REVIEW ARTICLE

A review on targeted drug delivery through macrophages

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Abstract

Macrophages are present in many tissues of the body which includes lymphohematopoietic organs, skin, gut, and nervous system. They are found in blood and extra-vascular system and undergo migrations. They are produced mainly from the bone marrow. The macrophages produced in the body enter the tissues and perform the functions like homeostasis. When an injury or infection occurs, these macrophages along with monocytes accumulate on the infection site and perform the defense mechanism thereby produce the immunity with the help of other cells and humoral product. Utilizing the macrophages as delivery system appears to have great importance in improving the therapeutic efficacy of the enclosed drug. The drug diffuses through different membranes and releases at the site of infection. This review discusses the advantages of targeted drug delivery through macrophages, drug loading methods, and its applications.

Key words: Applications, drug loading methods, macrophages, targeted drug delivery

INTRODUCTION

Monocytes originate from the bone marrow and circulate in the bloodstream for about 8 h, during this period, they are increased in their size. Then, they migrate into the tissues and differentiate into the tissue-specific macrophages. These macrophages are 5–10-fold larger than monocytes with increased phagocytic ability. They can also produce higher level of lytic enzymes. Macrophages are present all over the body, but depending on the tissue location, they are named as shown in Figure 1.

Not all macrophages get fixed to particular tissues, in fact, a large number of them retain their motility and function as free or wandering macrophages. They can be activated by a variety of stimuli into an immune response. Such stimuli include phagocytosis of particular antigens and interactions with other foreign substances, cytokinins, etc. Macrophages are capable of ingesting and digesting of whole microorganism. The activated macrophages produce a number of cytotoxic and microbicidal molecules (superoxide anion, hydroxyl radicals, single oxygen, hydrogen peroxide, hypochlorous acid, nitric acid, and nitrous oxide) that are important for the destruction of microorganisms.[1]

Most of the conventional dosage forms exhibit adverse effects due to the unwanted drug distribution. Hence, there is a need to develop a suitable drug delivery system that distributes the active drug molecule to the site of action only, without effecting other healthy tissues or organs. Targeted delivery is a method of delivering drugs to a specific target site. This improves efficacy of treatment by reducing side effects of the drug.[2]

These targeted drug delivery systems are chosen over conventional drug delivery system due to three main reasons. They are:

Pharmaceutical Reason

Decrease in the frequency of dosages taken by the patient having a more uniform effect of the drug, reduced side effects.

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Pharmacokinetic Properties

Conventional dosage form shows poor absorption, shorter half-life, and large volume of distribution.

Pharmacodynamic Properties

The conventional dosage forms have low specificity and low therapeutic index as compared to targeted drug delivery systems. Due to these reasons, targeted drug delivery system is preferred over conventional drug delivery systems.[3]

Needs of Targeted Drug Delivery Systems

Targeted drug delivery is considered to a specific organ or a cell or group of cells, which needs treatment. Drug carrier is one of the specialized molecules required for effective transportation of loaded drug to specific site. The drug carrier should be biodegradable or readily eliminated from the body without causing any damage to the body. The main goal of this system is to deliver certain amount of drug to the targeted area within the body. This will help to maintain the required plasma and tissue level drug concentration in the body, therefore avoiding any damage to the healthy tissue by drug.[4]

Advantages

- Targeted drug delivery system minimizes the side effects and toxicity
- The amount of drug administered decrease during the treatment period
- It avoids the first pass metabolism thereby reduces the drug degradation
- Drug bioavailability increases and fluctuations in plasma drug concentration decrease
- It also has beneficial effect on permeability of proteins and peptides
- These all factors in combination cause reduction in dosage frequency and hence reduce the cost of expensive drug.

Disadvantages

- With the targeted drug delivery, it becomes difficult to target the tumor cells present in the disease condition
- Advanced techniques and highly qualified individuals are required
- Sometimes, it may cause toxicity and difficult to maintain stability of dosage forms at the specific site.[5]

So as to overcome the above disadvantages of targeted drug delivery systems, a new approach has been discovered, that is, macrophage-mediated drug delivery system.

ADVANTAGES OF MACROPHAGE-MEDIATED DRUG DELIVERY SYSTEMS

Extended Half-life

The lifespan of macrophages is about several months to years, which is longer than the circulation time of normal drug carriers in the body. Macrophages are immune circulating cells, which are a part of mononuclear phagocyte system (MPS), therefore, macrophage-mediated drug delivery systems can be recognized as "self" by the host immune system.[6] Hence, drug-carrying macrophages can escape host defense mechanisms, thereby extending a drug's circulation time and half-life. Therefore, macrophages can be used effectively as drug carriers to prolong the circulation time and half-life of drugs, reducing the frequency of drug administration to the patients.

Biocompatibility and Biodegradability

Drug delivery systems based on polymers have always attracted attention because of their biomedical applications. However, the existing polymer materials are not completely inert in nature, which prevents them in using as drug carriers for clinical applications.[7] Therefore, macrophages have been used to replace polymer materials due to their higher biocompatibility and ability to be fully metabolized into safe non-toxic products.

Enhancement of Drug Stability

Macrophages are immune cells, can escape phagocytosis by MPS, loading drugs into these immune cells can also protect the drugs from phagocytosis. Moreover, the macrophage cell membranes can protect them from premature inactivation of loaded drug and degradation by endogenous factors.[8] Therefore, biomimetic drug delivery system based on macrophage membrane-wrapped drugs can increase drug stability and reduce immunogenicity.

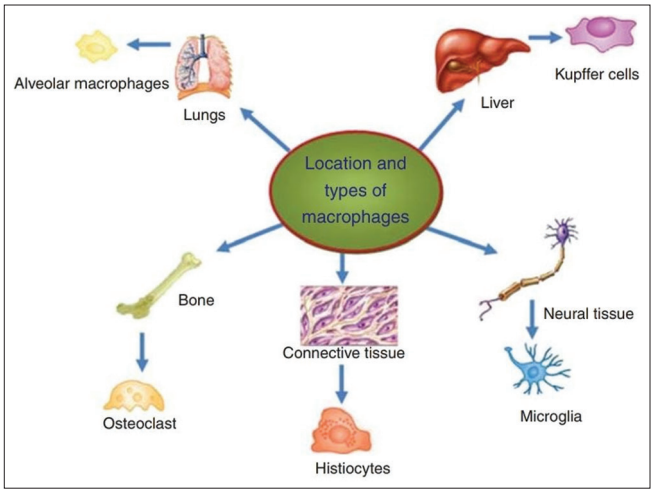

Figure 1: Locations of various macrophages

Prolonged Drug Release

Macrophage cell membranes are semi-permeable and flexible in nature and are composed of phospholipids. Their main functions are, to selectively exchange substances, discharge metabolic waste, absorb nutrients, and to secrete and transport proteins.[9] Drug loading into macrophages can result in sustained release of drug in slow and continuous manner. Therefore, macrophage-mediated drug delivery systems can be used to prolong the release time of a drug effectively, improve drug efficacy, and significantly reduce the fluctuation of drug concentration in the blood plasma.

Improved Drug Targeting Ability

The specific targeting ability of most drugs is low, which results in causing toxic side effect. Loading these drugs into nanoparticles (NPs) may reduce such effects, but naked NPs can be easily phagocytosed and cleared by MPS present in the body, as they lack active targeting properties. Macrophages, as immune cells, deliver the drug to inflammatory sites and tumors, while helping the drug to avoid the body's defense mechanisms.[10] Macrophage-mediated drug delivery systems display the same surface receptors and proteins as the parent macrophages from which they were produced, and these surface proteins can interact with desired targets. Therefore, macrophages can significantly deliver the drug to the specific target site without causing any toxic effects.

Versatile Drug Carrier

Several studies have proven that macrophages can be used efficiently to deliver a wide variety of substances. For example, macrophage membranes can encapsulate poly (lactic-co-glycolic acid) NPs,[11] liposome's,[12] chitosan,[13] Au NPs,[14] Fe_3O_4 NPs,[15] and SiO_2 NPs.[16] Macrophages can also be loaded with various small-molecular drugs under conditions that preserve their biological activity.

DRUG-LOADING METHODS FOR MACROPHAGE-MEDIATED DRUG DELIVERY SYSTEMS

Loading of drug into macrophages is the key step in the delivery system. The drug which is to be delivered to the target site is loaded into the macrophages using different loading methods, which are given below.

Incubation

To load drugs into macrophages, incubation is the most commonly used method. They are incubated with drugs or drug-loaded NPs under appropriate culture conditions needed by them, and the macrophages engulf the drug.[17,18]

Adhesion

With the aim of developing a drug delivery system that can successfully encapsulate and release the drugs in a controlled manner, scientists used a phagocytosis-resistant "cellular backpack," which is a thin film prepared through a layer-by-layer spray deposition technique.[19,20] This method did not affect macrophage health or proliferation, which shows that the approach does not show any undesired toxicity. Macrophages with cellular backpack for targeted drug delivery to the brain are shown in Figure 2.

Electroporation

Electroporation is the method which increases drug-loading capacity without phagocytosis. In this method, the macrophages are suspended in electroporation buffer containing the drug and transferred to an electroporation tube. The macrophages are electroporated and the drug diffuses into the cells through the small pores. This method is reproducible, fast, and cost effective. The process of electroporation is shown in Figure 3.

ENCAPSULATION METHODS

Encapsulation within Macrophage Membrane

Macrophage membrane has 1000 types of proteins in its structure, in which many of them recognize specific inflammatory factors and tumor cells and plays an important role in biological functions.[21] During the process of extraction and purification, the structure and activity of these proteins must be retained to ensure that the drug delivery system acquires macrophage properties.[11] The macrophage membrane extraction requires a combination of hypo-osmotic swelling, mechanical destruction, and many gradient centrifugation steps to separate enraptured and cell contents. Degradation of membrane proteins should be prevented by adding protease inhibitors and the entire process should be performed under low temperatures to prevent the inactivation of these membrane proteins.[11]

After that, the surface of drugs or drug-loaded NPs is wrapped by these membranes using ultrasonication or mechanical extrusions, and the core structures can be observed using transmission electron microscope. This drug delivery system combines the drug core with macrophage membrane, which helps in rapid clearance of drug in the body and also exploits the functional properties of the membrane.[22]

Encapsulation Inside Macrophage-derived Vesicles

To avoid the complex and inefficient macrophage membrane extraction method, which involves cell fragmentation,

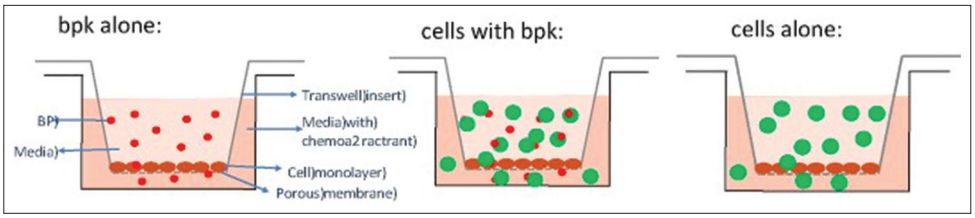

Figure 2: Macrophages with cellular backpack for targeted drug delivery to the brain

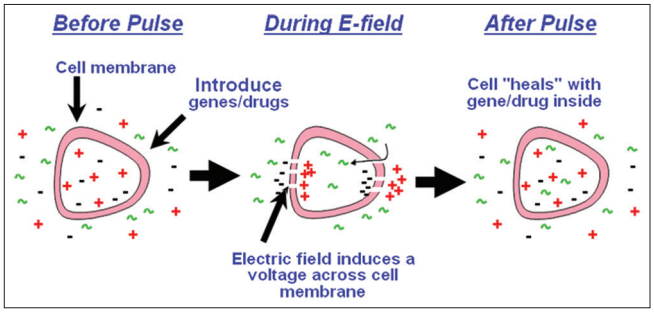

Figure 3: Process of electroporation

density gradient centrifugation, and ultracentrifugation steps, macrophage-derived vesicles can be used.[23,24] Macrophages are cultured in serum-free medium and stimulated using cytochalasin B to secrete macrophage-derived vesicles.[6] The obtained vesicles are purified through multiple gradient centrifugation cycles. Using this approach, scientists have encapsulated drug-loaded NPs into vesicles for rheumatoid arthritis treatment. Proteomic analysis study shows that their membrane proteins are similar to those of the macrophage membrane, suggesting that their biological activities would also be similar.

Extracellular vesicles (EVs) generated from macrophages: A broad set of pathological and therapeutic mediators in a variety of diseases:

EVs are group of membrane-enclosed vesicles which are naturally released by nearly all types of cells. Depending on their origins and sizes, they are divided into multiple subtypes, such as exosomes, micro vesicles, apoptotic bodies, exomeres, and large oncosomes. EVs package proteins, nucleic acids, and metabolites of parental cells, and are thought to show similar properties to their parent cells.[25] Recently, EVs have been recognized as important information carriers that transfer their cargos from parent cells to recipient cells, modulating the physiological and pathological processes in recipient cells. The functions of EVs derived from macrophages in various disease states have been investigated, and increasing evidence states that these EVs play key roles in the diseases progression. Thus, a comprehensive understanding is needed of macrophage-derived EVs and their roles in the disease pathology and treatment. The biological and therapeutic effects of EVs from macrophages in various disease conditions are shown in Figure 4.

APPLICATIONS OF MACROPHAGE-MEDIATED DRUG DELIVERY SYSTEMS

Numbers of applications are present in the treatment and prevention of several diseases such as inflammation, acquired immune deficiency syndrome (AIDS), leishmaniasis, tuberculosis, gauche disease, and rheumatoid arthritis.

Anti-inflammatory Treatment

When an injury occurs, body's defense system causes inflammation. Inflammation causes release of large number of monocytes which move to the target site and differentiate into macrophages, which regulates inflammatory process.[26] They are used as therapeutic agents or carriers to target the inflammatory site and regulate the response.

Therapeutic Agents

When inflammation occurs, macrophages at the inflammation site produce a large number of pro-inflammatory cytokines including tumor necrosis factor, interleukin-6, and interferon-γ, which recruit more macrophages in the blood circulation. Macrophage-mediated drug delivery systems can preserve the key membrane proteins of the source cells, such as cluster of differentiation 14 and Toll-like receptors 4, as well as the related cytokine-binding receptors, which can be established using Western blot analysis. Indeed, the membrane derivatization process leads to the significant improvement of these molecules.[11] Hence, macrophage membrane-coated empty NPs are, like the source macrophages, bind endotoxins and cytokines, and inhibiting subsequent inflammatory cascades.

CLINICAL APPLICATIONS

Macrophages play a key role in the treatment of the following diseases.

Tuberculosis

Macrophages act as reservoirs for various microorganisms. *Mycobacterium tuberculosis* is the causative organism, coming from the air, resides for a long period of time in alveolar macrophages of lungs.[27]

Rheumatoid Arthritis

Rheumatoid arthritis, a chronic, systemic autoimmune disorder, is characterized by inflammation of the joints. Immune-activated synovial macrophage plays an important role in this inflammatory disease. The degree of joint inflammation and tissue degradation depends on the number and level of macrophage activation.[28,29]

Gaucher's Disease

Gaucher's disease, a rare genetic disorder, is related with functional deficiency of b-glucocerebrosidase activity and distinguished by the presence of lipid-laden macrophages in the liver, spleen, bone, and lungs. The effective enzyme replacement therapy for this disease depends on the ability to deliver b-glucocerebrosidase to macrophages, as these cells accumulate glycolipid in enzyme deficiency.[30,31]

AIDS

The HIV can enter the macrophage through binding of gp120 to CD4 and second membrane receptor, CCR5 (a chemokine receptor).[32] The capacity of macrophages to migrate inside the organ and to survive within tissues makes them potential carriers of HIV-1 INFECTION.[33,34] In fact, productive HIV-1 infection takes place independently of cellular DNA synthesis in macrophages.[35]

Macrophage-mediated NP Delivery Systems

A macrophage-mediated NP delivery system for antiretroviral drugs was first reported in the year 2006.[36] A similar method using mononuclear macrophages was also applied to transport therapeutic NPs to tumor site and also to deliver drugs for the treatment of Parkinson's disease. Nanoporous silicon particles were later encapsulated with purified mononuclear macrophage membranes, resulting in materials with macrophage-like functions. Depending on these encouraging findings, the application of macrophages as carriers for the delivery of drugs with different properties has been significantly expanded to study and treat various conditions such as cancer, inflammation, and HIV infection which are given in Table 1.

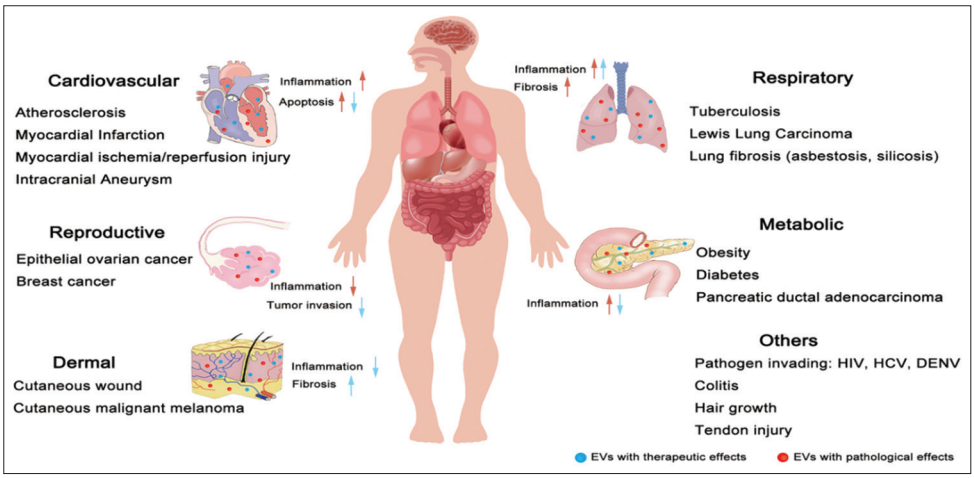

Figure 4: Extracellular vesicles from macrophages in various diseases

Table 1: Applications of macrophages in the treatment of various disease conditions

Source	Drug delivery system	Drug	Treatment	Reference
Bone marrow-derived macrophages	Liposome PEI-PEG	Indinavir Catalyze	Human immunovirus Parkinson's disease	[17,36]
Peritoneal macrophages	Liposome	Doxorubicin	Anti-tumor, imaging angiography	[37]
Alveolar macrophages	Gold-silica nanoshells	-	Glioma, PTT	[18]
Mononuclear macrophages	Gold nanoshells Amphiphilic bola-pattern polymers	- Paclitaxel	Anti-tumor Breast cancer	[38,22]

CONCLUSION

Macrophages are specialized cell which help in detection, phagocytosis, and destruction of any foreign substances which cause harm to the body. They perform key role during injury, inflammation, and cancer. Macrophage-mediated drug delivery offers many advantages over conventional drug delivery methods, but the heterogeneity among macrophages used to build such delivery systems has limited their clinical application. Moreover, how macrophages interact with drug is imprecise, so it is difficult to predict whether the drug will be degraded by endolysin. To make sure that macrophage-mediated delivery systems preserve the inherent functions of macrophages, membrane surface proteins must be protected during extraction of the macrophage membrane, and more research is needed into how to attain this. Storing macrophages in a way that preserves their biological activity remain a major barrier to large-scale production. Future work aim at these challenges may lead macrophage-mediated drug delivery closer to the clinical use.

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