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## THE MACROMOLECULAR AGGREGATE AS A DRUG CARRIER

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**Abstract:** Traditionally, a drug is expected to be biologically active and at the same time be able to ensure some sort of tissue or organ specificity. The latter property is necessary to avoid undesirable side effects when toxic drugs are being used. Such requirements are difficult to achieve only by changing the chemical formula of the drug. For these reasons, within the last few years a new pharmacological concept has been developed regarding delivery of biologically active compounds by the use of macromolecular aggregates. The purpose-specific design of macromolecular aggregates, able to deliver drugs to a desired location, is based on the assumption that different functions can be assigned to the separate chemical entities forming the aggregate. With the help of such an aggregate, the biologically active compound can be designed with solely its pharmacological potency in mind and without considering any limitations imposed by inaccurate delivery, such as undesired side effects. Specific molecules of the aggregate would ensure desired compound distribution within the organism. Furthermore, other molecules forming the aggregate should fulfill additional functions, e.g. protecting the drug from degradation. Additionally, aggregates formed from amphiphilic molecules should be capable of carrying drugs that are difficult to use as therapeutic agents due to low solubility in biological fluids (e.g. Taxol) or degradation (e.g. peptides, DNA). Such aggregates can be constructed from natural or/and synthetic compounds. Taken together, this creates possibilities of extending the spectrum of drug application and allows for the introduction of new technological modifications.

**Key Words:** Targeted Drug Delivery, Macromolecular Aggregates, Liposomes

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## INTRODUCTION

Traditional pharmacology assumes that a drug's intrinsic properties ensure its biological activity as well as its ability to reach the desired location. However, meeting both requirements (activity and delivery) is in most cases difficult to achieve. In order to ensure efficient drug delivery to the desired place, its biological activity frequently needs to be compromised due to modifications in its chemical structure. Such modifications are required when stringent solubility restrictions or the need to penetrate hydrophobic barriers [1,2]. Due to this, there is no reliable method that allows active compound behavior *in vivo* to be predicted, and in most cases the drug's biological potency *in vitro* cannot be used as a good indicator of its effectiveness *in vivo*.

Drugs which affect specific metabolic processes in selected cells need to be delivered intact to a well defined location in quantities sufficient enough to give the desired effects. To achieve this goal, compound chemical structure has to be modified appropriately in order to enable the drug to cross a number of physiological barriers. For example, if a specific cell surface receptor is not available, in order to penetrate the cell plasma membrane the drug needs to be able to partition into the lipid hydrophobic environment. However, chemical structure modifications may change the drug's properties to such an extent that it ceases to be a useful therapeutic agent. This is particularly a problem when the active compound is a macromolecule vulnerable to degradation *in vivo* and of not easily manipulated chemical structure, e.g. DNA or proteins [3-5]. Hence, the construction of a therapeutic agent with a chemical structure simultaneously ensuring proper bio-distribution and biological activity is difficult if not impossible, and other approaches have to be considered [6-10]. The incorporation of therapeutic agents into supramolecular aggregates opens the way to structural and functional modification without altering the active compound itself.

The aim of constructing supramolecular structures containing biologically active compounds is to improve the drug's pharmacological index by lowering dosage, extending circulation time and reducing the severity of side effects. In the construction of such a supramolecular aggregate, a number of stringent requirements need to be met. The aggregate should be stable, and ensure the protection and controlled release of the carried molecule. In addition, aggregate properties should improve the drug's bio-distribution following delivery and prevent tissue which are not the targets of the therapy from being exposed.

## THE MACROMOLECULAR AGGREGATE

The aggregate is an ensemble of noncovalently bonded macromolecules interacting via weak forces (hydrophobic, electrostatic and van der Waals interactions). Aggregates can be formed from lipids, proteins, nucleic acids, and

small organic molecules. Molecules ensemble into a single aggregate because they all possess molecular properties known as amphiphilicity. The main driving force responsible for aggregate formation is therefore the difference in the molecules' residual affinity to water. When the aggregate is assembled, a two-phase system consisting of hydrophobic and hydrophilic compartments is formed. Aggregate components with different affinity to water self-organize, simplifying formation procedures. The aggregate, due to its intrinsic instability (a consequence of weak intermolecular interactions) is very sensitive to changing environmental conditions. Its individual components can readily exchange between the aggregate and surrounding membranes. This process underlies time-dependent physicochemical property variations, which in turn decide about aggregate integrity and behavior in a changing environment (e.g. variable temperature, pH and ionic strength). Liposomes are examples of such supramolecular aggregates; they are formed from lipids and are already widely used in cosmetics and pharmacology as a nutrient and drug delivery vehicle. Liposome bio-distribution, interactions with body fluids and tissue specificity all depend on a given aggregate's properties, including its size, surface charge and surface-bound additives (synthetic polymers, polysaccharides, peptides and proteins). It has been shown that the pharmacological application of lipid aggregates not only improves the pharmacological index of existing drugs but makes it possible to apply compounds which are difficult to dissolve in the aqueous phase or which bear nonspecific toxicity in their free form [11].

### **MAJOR BARRIERS ENCOUNTERED BY THE DRUG WHEN APPLIED SYSTEMATICALLY**

In order to fulfill its function, a chemical compound needs to reach its target cell or organ without affecting other important body compartments. On its way, it has to penetrate various biological barriers and is subject to elimination by a variety of processes. Figure 1 is a schematic representation of the major barriers encountered by the drug en route to its destination.

To deliver the drug to the target tissue or organ, proper drug formulation and delivery methods need to be selected. It should be kept in mind that drug application procedure in most cases is the only possible way of achieving any sort of specificity when the drug is administered in its free form. For example, drugs delivered by inhalation are predominantly active in the lungs [9,12]. In some cases drug intake efficiency is extremely low, as in the case of drugs used in treating eye diseases. Biologically active molecules applied in the form of aqueous dispersion are quickly eliminated by the eye's drainage system, causing a risk of systemic toxicity. The association of such compounds with charged liposomes enhances their intake by an order of magnitude [13].

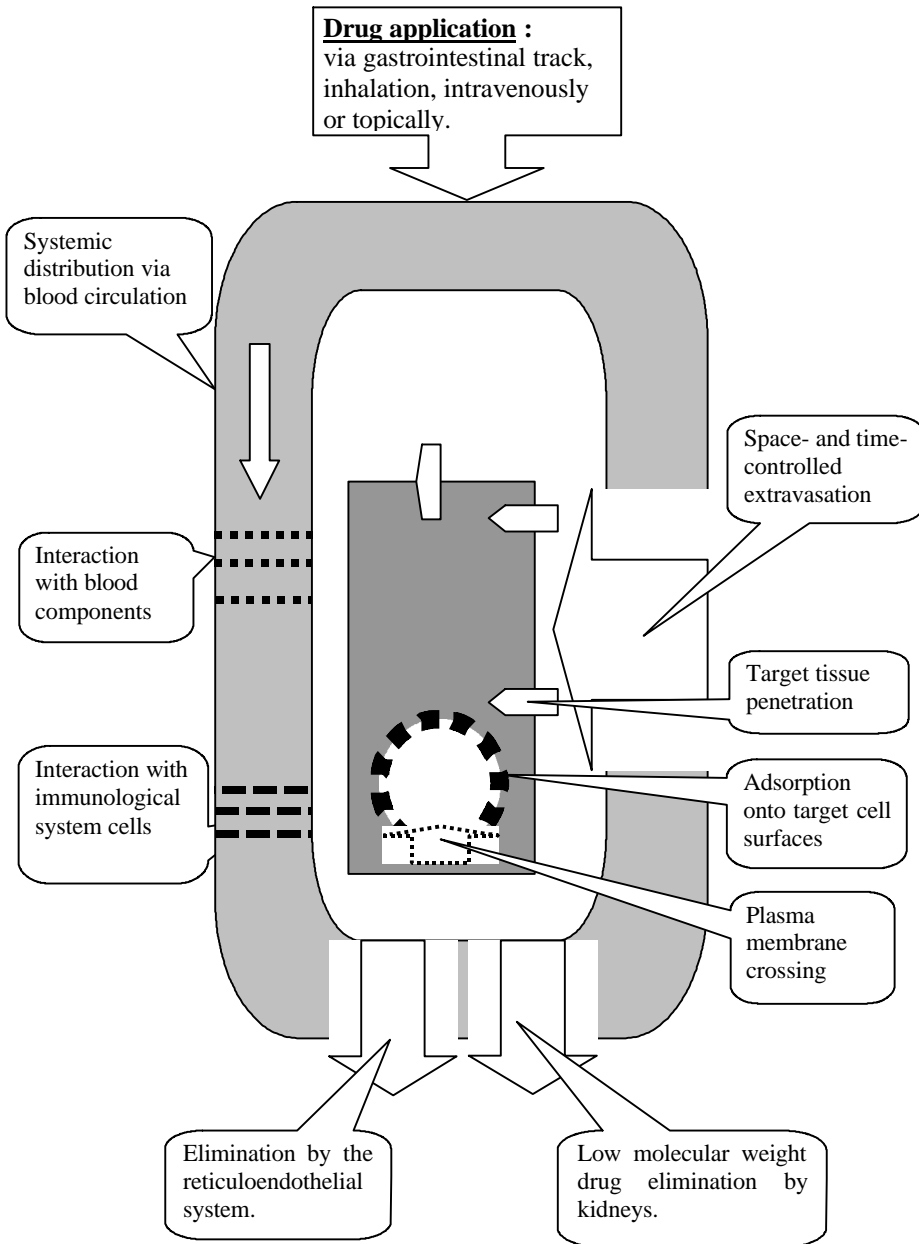


Fig.1. A schematic diagram representing major barriers encountered by the drug on its way to its target cells.

## **DRUG INTERACTION WITH BLOOD COMPONENTS**

When the drug enters circulation, it can interact with various blood components. Blood cells non-specifically adsorb free drugs, whereas phagocytic cells engulf large aggregates, in both cases affecting their availability to the target tissue [14]. Lipoproteins and other supramolecular endogenous blood aggregates are capable of accumulating amphiphilic and hydrophobic molecules serving as their reservoirs. Serum proteins (immunoglobulins and albumins) adsorb amphiphilic drug molecules in the free form, themselves being attracted to the macroaggregate surface, especially if the latter is negatively charged [15]. For this reason, free drug distribution between various blood components is difficult to predict and impossible to control. However, when the compound is delivered in a properly designed aggregate, its fate in circulation can be predicted with reasonable certainty. For example, large liposomes with negatively charged surfaces are preferentially accumulated inside macrophages, whereas liposomes covered with polymers demonstrate increased circulation time. Flexible chains of polyethylene glycol or rigid chains of polysaccharides, covalently attached to the liposome surface, form steric barriers which prevent serum proteins (immunoglobulins or apsonins) from adsorption onto their surface. Such liposomes are therefore unrecognisable by the immunological system, which allows them to persist in the blood stream for an extended period of time [16].

## **NONSPECIFIC DRUG ELIMINATION FROM CIRCULATION**

Chemical molecules with a molecular weight lower than 20 kDa are rapidly eliminated from circulation by the kidneys. This process is so efficient that on average the drug is removed from the blood within minutes. This proficiency necessitates the increase of free drug dosage, so that its concentration in the blood exceeds the therapeutic level for a significant period of time. When the amount of the drug is high and its non-specific toxicity is significant, the risk of kidney failure is substantial (in addition to other possible side effects). Such problems can be straightforwardly solved by toxic drug encapsulation in aggregates of a proper size to make them resistant to kidney elimination.

The drug, whether free or part of an aggregate, needs to leave the blood vessel in order to reach the tissue where it is intended to act. The endothelium forms a formidable barrier for the drug, especially when it is water soluble or delivered with the help of a supramolecular aggregate. However, the inability of macroaggregates to leave normal blood vessels can be utilized to deliver specific compounds to tissues in regions where vessels are leaky, i.e. regions of infection, inflammation or where a solid tumor is growing [17,18]. Such vessels are characterized by the presence of large openings between the endothelial cells and the absence of the basement membrane.

## **CELL SPECIFICITY AND CROSSING THE PLASMA MEMBRANE.**

In the target tissue, the free drug interacts specifically with targeted cells as well as non-specifically with other cells present in the region, whereas the aggregate, when properly labelled, can only bind to target cells. Specificity in drug delivery can be achieved, for example, by attaching a specific antibody to the aggregate surface.

Finally, when the drug is present in the direct vicinity of the target cell, it has to penetrate the plasma membrane in order to give the desired effect. Many naturally occurring molecules can be selectively transferred into the cytosol with the help of specialized proteins, but such a level of specificity is presently unachievable for most drugs. Delivering the therapeutic agent enclosed in an aggregate creates a number of possibilities. The aggregate can adsorb onto the surface of the target cell and release its contents into the direct vicinity, without disturbing the plasma membrane. It can fuse with the plasma membrane, delivering the active compound into the cytoplasm; or it can be internalised by the cell via an endocytic pathway.

## **SUMMARY**

This brief description of the barriers the therapeutic compound needs to overcome on its way to the target shows the intricacy of problems related to rational drug design. From a practical point of view, it is very difficult to construct a single molecule possessing properties that ensure its predictable distribution in the organism. Only rarely is the drug destination so easy to reach that its intrinsic properties ensure its reaching the target without affecting other tissues or organs. The problems become particularly difficult when the drug is toxic and the therapeutic window narrows, as in the case of cancer or fungal infection.

Substantial achievements in developing custom-made drug delivery vehicles already exist. For example, it has been established that, providing its size does not exceed 100 nm, liposome accumulates preferentially within the regions where blood vessels are stimulated to grow by tumour cells in the process called angiogenesis [17]. Such vessels are poorly formed, and characterized by the presence of large gaps between the endothelial cells and the absence of the basement membranes, which allows small aggregates (smaller than 150 nm) to leave the blood stream. Such a strategy has been applied in the liposomal formulation of doxorubicin. To ensure specific drug delivery to target tissues other requirements have to be met as well. Size-dependent accumulation requires the aggregate to persist in circulation for a certain period. This has been achieved with the help of hydrophilic, highly flexible polymers, like poly(ethylene glycol) or polysaccharides, which are covalently attached to the

aggregate surface [19-21]. Consequently, the liposome is large enough to not be eliminated by the kidneys and surface-located steric barriers prevent opsonization and elimination by the reticulo-endothelial system.

The idea of loading therapeutic agents into macromolecular structures is becoming widely recognised as a method of improving their effectiveness, reducing side effects and lower total drug dosage. Macroaggregates are already used to develop new toxic drug formulation methods for such diseases as cancer, HIV and persistent viral, bacterial and parasite infections [22-25].

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