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RECENT TRENDS IN DRUG DELIVERY SYSTEMS: LYMPHATIC DRUG TARGETING AND TRANSPORTATION

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Targeting of drugs to lymphatic system is useful in many ways. Targeting to lymphatic system may be done not only to treat certain pathological conditions like tumors, infections and inflammations but also to detect tumor metastases. Controlled delivery of vaccines through oral route can also be achieved with lymphatic targeting. Targeting to lymphatic system is also useful for per oral delivery of the newer generation peptides and a protein that are currently being examined for their therapeutic utility, and also for those drugs which undergo extensive hepatic first-pass metabolism. Considering the wide range utility of lymphatic targeting, this article attempts to give an insight into various routes through which this can be achieved, transportation of drugs through lymphatic system, carriers used in lymphatic drug targeting and its various applications.

Keywords: *Lymphatic targeting; Cancer therapy; Controlled delivery of vaccines; Microparticulate delivery sytems; Liposomes*

Introduction

Lymphatic drug targeting refers to targeting of drugs and therapeutic agents into the lymphatic system for the drug action in the lymphatic system itself or for their transportation in the lymph to specific tissues of interest. Targeting of drugs to lymphatic system is usually attained by utilising carries like microspheres (1), nanoparticles (2) and liposomes(3). The first objective of targeting is exemplified by the targeting of certain anti-cancer agents in some lymphomas and in tumor detection in lymph nodes using radio labeled liposome(4) and the other objective is exemplified by the transport of antiinflammatory agents to the site of inflammation (1), the per oral uptake(2) and the transport of certain macromolecules by subcutaneous administration(5). Drug targeting allows a substantial reduction in the amount of drug needs to be administered. It is of interest to point out that Koff *et al.*(6), while studying the effect of immunomodulators encapsulated in liposomes on macrophages, found about 800 times lower dose of modulator encapsulated in liposomes in comparison to free modulator. This order of magnitude would reduce the dose of steroids in arthritis treatment to safe levels. Moreover, peroral delivery of macromolecules, if they can be transported via lymphatics into the systemic circulation would be very advantageous where chronic therapy is indicated. A brief anatomy of the lymphatic system (7,8) is given below for a better perspective. Lymph vessels offer an alternative pathway for the return of the tissue fluid to blood stream and they possess several distinguishing characters:

1) They are small, delicate, thin walled vessels that tend to run side by side as leashes of vessels rather than to unite to form large trunks as do blood vessels.
2) They are equipped with innumerable valves.
3) The flow of lymph is very sluggish but is increased in activities which massage the vessel walls.

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4) They are principally associated with skin, membranes and glands.
5) They are absent where blood vessels are absent and are not found in the nervous system, including the eye.
6) Along their courses are found lymph nodes (glands) through which the lymph must pass. Lymph nodes resemble spleen in structure and are filters.
7) After passing through at least one and often through more than one node, the lymph is finally emptied into the venous blood stream at the root of the neck. Lymph nodes are extremely important as the main battlegrounds where the body fights the infection organisms and they vary in size from pinhead to a large bean. They are found along the courses of the principal lymph vessels and are particularly numerous at the roots of the limb-arm pits and groin (axillary and inguinal nodes), at the side of the neck (cervical nodes), and along the courses of the abdominal aorta and its branches. Lymph vessels or lymphatics enter a node at several points on its periphery as afferent vessels. The lymph collected by the lymph capillaries of the intestinal villi is known as chyle. The lymph vessels from these villi empty by intestinal trunks into a reservoir known as *cisterna chyli*.

Lymphatic drug targeting through the oral route

Oral route has been known since 1891 following Munk's classical experiments with a patient fitted with a lymph fistula at the thigh(9) that triglycerides could be absorbed in part appearing in the lymph as chylomicra and draining *via* the thoracic duct into the circulation. The possibility of uptake and absorption of nanoparticles and microparticles by the gastrointestinal tract has been a controversial issue although there is now accumulated evidence that it can and does occur(10-15).

The stomach, intestines and related organs of the gastrointestinal tract (git) are drained along the lymphatics and through nodes lying in the mesenteries and omenta with the vessels supplying these organs. These nodes are finally drained into the *cisterma chyli*. There are regions in the git, especially in small intestine, called Payer's Patches and Gut Associated Lymphoid Tissue (GALT) which drain into the lymph vessels. These are useful for the transport of particulates from git into the lymphatic system. Alpar *et al.*(1) claimed that about 39% of administered polystyrene latex particles in the size range of 100 nm to 3 mm were taken up by the rat git *via* the GALT and the particles, with the exception of 3 mm size, were subsequently transported into the liver, albeit in low numbers. The polystyrene particles were seen in discrete anatomical compartments and structures such as the Payer's patches. Absorbed microspheres then traversed the mesentery *via* the mesenteric lymph nodes and were transported from the lymphatic circulation into the venous circulation and subsequently into the liver.

The "porosity" of the epithelial membrane to particulates could be due to the macrophagic and pinocytic activity of M-cells overlying the Payer's Patches (16,17). Dange *et al.*(18,19) reported that insulin administered through polyalkyl cyanoarylate nanoparticles (220 nm) resulted in decreased blood glucose levels. Recently(20) prodrug approach was also utilized to promote drug (zidovudine) transport through intestinal lymph in rats after intraduodenal administration of 15 mg/kg drug as a micellar lipid solution.

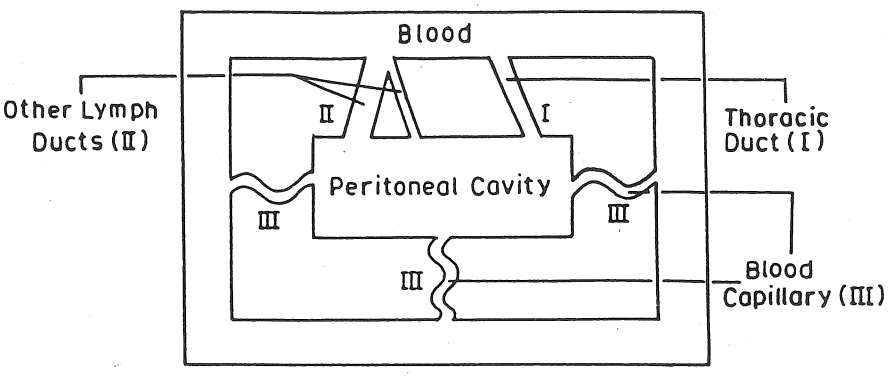

Fig. Schematic diagram for drug absorption from the peritoneal cavity. Key: I and II represent the lymphatic system and III represent splenic blood capillaries.

Lymphatic drug targeting through parenteral routes

Various parental routes like subcutaneous (sc), intramuscular (im) and intraperitoneal (ip) have been tried for lymphatic targeting. Fig depicts a schematic diagram showing the movement of an intraperitoneally administered drug. Most compounds of relatively small molecular weight are exclusively absorbed via splenic blood capillaries(III) into the portral vein. In contrast, the lymphatic system (I and II) can be a major absorption route for compounds impermeable to capillary membranes because of their large molecular size. Several lymphatic networks originate from the peritoneal cavity. These have been grouped into two major networks: one is the thoracic duct(I) which drains the retroperitoneal region and which enters the left subclavian vein; the other is the right lymphatic duct (II) which collects lymph from parasternal and intercoarstal paravertebral lymph ducts, which drain mainly through the diaphragm, finally flowing into the right subclavian vein (21,22).

A detailed discussion of the mechanisms and factors affecting drug absorption after sc and im drug administration was given by Ballard(23). From a macroscopic point of view, many drugs in solution injected into sc or im sites behave as if their absorptions were taking place passively by diffusion. For most drugs it has not been determined whether it is through capillaries, lymphatics, or both. Although there are other factors such as area of the drug depot formed after injection, volume of the injection, drug concentration, pH and biological factors such as body movements, disease conditions, age, anatomical region and the tissue condition at the site of injection affect the rate of diffusion, the molecular size of the drug or the particle size of the carrier determines whether it will be absorbed via the capillaries or the lymphatics.

Supersaxo *et al.*(24) reported a linear relationship between the molecular weight of a drug and the proportion of the dose absorbed by the lymphatics which drain the site of sc application in the investigated MW range. Molecules, with MW>16,000 are absorbed mainly by the lymphatics but compounds with MW<1000 are hardly absorbed at all by the lymphatic vessels. An increasing tendency towards lymphatic absorption was determined for molecules with a MW between 1000 and 16000.

Lack of basal lamina around lymphatic capillaries may be of major func-

tional importance in facilitating access of interstitial macromolecules to the lymphatic system(25). In addition, adjacent endothelial cells of terminal lymphatic may have gaps from 20 to more than 100 nm. These gaps make it possible that macromolecules and even large particles of up to perhaps 1 mm diameter may enter the lymphatics(26,27).

Although passive diffusion is an important mechanism for drug absorption, the process of endocytosis may also be involved in drug absorption from sc and im sites. Rees and co-workers(28) studied the systemic distribution of dimethyl polysiloxane, following sc administration, in mice. Authors suggest that mechanism of absorption is most likely by the process of endocytosis by histocytes. Weinstein also confirmed cellular uptake within one hour of dosing using fluorescence microscopy(29). Images were seen that were consistent with endocytosis of the fluorescent lipid vesicles administered.

Beresford and co-workers(30) showed that most of the absorption of iron-polysaccharide complexes injected into the rabbit muscle tissue occurred during the initial 72 hours. The absorption during this time was mediated in part by lymphatic transport of the iron complexes.

Prodrug concept in lymphatic targeting

Prodrugs, by definition, are the inactive derivatives of the active drug molecules. They are inactive *per se*, but are converted into the active form once inside the body. Recently, the use of macro-molecular carriers for the selective targeting of antitumor agents has been advocated with increasing frequency. Numerous reports on the conjugation of agents such as anthracyclines (31-33), alkylating agents (34) and methotrexate (33,34) with carriers such as DNA(31), polypeptides (32,34,35) or polysaccharides (33,36) are available. In a series of investigations, Hashida *et al.* synthesized the conjugates of Mitomycin-C (MMC) with agarose beads (37), dextran (38-40) and polypeptides (53), and obtained enhanced anti-tumor activity. The absorption of these prodrugs into capillaries or lymphatics is governed by factors that affect the absorption of other drugs and carriers. Since these prodrugs, such as Mitomycin-C dextran (MMC-D), are high molecular weight compounds, they have a high chance of getting absorbed through lymphatics. Design of prodrugs which liberate the drug spontaneously in the chemical environment of the lymphatics or otherwise would be useful in treating various lymphomas, tumor metastases and other inflammatory conditions of the lymphatics.

Drug delivery systems used in lymphatic targeting

As far as the drug delivery to the lymphatics is concerned, three main delivery systems have been used: microspheres or microcapsules; nanospheres or nanocapsules or nanoparticles or nanosomes and liposomes (all collectively known as colloidal drug delivery systems)(41). While microspheres and nanospheres have been tried for both oral and parenteral delivery, liposomes have been tried mainly for parenteral targeting. The methods of preparation of liposomes has been referred to in our recently published article(42). Preparation of microspheres(43) and nanoparticles(41) have been referred to elsewhere.

Applications of lymphatic drug targeting

1) *Controlled delivery of vaccines*

Oral immunisation has obvious advantages if absorption of the colloidal carriers for vaccines could be achieved in useful quantities. There

is a pressing need to develop effective single dose vaccines that would reduce the cost by eliminating the booster shoots while broadening the coverage. Polymeric controlled release technology combined with lymphatic targeting offers the potential of meeting this need.

Eldridge *et al.* (44) studied the controlled release of vaccines in the GALT. They found that orally administered biodegradable microspheres targeted the Peyer's Patches and good control over the release rate was obtained. They demonstrated that the particle size of *Staphylococal enterotoxin B(SEB)* toxoid has a significant impact on the onset and intensity of antibody production. In a recent study (45) enhanced lymph node delivery and immunogenicity of hepatitis B surface antigen was found when antigen entrapped in galactosylated liposomes was administered im in rats as compared to nonliposomal antigen.

2) *Treatment of inflammation*

Inflammation accompanies a range of pathological disorders such as infections and rheumatic diseases. A characteristic feature of inflammation is oedema. With infiltration of leukocytes from the vascular compartment into tissues and exudates, leukocytes enter the synovial fluid through gaps that open between epithelial cells in blood vessels under the influence of inflammatory mediators. Alpar *et al.*(1) studied the distribution of latex microspheres *in vivo*, as a potential passive targeted system for the treatment of inflammation. Microspheres administered orally were found in the circulation and in inflamed tissues and exudates of inflammatory air pouches in rats. Oral absorption was also found in a rabbit. Particles administered directly into the circulation also penetrated the air pouch tissues and fluids.

3) *Detection of tumor metastases*

One of the major problems of neoplastic diseases is the metastasising of the neoplastic cells. Metastases refers to the dislodgment of the neoplastic cells from their originating site, circulating in the body and finally getting lodged at a different place in the body to create secondary growth in the organ or tissue. Lymphatic circulation is one of the major routes of movement for metastases. Liposomes can be used to detect metastases in lymph nodes(4). It was shown that ^{99M}Tc labeled liposomes could be used for regional lymph node imaging and that nodes involved in metastatic spread showed a suppression of uptake of labeled liposomes made from phosphatidly choline and cholesterol (46).

4) *Cancer therapy*

One of the major disadvantages of chemotherapy of cancer is the non-specific action of the potent chemotherapeutic agents used. This leads to severe side effects and a huge wastage of the dose. To overcome these defects, it would be beneficial to concentrate their cytotoxicity at the tumor site and to minimise the burden to other tissues by modifying the biological and pharmacokinetic properties.

One possible approach to alter the biopharmaceutical behavior would be the derivatisation into a latent form with a high molecular weight (47). This was exploited by Hashida *et al.* (38,39) who developed a macromolecular derivative of MMC, MMC-D conjugate and examined its pharmacodynamic prop-

erties. They found that MMC-D exhibited improved antitumor activity in the *ip-ip* system against murine tumors thriving mostly in the peritoneal cavity such as B16 melanoma and *Ehrlich ascites* carcinoma. Free MMC was detected for several days in plasma and urine of mice given *ip* administration of MMC-D, and it was suggested that persistent retention of this compound in a specific locality as a potential source of free MMC was responsible for its therapeutic efficiency (38).

Another approach to treat tumors involves the use of endogenous proteins, the so-called biological response modifiers (48). These agents affect the patient's biological response to a neoplasm benefically. Recombinant DNA technology has greatly facilitated the identification and production of a number of human proteins like interferons, interleukins and erythropoeitins etc. with potent effects on the function and growth of both normal and neoplastic cells.

Poste (49) proposed that effective therapy with these proteins requires a delivery system that leads to a distribution of the drug in the body approximating the natural distribution. In this regard one has to distinguish between endocrine and paracrine molecules(24). Endocrine agents are released into the blood stream at the site of production and are then distributed within the blood to reach their target tissues at distant sites. Therefore, systemic administration such as iv application is a suitable way to administer such drugs. In contrast, most paracrine mediators act only over limited regions and are often rapidly inactivated to prevent their action at distant sites. In addition, many of paracine mediators act in a cascade with complicated interaction between mediators, leading to positive and negative feed back regulations. As a result, site directed delivery with a release profile which mimics the complex chronopharmacological behavior of such cascades should be used for optimal therapy. Interferons and IL-2, can be considered as paracrine agents. Their physiological action is limited mostly to lymphoid system. Therefore, Bocci(50,51) has proposed that lymphatic delivery of lymphokines might improve their therapeutic efficiency. Supersaxo *et al.*(24) suggested that the sc administration of endogenous mediators with MW above 16000 and whose targets are lymphoid cells may represent a suitable delivery strategy.

5) Treatment of infections

Lymphatic targeting or transportation can be used beneficially to treat infections of the lymphatics and reticuloendothelial system. This has been widely used for treatment of parasitic diseases of the liver. Liposomal encapsulated antimonial drugs have been used to treat leishmaniasis, a parasitic infection that affects the macrophages (52). Similarly, liposome encapsulated immuno modulators have been used to stimulate macrophages(6).

6) Peroral delivery of macromolecules

Macromolecules such as peptides and proteins are being used extensively in therapeutics(53). Oral adminstration is the preferred route for most drugs. However, due to their size, susceptibility to enzymatic degradation, and in most cases, low lipophilicity, peptides and proteins

are prevented from entering the blood circulation in significant amounts. One way of improving the peptide absorption in git would be to target the drug to regions where enzymatic activity is low or to regions where macromolecules can be absorbed in spite of their large mw. Colon-specific delivery is an example of the former while targeting of peptides or their carries to Payer's patches is an example of the latter. Cyclosporin, a cyclic peptide (MW 1203) is lipophilic and normally administered in an oil-based vehicle or as a microemulsion and the bioavailability of the drug in such formulations is approximately 30% which may, in part, be due to lymphatic absorption(54). Aprahanian *et al.* (14) reported transmucosal absorption of 100-200 nm diameter polyalkyl cyanoacylate nanoparticles containing insulin which decreased the glucose levels significantly.

Conclusions

It can now be appreciated that lymphatic drug targeting and transportation can be an important phenomenon for the absorption and delivery of drugs. While lymphatic targeting and transportation may be aimed at specifically as in the case of carriers for peptides for avoiding hepatic first pass metabolism, for tumor detection and its treatment or for the treatment of inflammations, it may also be normal route of absorption even when not aimed at. Some conventional drugs with high lipophilicity may be incorporated into chylomicrons inside the intestinal epithelial cells and be absorbed into the systemic circulation via the lymphatic system. The use of carrier systems such as microspheres and nanoparticles to target specific areas of git such as Payer's Patches have been tried so that peroral delivery systems can be developed for therapeutic molecules, which are used for chronic treatment in conditions like diabetes, rheumatic disorders and others. Oral immunisation is another incentive for developing peroral formulations that target the lymphatics. Parental routes such as sc, im and ip offer the alternative routes for lympatic targeting. Liposomes have been the mainstay of targeting through this route although microspheres and nanocapsules have also been tried. Thus, the selection of the route and the carrier for lymphatic targeting depends on various factors such as the drug's physicochemical nature, intended final target and the application for which it is being developed. The option of using prodrugs must also be kept in mind when a particular system is being developed. Although good deal of work has been done since 1960s and some systems have achieved a good amount of success, more research is needed in the area of lymphatic targeting for developing practical systems that can be used clinically. This is especially true for the latest therapeutic molecules produced by recombinant DNA technology.

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