3. DISTRIBUTION OF PROTEIN THERAPEUTICS

Once a molecule reaches the blood stream, it encounters the following processes for intracellular biodistribution: distribution within the vascular space, transport across the microvascular wall, transport through the interstitial space, and transport across cell membranes. The biodistribution of macromolecules is determined by the physicochemical properties of the molecule, and by the structural and physicochemical characteristics of the capillaries responsible for transendothelial passage of the molecule from the systemic circulation to the interstitial fluid. In addition, the presence of receptors determines the biodistribution to certain tissues, including extracellular association and/or intracellular uptake. Capillary endothelia are of three types, in increasing order of permeability: continuous (nonfenestrated), fenestrated, and discontinuous (sinusoidal) (10,42). The most likely dominant mode of transport of macromolecules in nonfenestrated capillaries is through interendothelial junctions. Through these junctions, there are two modes of transport (43): the convective transport, often the most important for macromolecules, is dependent on a pressure difference between the vascular and interstitial spaces and the diffusive transport is driven by a concentration gradient.

Capillaries selectively sieve macromolecules based on their effective molecular size, shape, and charge. Because of the large size of proteins, their apparent volume of distribution is usually relatively small. The initial volume of distribution after intravenous injection is approximately equal to or slightly higher than the total plasma volume. The total volume of distribution is generally up to two times the initial volume of distribution. Although this is sometimes interpreted as a low tissue penetration, it is difficult to generalize. Indeed, adequate concentrations may be reached in a single target organ because of receptor-mediated uptake, but the contribution to the total volume of distribution may be rather small.

In addition to size, it appears that the charge-selective nature of continuous capillaries and cell membranes may also be important for the biodistribution of proteins. Information for this is available from studies with different types of Cu, Zn-superoxide dismutase (Cu, Zn-SOD), which are similar in molecular weight (33 kDa), but have different net surface charges, and are isolated from different species (44). Tissue equilibration of the positively charged sheep Cu, Zn-SOD was much faster than for the negatively charged bovine Cu, Zn-SOD. In addition, the positively charged Mn-SOD equilibrated much faster than the negatively charged human Cu, Zn-SOD, although Mn-SOD is much bigger (88 kDa). A trend towards increasing anti-inflammatory activity, for which interstitial concentrations are important, was observed with increasing isoelectric point. It was suggested that the electrostatic attraction between positively charged proteins and negatively charged cell membranes might increase the rate and extend of tissue