therefore, is not practical.

Phagocytosis is believed to be the major mechanism responsible for removing foreign particulate matter or bacteria from blood circulation and for their accumulation into the MPS organs (Tabata and Ikada, 1991). One of the major phagocytic mechanisms of removal of particles from circulation is phagocyte-mediated uptake of antibody-bound particles through recognition of the Fc portion of the bound antibody. However, some bacteria can escape phagocytosis by various ways. *Staphylococcus aureus* Cowan I produces a cell wall protein, called protein A, which binds to the Fc portion of IgG, leaving the Fab portion free (Forsgren and Sjoquist, 1966). Because the IgG that is bound to the bacteria has no outward representation of Fc moieties, it can therefore escape phagocytosis (Van Oss, 1986). It is now well documented that protein A binds to various subclasses of IgG from most mammalian species, including the mouse (Chalon et al., 1979). Since phagocytes possess cell membrane receptors to recognize only the Fc portion of antigen-bound antibodies, protein A antibodies should not be recognized by these receptors. This concept leads to the hypothesis that phagocytic uptake of protein A-coated particles will be decreased, resulting in a longer blood circulation time of the particles.

Amino-modified polystyrene particles having a particle size of 240 ± 2 nm were obtained from Polysciences (Warrington, PA) as a 2.5% w/v aqueous suspension. The particle size was determined by the vendor using the centrifugal photosedimentation technique on a Brookhaven Particle Size Analyzer, and was determined in our laboratory to be 300 ± 38 nm using photon correlation spectroscopy on a Coulter type N4MD instrument (Hialeah, FL). The particle size was monitored in our laboratory only for the purpose of keeping track of the change in particle size, if any, during the process of labeling and coating. For the purpose of data treatment and discussion, the vendor-specified size has been used. [^{14}C]Formaldehyde was obtained from NEN Research Products, and sodium cyanoborohydride from Aldrich Chemicals. Protein A (from cell walls of *S. aureus*, Cowan strain, chromatographically purified grade) and glutaraldehyde (70% aqueous solution) were obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were of analytical grade and were obtained from Sigma Chemical Co. or from Fisher Scientific, Pittsburgh, PA.

Male Swiss-Webster mice weighing 33–36 g (Taconic Farms) were conditioned to the housing environment, which consisted of a 12 h light/12 h dark cycle. The rats were fed a standard laboratory diet and drinking water.

Amino-modified polystyrene nanoparticles were coated with protein A by covalently binding the protein to the particle surface using a modified two-stage method for cross-linking with glutaraldehyde (Otto et al., 1973). The first step is the activation of nanoparticles with glutaraldehyde; the second step is the coupling of the activated nanoparticles with protein A.

In the first step, the suspension of nanoparticles (0.5% suspension in a 0.02 M phosphate buffer solution, pH 7.0) was added at a rate of approximately one drop every 15 s to a 35% solution of glutaraldehyde (in 0.02 M phosphate buffer solution, pH 7.0) using a syringe dispenser (Sage Instrument, Cambridge, MA). The unbound glutaraldehyde was remove by washing the nanoparticles in a buffer solution three times followed by ultracentrifugation at 50 000 rpm, at 4° C for 30 min. The activated nanoparticles were suspended in a 0.01 M phosphate buffer solution, pH 7.0, in the presence of 0.02% of sodium azide to give a final concentration of about 0.5%. The size of the activated particles was determined by photon correlation spectroscopy (Coulter model N4MD, Hialeah, FL) to ensure that there was no aggregation. In the second step, which followed immediately after washing, the activated particles were added dropwise to a solution of 1% protein A in 0.01 M phosphate buffer solution containing 0.02% sodium azide using the syringe dispenser and addition rate as in the first step. 3 ml of

freshly prepared 3.2 M sodium cyanoborohydride was then added into this mixture and gently agitated for 1 h. The protein A-coated particles were washed by ultracentrifugation as in the first step and resuspended in a 0.01 M phosphate buffer, pH 7.0 in the presence of 0.02% of sodium azide to provide a final particle concentration of about 2.5%. The size of the final protein A-coated particles indicated that aggregation did not occur. Radiolabeling of the protein A was conducted immediately after this step.

The protein A-coated and uncoated particles were radiolabeled by the reductive alkylation method using [^{14}C]formaldehyde and sodium cyanoborohydride, adapted from the method for ^{14}C labeling of proteins (Dottavio-Martin and Ravel, 1978). Briefly 25 μCi of [^{14}C]formaldehyde in 0.25 ml of 0.04 M phosphate buffer, pH 7.0 was added into 5 ml of 2.5% suspension of protein A-coated nanoparticles. After 3 h of gentle agitation, 1.25 ml of freshly prepared 3.2 M sodium cyanoborohydride was added to this reaction mixture; stirring was continued for another 3 h. The resulting ^{14}C-labeled protein A-coated nanoparticles were washed by ultracentrifugation as above. The size of the particles was remeasured using photon correlation spectroscopy to rule out any aggregation during labeling.

Covalent binding of protein A to the particles was verified by incubating the particles in a solution of 0.1% non-radioactive protein A. This was carried out at room temperature with gentle stirring for 24 h. The particles were removed by ultracentrifugation at 50000 rpm at 4° C for 1 h. The supernatant fluid showed no radioactivity, indicating that no exchange had taken place between the bound (radioactive) protein A and protein A in solution. The number of protein A molecules per particle was determined by analyzing the initial and the final amount of free protein A in the reaction mixture using the BCA method of protein assay (Pierce, Rockford, IL). The number of particles used in the coating can be calculated using the percent concentration of particle suspension, density, and size.

Prior to injection, the suspension of labeled protein A-coated nanoparticles was made isotonic by adding dextrose solution to give a final dextrose concentration of 5%. Particle size of the nanoparticles was measured by photon correlation spectroscopy prior to injection to rule out any aggregation.

The isotonic suspension of the labeled protein A-coated nanoparticles was rapidly injected intravenously into the tail vein of mice. Five animals were used for each time point. The number of particles injected was about 2.19×10^{11}. At times 1, 2, 3, 5, 10, 15, and 30 min after injection, the animals were anesthetized and then killed by decapitation; blood, liver, spleen, and lungs were collected.

Each of the blood, liver, spleen and lung samples was individually weighed and solubilized in a tissue solubilizer (Solvable, NEN Research Products) at 50° C for 12 h, yielding clear fluids. An aliquot was taken from each of these samples and was decolorized by adding 30% solution of hydrogen peroxide to minimize color quench (in the presence of the tetrasodium salt of EDTA to reduce foam formation). Scintillation cocktail was added to each of the decolorized samples and the solutions were then neutralized by adding 0.5 M HCl solution. After storing the samples in the dark for 24 h, the radioactivity was measured using a liquid scintillation counter. The standard quench curve and background for each organ was incorporated into the scintillation counter during measurement of the samples.

Dose preparation, dose administrations, and organ sample preparation for constructing standard quench curves and determining backgrounds for each type of organ were exactly the same as those for the ^{14}C-labeled particles described above, except that non-radioactive amino-modified polystyrene particles were used instead of ^{14}C-labeled amino-modified polystyrene particles.

The organ distribution and blood profile of protein A-coated nanoparticles are shown in Fig. 1. Blood, liver, spleen, and lungs patterns of protein A-coated vs non-coated nanoparticles are presented in Fig. 2 and 3. In general, protein A coating of amino-modified polystyrene nanoparticles does not dramatically modify their organ distribution and blood profiles. A higher number of coated particles was found in the blood for the first 3 min after injection. The slopes of the

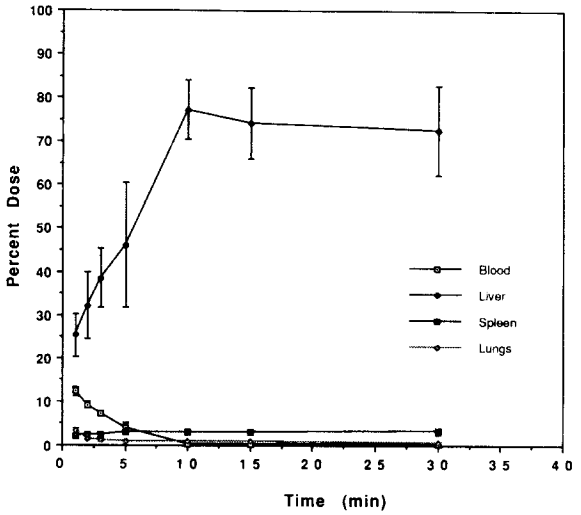

Fig. 1. Body distribution of protein A-coated nanoparticles. Concentrations in blood are in percent of dose per g of blood. Concentrations in organs are in percent dose per whole organ. Bars are standard deviations for five studies.

curves for blood concentration of coated and uncoated particles are not significantly different. However, the curve for the coated particles appears to be shifted to the right. This can be an indication that the onset of phagocytosis is delayed for the coated particles. At 5 min after injection, 3.9% of the administered protein A-coated particles were present per g blood as compared with 2.6% for the uncoated particles. However, only a small fraction of the injected particles were found in blood at 10 min after injection, for both the coated and uncoated particles.

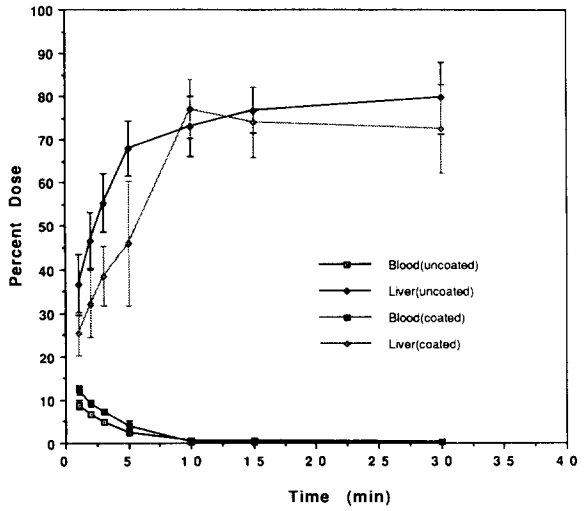

Fig. 2. Blood and liver profiles of protein A-coated vs uncoated particles. Concentration is in percent dose per g blood. Bars are standard deviations for five studies.

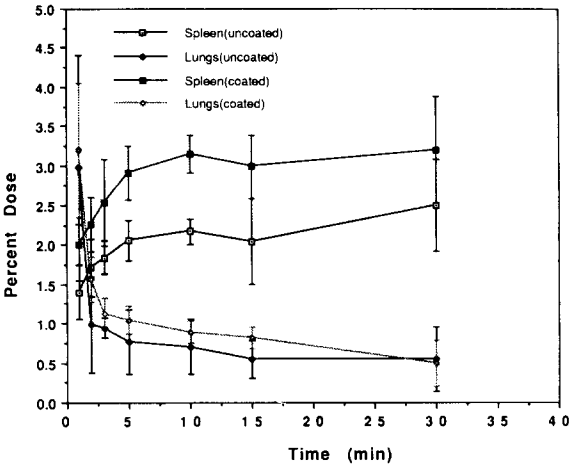

Fig. 3. Spleen and lung profiles of protein A-coated vs uncoated particles. Concentration is in percent dose per whole organ. Bars are standard deviations for five studies.

There are no significant differences in the spleen and lung uptake between coated and uncoated particles. In both cases, accumulation stays below 3.5% at all times. However, in general, the liver accumulation of the coated particles is less than that for the uncoated particles during the first 5 min. Therefore, it appears that the change in circulation time for the two kinds of particles resulted from the difference in the liver distribution.

Many factors could account for the fact that the change in circulation time for coated particles is small. Among these is the possibility that the binding affinity of protein A with IgG, which is reported to be fairly strong in solution (Chalon et al., 1979), may have changed as a result of the change in protein A conformation after its binding to the particle surface and ^{14}C labeling. Also, the density of the particle surface amino groups may not be high enough to allow a complete particle surface coating of protein A. The rela-

tively large size of protein A (Mol. Wt 42000) may cause steric hindrance to prevent more free protein A from approaching the particles leading to an incomplete coating. These factors could lead to insufficient attachment of IgG to the protein A-coated particles.

The ratio between the number of protein A molecules bound to a particle and the number of surface amino groups available on a particle is low (about 6%). This is not surprising since one molecule of protein A may bind more than one surface amino group on the particle.

It should be noted that even small changes in circulation time immediately following administration of particles may be important from a drug targeting standpoint because they will allow greater circulation of particle through the non-MPS organs. Therefore, the particles may have a better chance to be taken up the organs of interest (Poste, 1983).

References

Arturson, P., Laakso, T. and Edman, P., Acrylic microspheres in vivo: IX. Blood eliminatin kinetics and organ distribution of imcroparticles with different surface characteristics. *J. Pharm. Sci.*, 72 (1983) 1415–1420.

Chalon, M.P., Milne, R.W. and Vaerman, J.P., Interactions between mouse immunoglobulins and staphylococcal protein A. *Scand. J. Immunol.*, 9 (1979) 359–364.

Dave, J. and Patel, H.M., Differentiation in hepatic and splenic phagocytic activity during reticuloendothelial blockade with cholesterol-free and cholesterol-rich liposomes. *Biochim. Biophys. Acta*, 888 (1986) 184–190.

Dottavio-Martin, D. and Ravel, J.M., Radiolabeling of proteins by reductive alkylation with [^{14}C]formaldehyde and sodium cyanoborohydride. *Anal. Biochem.*, 87 (1978) 562–565.

Dunn, S.E., Brindley, A., Davis, S.S., Davies, M.C. and Illum, L., Polystyrene-poly(ethylene glycol) (PS-PEG 2000) particles as model systems for site specific drug delivery. 2. The effect of PEG surface density on the in vitro cell interaction and in vivo biodistribution. *Pharm. Res.*, 11 (1994) 1016–1022.

Forsgren, A. and Sjoquist, J., protein A from *S. aureus*: I. Pseudo-immune reaction with human globulin., *J. Immunol.*, 97 (1966) 822–827.

Freise, J., Muller, W.H. and Magerstedt, P., Uptake of liposomes and sheep red blood cells by the liver and spleen of rats with normal or decrease function of the reticuloendothelial system. *Res. Exp. Med.*, 178 (1981) 263–269.

Illum, L., Davis, S.S., Wilson, C.G., Thomas, N.W., Frier, M., Hardy, J.G., Blood clearance and organ disposition of intravenously adminustered colloidal particles. The effect of particle size, nature and shape. *Int. J. Pharm.*, 12 (1982) 135–146.

Kanke, M., Simmons, G.H., Weiss, D.L., Bivins, B.A., DeLuca, P.P., Clearance of ^{141}Ce-labeled microspheres from blood and distribution in spefific organs following intravenous and intraarterial administration in beagle dogs. *J. Pharm. Sci.*, 69 (1980) 755–762.

Kao, Y.J. and Juliano, R.L., Interactions of liposomes with the reticuloendothelial system. Effects of reticuloendothelial blockade on the clearance of large unilamellar vesicles. *Biochim. Biophys. Acta*, 677 (1981) 453–461.

Lazar, G., The reticuloendothelial-blocking effect of rare earch metals in rats. *J. Reticuloedothelial Soc.*, 13 (1973) 231–237.

Loegering, D.J., Intravscular hemolysis and RES phagocytic and host defense functions. *Circ. Shock*, 10 (1983) 383–395.

Moghimi, S.M., Porter, C.J.H., Muir, I.S., Illum, L. and Davis, S.S., Non-phagocytic uptake of intravenously injected microspheres in rat spleen: Influence of particle size and hydrophilic coating. *Biochem. Biophys. Res. Commun.*, 177 (1991) 861–866.

Otto, H., Takamiya, H. and Vogt, A., A two-stage method for cross-linking antibody globulin to territin by glutaraldehyde. Comparison between the one-stage and the two-stage method. *J. Immunol. Methods*, 3 (1973) 137–146.

Patel, K.R., Li, M.P., Baldeschwieler, J.D., Supression of liver uptake of liposomes by dextran sulphate 500. *Proc. Natl. Acad. Sci. USA*, 80 (1983) 6518–6522.

Poste, G., Liposome targeting in vivo: problems and opportunities. *Biol. Cell.*, 47 (1983) 19–38.

Roerdinck, F., Dijkstra, J., Hartman, G., Bolscher, B. and Scherphof, G., The involvement of parenchymal, Kupffer, and endothelial liver cells in the hepatic uptake of intravenously injected liposomes. *Biochim. Biophys. Acta*, 677 (1981) 79–89.

Souhami, R.L., Patel, H.M. and Ryman, B.E., The effect of reticuloendothelial blockade on blood clearance and tissue distribution of liposome. *Biochim. Biophys. Acta*, 674 (1981) 354–371.

Tabata, Y. and Ikada, Y., Phagocytosis of polymeric microspheres. In Szycher, M. (Ed.), *High Performance Biomaterials*, Technomic, Lancaster, 1991, pp. 621–646.

Troster, S.D., Wallis, K.H., Muller, R.H. and Kreuter, J., Correlation of the surface hydrophobicity of the ^{14}C-poly(methyl methacrylate) nanoparticles to their body distribution. *J. Controlled Release*, 20 (1992) 247–260.

Van Oss, C.J., Phagocytosis: An overview. *Methods Enzymol.*, 132 (1986) 3–15.

Van Oss, C.J., Phagocytosis as a surface phenomenon. *Annu. Rev. Microbiol.*, 32 (1978) 19–39.

Van Rooijen, N. and Van Nieuwmegen, R., Elimination of phagocytic cells in the spleen after intravenous injection of liposome-encapsulated dichloromethylene diphosphonate. An enzyme-hystochemical study. *Cell Tissue Res.*, 238 (1984) 355–358.