Journal of Controlled Release 193 (2014) 241–256

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Nano-chemotherapeutics: Maximising lymphatic drug exposure to improve the treatment of lymph-metastatic cancers

Gemma M. Ryan, Lisa M. Kaminskas, Christopher J.H. Porter *

Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Pde, Parkville, VIC 3052, Australia

ARTICLE INFO

Article history:
Received 11 March 2014
Accepted 23 April 2014
Available online 5 May 2014

Keywords:
Drug delivery systems
Lymphatic
Metastasis
Chemotherapeutic
Lymph node

ABSTRACT

Nano-sized drug delivery systems incorporating chemotherapeutic drugs ("nano-chemotherapeutics") have been widely employed for the treatment of solid tumours. The dimensions of nanoparticulate drug delivery systems also make them ideal vectors for improving drug exposure to the lymphatic system, potentially enhancing the treatment of lymph-resident metastases. This review examines the physical properties of nanoparticulate drug delivery systems that promote lymphatic exposure and lymph node retention, and discusses methods for improving lymphatic access. Drug delivery systems that have been investigated for the treatment of lymph node metastasis are also reviewed, and recent advances towards active targeting approaches for lymphatic metastases highlighted.

1. Current treatment strategies for lymphatic metastases and the limitations of these approaches

Tumour metastasis is the primary cause of cancer-related mortality. Wide intercellular pores between adjacent lymphatic endothelial cells and an incomplete underlying basement membrane impart the lymphatic system with properties that facilitate the invasion and metastatic spread of cancer cells. As such, the network of lymphatic vessels is a primary route for the metastatic dissemination of a wide variety of cancers and the arrest of disseminated cancer cells within sentinel lymph nodes draining a solid tumour is of prognostic significance in a large number of cancers [1,2]. Lymph node-resident metastases also have the potential to act as a reservoir for cancer cells, resulting in spread beyond the initial metastatic site and advancement of the malignancy [3–5]. The eradication of cancers within the lymphatic system is therefore a key treatment goal in a range of cancers and an important determinant of patient prognosis.

Treatments for lymph node metastases vary, but commonly involve surgical removal of the sentinel lymph nodes (lymphadenectomy) and radiotherapy. These are invasive procedures and are associated with side effects including pain, some loss of movement and lymphedema [6]. Nonetheless, the relatively limited range of effective treatment options for metastatic cancer dictates that collateral effects such as these are usually deemed acceptable. The major limitation of lymphadenectomy, however, is that data supporting the effectiveness of this approach is limited and in most cases there is a poor correlation between the removal of the sentinel lymph nodes draining a tumour and improved survival [7]. This is particularly true in advanced cancers where metastases have spread beyond the sentinel nodes.

A further obstacle to the treatment of lymph node-resident cancers is the delivery of sufficient quantities of a chemotherapeutic to the lymphatics and lymph nodes in order to facilitate cancer killing, whilst limiting toxicity elsewhere. In this regard, the biopharmaceutical properties of small-molecule chemotherapeutics commonly dictate poor lymphatic uptake [8,9], precluding effective treatment of lymph node resident tumours. For example, a recent study in rats has shown that the maximum concentration of doxorubicin in the lymph of animals given a 1 min intravenous (IV) infusion of the drug is approximately 8-fold lower than in plasma [8]. Drug formulations or delivery systems that promote lymphatic targeting therefore provide significant opportunity for improved treatment options in metastatic cancer. This concept is supported by recent work showing that improving the lymphatic exposure of small molecule chemotherapeutics or proteins may lead to a significant reduction in the growth rates of lymphatic metastases [9,10].

Nano-chemotherapeutics have been widely investigated for their use in cancer therapy to improve drug delivery to solid tumours [11], and several formulations are approved for clinical use [12]. In most cases, the advantages of nanoparticulate approaches to tumour delivery stem from their ability to enhance plasma circulation time (often as a result of avoidance of clearance by the mononuclear phagocyte system (MPS) due to the presence of polyethylene glycol (PEG) polymer chains on the particle surface) and to simultaneously reduce free drug concentrations in the systemic circulation. In the vicinity of most solid tumours, angiogenesis is increased in order to maintain nutritional supply to rapidly dividing cells. As such tumour associated blood vessels are often poorly formed and have relatively wide fenestrations. This allows long

* Corresponding author. Tel.: +61 3 99039649; fax: +61 3 99039583.
E-mail address: chris.porter@monash.edu (C.J.H. Porter).

http://dx.doi.org/10.1016/j.jconrel.2014.04.051
0168-3659/

circulating drug delivery systems to more effectively extravasate at the tumour site and forms the basis for the enhanced permeability and retention (EPR) effect, first described by Matsumura and Maeda in 1986 [13] and since exploited by many research groups to enhance delivery to solid tumours [14]. However, dispersed cancer cells and lymphatic micrometastases with volumes less than 10 mm^3 are poorly vascularized and therefore passive targeting by EPR is difficult [15].

In some cases, nano-chemotherapeutics may also assist in overcoming multi-drug resistance (MDR), a property that many cancers develop following prolonged exposure to high drug doses. The mechanisms by which MDR develops include increased expression of drug efflux proteins (such as P-glycoprotein) that 'pump' drug out of cancer cells, reducing intracellular concentrations below lethal levels [16,17]; decreased expression of the drug uptake transporters required to deliver drugs to the intracellular environment, thereby reducing drug internalisation [18] and inhibiting intracellular pathways; and activation/deactivation of gene mutations, resulting in cellular resistance to apoptosis [17]. Nano-chemotherapeutics enhance the responsiveness of MDR cancer cells to drug therapy by multiple mechanisms [19], including the inhibition of, and competitive binding to, drug efflux pumps such as P-gp [20]; direct interaction with cell membranes inducing structural changes such as greater fluidity and differences in the composition of lipid rafts [21] leading to enhanced cellular drug entry; and promotion of drug uptake *via* endocytotic mechanisms [22] that avoid efflux process. The utility of nanoparticle-based delivery systems can also be enhanced by the attachment of targeting ligands to the surface of the particle, enhancing cellular specificity and in most cases stimulating receptor mediated internalisation into cancer cells.

Nanoparticulate drug delivery systems therefore provide a range of potential advantages for systemic chemotherapy [23]. Many of these advantages are equally beneficial for the treatment of lymph-resident metastases, however, the physical dimensions of nanomaterials also provide an additional benefit since they inherently promote drainage into the lymphatic capillaries after interstitial (subcutaneous (SC), intradermal (ID), intramuscular (IM) *etc.*) administration, and in some cases appear to promote lymph access across systemic capillary beds after IV administration.

Several approaches to enhanced drug delivery to the lymph have been described including access to the intestinal lymphatics after oral administration [24,25] and access to the peripheral lymphatics after parenteral administration [8,25]. With the potential exception of some gastrointestinal or mesenteric cancers, parenteral approaches to lymphatic targeting are of most utility for the treatment of localised or disseminated metastatic tumours and are the focus of this review. Some of the published work to this point has explored non-drug-loaded delivery systems [26,27], however the goal of these studies, *i.e.* to achieve greater concentrations of therapeutic within the lymphatic system, is consistent with improved therapeutic endpoints. As such, this review describes recent advances in lymphatic targeting using nano-sized drug delivery systems and highlights approaches that have led to enhanced treatment wherever possible.

2. The lymphatic system

The lymphatic system has some similarities to the vascular system, but unlike the blood circulation, lymph flow is unidirectional and retrieves excess extracellular fluid, macromolecules, foreign cells and antigens from the periphery and returns them to the systemic circulation. The lymphatics also provide a conduit for the trafficking of lymphocytes. Thus, as blood circulates throughout the capillary beds, fluid and plasma proteins extravasate from the vasculature into the surrounding interstitium. Although some fluid re-enters the systemic circulation *via* the post capillary venules [28], a proportion is collected by blind-ended initial lymphatic capillaries. These vessels comprise a single layer of endothelial cells that lack a prominent basement membrane. The initial lymph capillaries contain no muscle fibres, but are bound to the surrounding extracellular matrix by anchoring filaments. This forms a dynamic relationship between the lymphatics and the surrounding environment, facilitating the opening of cellular junctions and fluid entry into the lymphatics with movement and increases in interstitial pressure (Fig. 1).

From the lymph capillaries, lymph fluid drains into lymphatic collecting vessels where it passes through at least one, and usually many, lymph nodes. Lymph enters the nodes through one of many afferent channels, is filtered *via* node-resident macrophages, lymphocytes and reticular fibres, and exits *via* a single efferent vessel. Collecting vessels converge into lymphatic trunks, which in turn empty into one of two lymphatic ducts that deposit lymph directly into the systemic circulation at the junctions of the left and right subclavian and internal jugular veins in the neck.

Lymph is transported towards the neck *via* the contraction of smooth muscle that is present in the walls of all lymphatic vessels (excluding the initial lymphatics) and indirectly *via* contraction of skeletal muscles and by respiration [29]. Drug molecules that are introduced into the interstitial environment by *e.g.* SC injection therefore have the potential to be taken up by not only the blood capillaries, but also by the lymphatic capillaries from where lymph flow transports drug (or a drug delivery system) back to the systemic circulation *via* a series of lymph nodes.

3. Mechanisms of lymph node metastasis

The mechanisms of lymph node metastasis are still incompletely understood. Several hypotheses have been proposed, however most remain controversial with differences between tumour types complicating conclusions. In spite of these uncertainties, there are a number of factors that are acknowledged to play key roles in the spread of malignant disease. In the metastatic cascade, cells must first disseminate from the primary tumour, and migrate towards blood or lymphatic vessels. Cells then travel towards distal organs, and must finally arrest and adhere at distal sites. Initial metastatic arrest within lymph nodes is typically within the nodes sentinel to the primary tumour, followed by secondary nodes and then increasingly towards distal nodes. As such, sentinel node biopsy is often used as a predictive factor for lymph node involvement in cancer. As the lymph nodes are often the initial point of metastatic cell growth, sentinel node biopsy is also useful in many cases for determining the malignancy of the disease.

The preference for cells to disseminate *via* the blood or the lymph may reflect one of many factors, including mechanisms attracting cells to a particular vascular or lymphatic environment (chemokine signalling), the viability of cells under high shear forces within the vasculature vs lower shear forces in the lymph, and the physical availability of blood *versus* lymph capillaries [30]. The lymphatic system may provide a more favourable route for metastatic spread than the vascular system for reasons that include preferential cell access across the more permeable lymphatic endothelium, wider lymph vs blood vessels, and slower flow rates and reduced pressure in the lymph when compared to the blood circulation, reducing cell damage by shear stress and mechanical deformation. In addition, cell viability may be higher in the lymph, which is comprised of similar components to the interstitial fluid, whereas serum toxicity can reduce cancer cell viability in the blood [28] (Fig. 2).

3.1. Lymphangiogenesis

In the case of lymph node metastasis, the formation of new lymphatic vessels (lymphangiogenesis) is a common (but not essential) precursor to malignant spread. Lymphangiogenesis occurs not only at the primary tumour but also at metastasis positive lymph nodes and sentinel lymph nodes prior to the arrival of metastasising cancer cells [31–33]. Lymphangiogenesis at the sentinel lymph node has been correlated with an increased incidence of lymph node metastasis [31].