The Lysosome/Endosome Membrane: A Barrier to Polymer-Based Drug Delivery?

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SUMMARY: Drug delivery by means of polymer conjugates that are internalized into cells by endocytosis is now a viable therapeutic approach. Successful deployment of this model depends upon release of the free drug within the endosome/lysosome compartments and its efflux into the cytoplasm. The latter process involves the drug crossing the endosome/lysosome membrane, which is known to be impermeable to all large and many small molecules and which is equipped with numerous substrate-specific transporters that allow metabolites across. Passive diffusion is the only viable mechanism for most xenobiotics to cross the endosome/lysosome membrane. Studies are reported on the permeability of the rat liver lysosome membrane. These demonstrate that permeance of molecules correlates inversely with their hydrogen-bonding capacity, a function that can be calculated from inspection of structural formulae. It is deduced that drug molecules containing cationic and/or anionic functional groups, or numerous hydrogen-bonding moieties such as hydroxy, ether or carbonyl, will cross the lysosome membrane unacceptably slowly, but that many drugs will cross at a satisfactory rate. This conclusion is supported by the rather meagre data available on membrane permeability of lysosomes *in situ* within cells. Systematic experimental studies on the endosome membrane are lacking, but there is every reason to suppose that its permeability is similar to that of the lysosome membrane.

Introduction

It is a pleasure and a privilege to contribute to a symposium marking Jindrich Kopecek's sixtieth birthday. He and I met for the first time in 1978 in Dresden, where we planned and initiated a research collaboration that has proved unexpectedly fruitful and has led to the development of a family of drug-macromolecule conjugates with proven clinical efficacy. My involvement with the project lasted until the mid-1980s, when my colleague Ruth Duncan took over its academic leadership. I am full of admiration for the persistence and dedication with which she and others have effected the translation of an interesting scientific notion into a practicable therapeutic tool.

I have elsewhere[1] described the origins of my collaboration with Jindrich. I will confine myself here to two remarks. The first is that working with him and Pavla was always stimulating and enjoyable. I shall not forget my many visits to Prague, mostly in the winter, with long days in the Institute of Macromolecular Chemistry and weekend days cross-country

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skiing. I also want to acknowledge my and Jindrich's debt to Helmut Ringsdorf, who introduced us to each other and with characteristic generosity suggested that we should consider collaborating. I am indebted to Helmut and to Jindrich for introducing me to polymer science and infecting me with their enthusiasm for this discipline.

It is also a pleasure to return to Prague and to the Institute. In company with many other participants in this symposium, I was a speaker at the 1984 symposium here, and have vivid and pleasant memories of that occasion.

The year 2000 marks another important anniversary: 25 years ago Ringsdorf published a landmark review[2] that included his vision for macromolecule-drug conjugates. That vision was for a water-soluble polymer to which a pharmacologically active agent was attached by a biodegradable linkage. Targeting moieties would also be attached to the polymer to direct the drug to the desired site of action in the body. The conjugates of chemotherapeutic agents with poly[*N*-(2-hydroxypropyl)methacrylamide] (polyHPMA) that arose from the collaboration between Keele University in England and the Institute of Macromolecular Chemistry in Prague took that concept and developed it in the light of emerging knowledge about how living cells handle macromolecules. The key issue was an appreciation that the chemical linkage between the drug and the macromolecule had to be resistant to extracellular enzymes, such as those in the blood plasma, but susceptible to the enzymes in the lysosomes.

Lysosomes and Endosomes

Lysosomes are intracellular structures surrounded by a membrane that separates their contents from that of the surrounding cytoplasm. They have been aptly described as the cell's stomach. Within the lysosomes are a collection of digestive enzymes that can degrade biological macromolecules to their monomer units. Thus proteins are broken down to amino acids (and a few resistant dipeptides) by a battery of proteases, and glycogen is degraded to glucose. The sequential action of several enzymes breaks down complex lipids and polysaccharides to their various building blocks. The interior of the lysosome is maintained at an acidic pH, although at around 5.5 this is not as low as the stomach's. Many of the lysosomal enzymes are maximally active at pH values below neutrality.

Lysosomes are the only intracellular site accessible to most exogenous macromolecules. This is because biological membranes, including the plasma membrane, are impermeable to them. However, they can enter cells by endocytosis, a process of membrane internalization that can engulf macromolecules attached to the cell or present in its environment. After endocytosis the macromolecules are delivered to the lysosomes. This process does not involve passage through membranes: like the gastrointestinal system the lysosomes are technically continuous with extracellular space.

The endosome compartment of cells is a staging post between endocytosis and the lysosome. If the lysosome is the cell's stomach, the endosome is the mouth. Although these analogies cannot be pressed too far, they have some validity. In the endosome substrates are sorted (rather as we 'sort' grapes or olives), and there is some substrate processing but little digestion. The endosome is also the compartment through which many of the lysosomal enzymes pass *en route* to the lysosomes themselves.

Many aspects of lysosome and endosome physiology were reviewed[3] in 1996.

The Lysosome Membrane

When the polyHPMA-drug conjugates were being designed in the early 1980s, attention focused on the properties of the linkage moiety. Oligopeptide linkers with graded susceptibility to the lysosomal proteinases were identified, so that drug would be released within the lysosome. Little thought was given to the subsequent release of the drug across the lysosome membrane into the cytoplasm. It was assumed that this would occur by passive diffusion.

During the 1980s numerous publications appeared demonstrating that the lysosome membrane possesses a wide range of metabolite transporters. Each of these porters is substrate-specific for one or at most a few of the products generated by the catabolic enzymes in the lysosomes. A porter exists for neutral sugars, such as glucose, and others for acidic sugars and *N*-acetylhexosamines. At least ten porters exist for amino acids, each with its own substrate specificity. The existence of these porters was taken as strong, albeit teleological, evidence that they are necessary for the metabolites in question to leave the lysosome at a physiologically adequate rate. Direct evidence for this conclusion is available in respect of a few of the porters. The best documented example concerns cystinosis, a human genetic disorder characterized by the accumulation of cystine in the lysosomes. Cystinosis results from the absence of a functional cystine-porter from the lysosome membrane. The mechanisms by which metabolites exit from, and in a few cases enter, the lysosome have been reviewed[4].

If passive diffusion is not an adequate mechanism for molecules such as glucose and alanine to cross the lysosome membrane, what of xenobiotics? Contemplation of this question, and of the high substrate specificity of the known lysosome membrane porters, raised a serious concern that drugs delivered to the lysosome as polymer conjugates might remain trapped there and be unable to exert their desired effect. It therefore became important to discover whether non-physiological molecules can cross the lysosome membrane by passive diffusion in the absence of a porter that recognizes them. And, if this route exists, what characteristics must a molecule possess if it is to use it?

Over the past decade we have sought to answer this question. Using an osmotic-protection methodology we have studied the ability of xenobiotics to cross the rat liver lysosome membrane *in vitro*. Our first study concerned non-electrolytes[5], and this was later followed by studies on cationic[6] and anionic[7] molecules. Each study sought to test a range of chemicals, so as to identify any relationship between ease of passage across the lysosome membrane, and to avoid molecules that resembled physiological species, so as to minimize the possibility that any translocation observed was due to a porter in the membrane.

The primary publications[5-7] and a recent review article[8] describe and discuss the experimental methods used. This material is not repeated here, and we concentrate on the results and their relevance for polymer-based drug delivery.

The data are consistent with a hypothesis that the lysosome membrane has permeability properties typical of phospholipid membranes, whether natural or synthetic. The permeance of a molecule depends upon the ease with which it can pass from an aqueous to a hydrophobic environment, and this in turn correlates with log P, where P is the oil-water partition coefficient. Molecular weight is at most a secondary and minor determinant of permeability.

In a classic study, Stein[9] analysed permeability data from a variety of sources and demonstrated an inverse correlation with the notional hydrogen-bonding capacity of molecules. Hydrogen-bonding capacity is a calculated, not a measured, parameter. Each functional group on a molecule is assigned a hydrogen-bonding capacity, and these are summed to yield a cumulative value for the entire molecule. Predicting permeance on the basis of hydrogen-bonding capacity has two considerable advantages over the use of log P. First it requires no experimental data, only a knowledge of the molecule's structural formula. Secondly, it is particularly useful for highly hydrophilic molecules, whose log P cannot be measured with any degree of accuracy.

A detailed discussion of the hydrogen-bonding capacities of the individual functional groups present in organic chemicals will be found elsewhere[8]. Three examples are the aliphatic hydroxy group (2), the uncharged primary amine group (2), and the charged carboxylate (10-12). On this basis the hydrogen-bonding capacity of glycerol would be 6; and of the citrate anion at least 32.

The experimental data on the permeability of the rat liver lysosome indicated[8] that molecules with hydrogen-bonding capacity up to 7.5 rapidly cross the lysosome membrane by passive diffusion; those with a value between 7.5 and 11.5 cross more slowly; those between 11.5 and 18.0 slowly to very slowly. Molecules with hydrogen-bonding capacity above 18.0 are

essentially non-permeant. Although these broad divisions are useful in practice, they have no theoretical significance; permeances of molecules fall on a continuous spectrum of values.

An important issue, however, is the correlation of apparent permeances across rat liver lysosome membranes in vitro with permeability across the lysosome membrane *in situ* within the living cell. Regrettably, there are few data available on permeances *in situ*, but the data that do exist are fully consistent with the conclusions summarized in the previous paragraph[8].

Doxorubicin was the first drug to be delivered to cells as a polymer-conjugate designed to be taken into cells by endocytosis and then cleaved in the lysosomes. As discussed elsewhere[8], the functional groups on the doxorubicin molecule give it a cumulative hydrogen-bonding capacity of 14.4 in the uncharged state, predicting a slow rate of efflux from the lysosome. The therapeutic efficacy of the lysosomally degradable polyHPMA conjugate is testimony to the adequacy of this rate of efflux in delivering doxorubicin to the cytoplasm.

The title of this lecture posed the question of whether the lysosome membrane is a barrier to polymer-based drug delivery. The answer, with respect to delivery by endocytosis of a lysosomally degradable drug-polymer conjugate, is that it is certainly a potential barrier, and that not all drugs are suitable for this mode of delivery. The use of hydrogen-bonding capacity to predict efflux rates of molecules from the lysosome is a simple and effective way of identifying which drugs have potential in this regard.

The Endosome Membrane

There has been great interest in recent years in the role of the endosome membrane in delivering exogenous DNA into the cytoplasm of cells, from whence it can access the nucleus. Viral vectors and polyelectrolytes promote this translocation, apparently by condensing the DNA and interacting with the endosome membrane, rendering it permeable to the DNA. There have, however, been no systematic studies of the intrinsic permeability properties of the endosome membrane. In the absence of any direct data, it is reasonable to adopt the null hypothesis that the these will be closely similar to those of the lysosome membrane as reported above.

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