ELSEVIER

International Journal of Pharmaceutics 162 (1998) 45–50

international journal of pharmaceutics

The macrophage as target or obstacle in liposome-based targeting strategies

Nico van Rooijen *, Annemarie Sanders

Deptartment of Cell Biology and Immunology, Faculty of Medicine, Vrije Universiteit, Van der Boechorststraaat 7, 1081 BT Amsterdam, The Netherlands

Accepted 7 November 1997

Abstract

Macrophages can be found in all organs of the body. For that reason, the majority of liposomes, intended for drug- or antigen-targeting in vivo, will find macrophages on their way. Phagocytosis of the liposomes followed by disruption of their phospholipid bilayers and release of the entrapped molecules will be the consequence. This capability of macrophages may be an advantage when these cells themselves are forming the targets of the liposomes. However in most cases, the activity of macrophages has to be considered an obstacle for liposome-based targeting strategies. The positive and negative effects of macrophages on liposomes as drug- and antigen-carriers will be discussed.

Keywords: Macrophages; Liposomes; Targeting

1. Introduction

Macrophages can be found in all organs of the body. Monocytes, their direct precursors, are released in the circulation from the bone marrow (De Bruijn, 1997). Via the circulation, these monocytes immigrate into the organs where they differentiate in resident macrophages.

From an evolutionary point of view, macrophages form an ancient cell population, representing the main host defense mechanism before the development of the immune system. During evolution, these cells have also acquired important functions in the regulation of humoral and cellular immune reactions (serving as accessory cells for the induction of acquired immunity against particulate antigens), and in the control of various nonphagocytic cells that participate in immune responses. As a consequence, in mammals, macrophages are multifunctional cells (Van Rooijen et al., 1996a,b).

* Corresponding author. Tel.: +31 20 4448075; fax: +31 20 4448081; e-mail: N.van_Rooijen.cell@med.vu.nl

0378-5173/98/$19.00
PII S0378-5173(97)00411-0

The capability of macrophages to clear non-self (e.g. microorganisms) and altered-self (e.g. aged erythrocytes and apoptotic cells) particles represents an important mechanism of homeostasis. Such particles are ingested by macrophages and are consecutively digested with the aid of their large panel of lysosomal enzymes. This capability of macrophages can be advantageous when particulate drug-carriers, such as liposomes are to be targeted to macrophages, for instance when macrophages are the targets for delivery of liposome-encapsulated antimicrobial agents (Alving, 1988), macrophage-activators (Fidler, 1992) or antigens (Van Rooijen, 1995). However the same capability of macrophages is also responsible for removal of the bulk of most particulate targeting devices with a non-phagocytic destination, for example liposomes used as drug carriers (Hu et al., 1996) or adenovirus vectors used for gene transfer (Wolff et al., 1997), as well as of nonautologous grafted cells such as human cells grafted into severe combined immunodeficient (SCID) mice (Fraser et al., 1995). Correspondingly, it has been shown that transient suppression of macrophage functions by administration of liposome-encapsulated drugs improves the efficiency of such treatments (Van Rooijen et al., 1997). In general, in vivo applications of liposomes can be divided into two categories: those taking advantage of phagocytosis and those for which phagocytosis forms an obstacle on the way to success.

In the present contribution, the influence of macrophages on liposome-based targeting strategies will be reviewed.

2. Macrophages as a target

2.1. Liposomes in the induction of immunity

Liposomes may be used for targeting of antigens to macrophages as a first step in the induction of immunity. However, liposomes may also be used to stimulate immunity by abrogation of the activity of suppressor macrophages.

Small soluble antigens such as serum albumins can be converted to particulate antigens by their association with liposomes. In vivo studies have shown that splenic phagocytic cells play a role in the induction of antibody responses against such liposomal antigens when intravenously injected (Su and Van Rooijen, 1989; see also a recent review by Van Rooijen, 1995). On the contrary, these phagocytic cells in the spleen had a negative effect on antibody responses against similar antigens when given in a non-liposome associated (soluble) form. Recent studies have shown that there is a phagocytic cell in the spleen that combines characteristics of both macrophages and dendritic cells (DC, Leenen et al., 1997). The phagocytic capability of this cell allows the endocytosis and processing of large liposome-associated antigens and their presentation to T cells as a first step in the induction of humoral immunity.

Depletion of alveolar macrophages in the lung by liposome encapsulated clodronate had a dramatic effect on the antibody response against intratracheally administered antigens (Thepen et al., 1989). Numbers of antibody forming cells in the lung-associated lymph nodes were strongly increased, a prolongation of the response was observed and, contrary to the response in normal animals, antibody forming cells were also observed in the lung tissues itself, when antigen was given after depletion of alveolar macrophages. Recently it was shown that also the cytotoxic T lymphocyte (CTL) response was strongly enhanced when antigen was given via the airways after depletion of alveolar macrophages (Wijburg et al., 1997). It has been confirmed that alveolar macrophages actively suppress the antigen presenting cell function of pulmonary dendritic cells in vivo (Holt et al., 1993). It is suggested that liposomes by themselves, i.e. without any encapsulated drug or antigen, may be applied to enhance the pulmonary immune response by blocking the alveolar macrophage-mediated immune suppression (Van Rooijen, 1993).

2.2. Liposome-encapsulated antimicrobial agents

It is well known that macrophages play an important role in the resistance against microorganisms by phagocytosis and intracellular killing. However, several microorganisms have evolved mechanisms that allow them to survive and even

multiply in macrophages (Alexander and Russell, 1992; Montgomery et al., 1993; Fenton and Vermeulen, 1996). As a consequence, some of the latter microorganisms were able to reverse the influence of macrophages from threatening to protective. For this reason, macrophages are the target for delivery of several liposome-encapsulated antimicrobial agents (Alving, 1988). Because macrophages have a mannose-receptor, the therapeutic efficacy of such liposome-encapsulated antimicrobial agents could be enhanced by incorporation of mannose residues in the phospholipid bilayers (Banerjee et al., 1994).

2.3. Liposome-mediated activation of macrophages

Activated macrophages have the capability to recognize and destroy neoplastic cells, without injuring non-tumorigenic cells. Both hydrophilic muramyl dipeptide (MDP), a component of the bacterial cell wall, and lipophilic muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE) are potent macrophage activators (Fidler, 1992). Taking advantage of phagocytosis as a natural fate of liposomes, Fidler and colleagues were able to activate macrophages in vivo to a tumoricidal state by administration of these immunomodulators in a liposome-associated form.

3. Macrophages as an obstacle

3.1. Approaches to prevent phagocytosis

Several particulate drug carrier devices have been proposed. Among these, liposomes may be considered the most versatile and promising drug delivery system (Gregoriadis, 1995). However, clearance by tissue macrophages prevents the bulk of all liposomes from reaching their targets. They are rapidly ingested and digested by macrophages and their encapsulated molecules may have an unwanted effect on macrophages and will be released in the circulation. Several modifications of the original liposome formulations, such as the incorporation of amphipathic polyethylene glycol (PEG) conjugates in the liposomal bilayers, have been proposed in order to reduce the recognition of liposomes by macrophages and the consecutive destruction of the former by the latter. It has been postulated that the protection of the PEG-liposomes is based on prevention of interactions between opsonins and liposomes (Torchillin and Papisov, 1994). However, a large percentage of these so called long circulating liposomes, will be ingested by macrophages (Litzinger et al., 1994; Oussoren, 1996).

Another approach to prevent phagocytosis focuses on the macrophages themselves. Transient suppression of phagocytosis can be achieved by temporary depletion of macrophages using liposome encapsulated drugs such as clodronate and propamidine (Fig. 1A,B, Van Rooijen and Sanders, 1994 and Van Rooijen et al., 1997). Liposome-mediated intracellular accumulation of the drugs disturbs the metabolism of the cells and activates their cell death program, which results in apoptosis (Van Rooijen et al., 1996a). The depletion is temporary because new bone-marrow-derived monocytes migrate to the depleted tissues

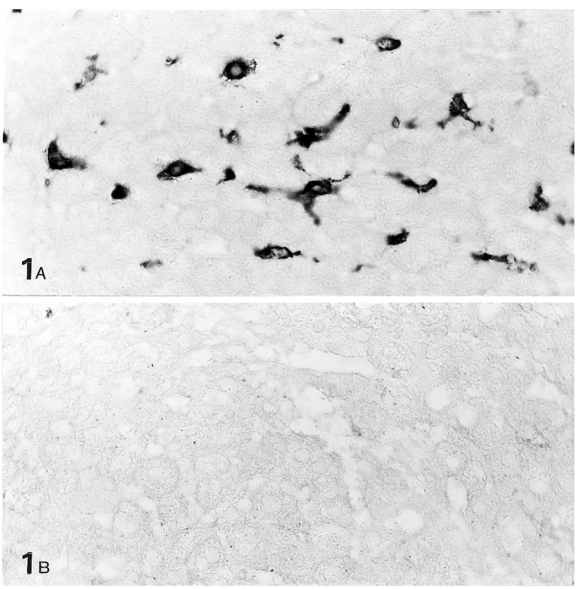

Fig. 1. Kupffer cells (macrophages) in rat liver stained with the monoclonal antibody ED2. (A) Normal rat liver. (B) Liver of a rat at two days after treatment with liposome encapsulated clodronate Note the absence of Kupffer cells after treatment with liposome-encapsulated clodronate.

and differentiate into new resident macrophages. Most resident macrophages will be replaced within 2 weeks. However in the spleen (Van Rooijen et al., 1989) and lymph nodes (Delemarre et al., 1990), repopulation with all compartment-specific macrophage subpopulations may take several months. Currently we are investigating whether liposome-encapsulated drugs can be used to suppress phagocytic activity without physical depletion of the macrophages themselves.

3.2. Macrophages as a target in order to avoid their action as an obstacle

Several studies have shown that depletion of macrophages by administration of liposome-encapsulated clodronate, followed by administration of particulate drug- or gene-carriers along the same route, strongly enhanced the efficacy of the latter treatment.

Depletion of macrophages by intravenous treatment with liposome encapsulated clodronate significantly prolonged the circulation time of consecutively administered liposomes, even when the latter liposomes were long circulating liposomes themselves (Hu et al., 1996). The (macrophage dependent) clearance function of the liver, with respect to liposomes, was almost completely abrogated 24 h after injection of the clodronate liposomes. Based on these results, it may be expected that the efficacy of drug targeting using particulate carriers may be improved by transient suppression of macrophage functions prior to administration of the drug carrier.

Other recent studies have shown that depletion of liver macrophages (Kupffer cells) by liposome encapsulated clodronate, prior to intravenous administration of an adenovirus vector, led to a higher input of recombinant adenoviral DNA to the liver, an absolute increase in adenovirus-mediated transgene expression and a delayed clearance of the vector DNA and transgene expression (Wolff et al., 1997). It might be anticipated that gene transfer mediated by cationic liposomes will benefit from the absence of macrophages in a similar way.

4. Administration routes of liposomes and organ specific effects

4.1. Intravenous administration

Since liposomes are not able to cross the endothelial walls of blood capillaries, intravenously injected liposomes will target their contents only to those macrophage (sub)populations that are in direct contact with the blood circulation, i.e. to the Kupffer cells in the liver and to several subpopulations of splenic macrophages (Van Rooijen and Sanders, 1996). It has been demonstrated that intravenously injected liposome encapsulated drugs can also be targeted to macrophages in the bone-marrow (Barbé et al., 1996). Kupffer cells in the liver and marginal zone macrophages, marginal metallophilic macrophages and red pulp macrophages in the spleen have access to intravenously injected liposomes (Van Rooijen et al., 1989).

4.2. Administration via the airways

Alveolar macrophages, constituting $\sim 80\%$ of the total macrophage population of the lung form a first line of defense against pathogens entering into the body via the airways. Since they are present in the alveoli of the lung, there is no natural barrier between these macrophages and liposomes entering from the airways. Liposomes can be administered via the airways, by intratracheal instillation (Thepen et al., 1989), by intranasal administration (DeHaan et al., 1996) or by application of aerosolized liposomes (Hashimoto et al., 1996). Interstitial macrophages, the remaining 20% of lung macrophages cannot be reached by liposomes administered along the airways.

4.3. Intraperitoneal administration

Intraperitoneally injected liposomes have access to all macrophages in the peritoneal cavity and the omentum. Since parathymic lymph nodes in the mouse drain the peritoneal cavity, marginal sinus macrophages and medullary macrophages in these lymph nodes also have access to liposomes

administered along this way. Ultimately, lymph from the peritoneal cavity is drained to the blood circulation. As a consequence, macrophages in the liver and in the spleen can be reached as well by intraperitoneally injected liposomes (Biewenga et al., 1995). Taken altogether, intraperitoneal administration of liposomes allows the liposomes to reach more macrophages than any other administration route.

4.4. Subcutaneous administration

Lymph nodes are extremely small organs, but there are many of them distributed over the body, from tens in mice to hundreds in men. They are involved in the induction of immune reactions against antigens in their draining areas. Macrophages lining the subcapsular sinus as well as those located in the medulla of lymph nodes can be reached by subcutaneously administered liposomes (Delemarre et al., 1990). Recently, it has been shown that large liposomes are much more efficacious for targeting to lymph node macrophages than their smaller counterparts (Oussoren, 1996). Liposomes that are not trapped by the lymph nodes, leave the nodes by the efferent lymph vessels and may reach the circulation after passing several consecutive lymph node stations in line. As a consequence, part of these liposomes may reach the macrophages in spleen and liver.

4.5. Direct intra-organ administration

Recently, it was shown that thymic macrophages can be affected by liposomes that were locally injected into the thymus (Rezzani et al., 1995). The numbers of testicular macrophages could be reduced by at least 90%, by local injection of clodronate liposomes into the testis of adult rats (Bergh et al., 1993). A single intra-articular injection with liposome encapsulated clodronate in the mouse knee joint caused the selective depletion of phagocytic synovial lining cells (Van Lent et al., 1993). The above results show that in some organs, locally injected liposomes will be able to diffuse from the site of injection in order to reach macrophages in the entire organ. This may not be expected to happen in organs with a densely woven tissue structure.

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