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EFFECT OF PARTICLE SIZE ON THE LYMPHATIC DISTRIBUTION OF ^{111}INDIUM-AMINOPOLYSTYRENE THROUGH INTRAPLEURAL ADMINISTRATION

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ABSTRACT

The study examined the impact of size on lymphatic particle distribution through intrapleural (ipl.) administration. Aminopolystyrene of three sizes, 0.29 µm, 2.18 µm, and 11.2 µm were radiolabeled with ^{111}Indium and their biodistributions were evaluated in rats after ipl administration. Animals received either particles of three different sizes (4 mg, 200 µCi/animal) or unconjugated ^{111}Indium as control. The percentage of injected dose (%ID) per organ or sample was determined for left (L) and right (R) mediastinal lymph nodes (LN), blood, lung, and pleural wash. The biodistribution of 2.18 µm ^{111}In-aminopolystyrene was further investigated at 6 h, 24 h, 48 h, and 72 h following ipl administration to examine the possible particle retention time. The 2.18 µm particles had significantly higher uptake in both LLN and RLN compared to other sizes. The systemic uptake was minimal. At 72 h, there was still 3.2 ± 3.2% and 2.1 ± 1.8% of injected dose retained in the LLN and RLN, respectively. Scintigraphic imaging revealed significant accumulation of the radioactivity in mediastinal nodes. Particle size has significant impact on lymphatic particle distribution through ipl administration. Approximately 2 µm seems to be a suitable size.

Keywords: particle size, lymphatic distribution, ^{111}Indium-aminopolystyrene, lymph node, lymphatics, pleural space

The lymphatic system is commonly involved in the dissemination of cancer and many other diseases such as infection. Effective delivery of therapeutic agents to the regional lymph nodes in malignancies poses a significant challenge to treatment success (1). The lymphatic system is a complex network of channels permeating throughout the body to drain fluids and proteins from the interstitial space to the lymph nodes and eventually into the blood circulation. The highly effective physiological function of the lymphatic system in clearing foreign particulate matter provides a solid rationale for utilizing micro- or nanoparticulates as drug transport vehicles for lymphatic targeting. Lymphatic targeted delivery using colloidal carriers has been studied extensively in several body compartments through different routes of administration such as subcutaneous (sc), intramascular, intraperitoneal (ip), and gastrointestinal (GI) tract (2). A consistent finding in these studies is that colloidal particles administered interstitially are mainly taken up by the regional lymphatic system and accumulate to varying degrees in the draining lymph nodes.

Studies have shown that the lymphatic delivery of colloidal agents depends strongly on the particle size (3). The effect of size on lymphatic distribution using different routes of administration has been studied using fluorescent or radiolabeled particulates (4-6). For subcutaneous administration, the optimum size is reported to be between 10 nm and 50 nm. Particles larger than a few hundred nanometers in diameter are preferentially retained at the site of injection (7). Interestingly, when particles are administered intraperitoneally, particle size within the nanometer range becomes less important because drainage is simply from a cavity into the surface lymphatics; hence, uptake is not limited by diffusion through the interstitial space (8). The open junctions on the lymphatic wall are the only size restrictions to lymphatic uptake from the peritoneal cavity (9). Liggins et al (10) investigated intraperitoneal administration of paclitaxel loaded poly(L-lactic acid) microspheres of two size ranges 1-40 μm and 30-120 μm in rats to prevent surgical tumor spill and peritoneal carcinomatosis. Only one or two microspheres with diameters greater than 10 μm were observed among 200-400 microspheres observed in the lymph nodes of each rat. Majority of particles retained in the lymphatic tissue are in small micrometer sizes. These findings indicate that the particle size suitable for lymphatic distribution may vary depending on the route of administration chosen.

Thoracic lymph nodes, especially mediastinal lymph nodes, are frequently involved in tumor metastasis, particularly in lung cancer and represent a difficult target for systemic drug delivery. The feasibility to direct therapeutic agents to regional lymph nodes could have significant therapeutic potential. The transport of therapeutic agent to mediastinal lymph nodes before the disease spreads to distant sites may result in a more successful treatment and control of the disease. Emulsions, liposomes, and nanospheres have been described as potential drug carrier systems that can provide a considerable reduction of undesirable side reaction of the free drug (1). Our study, along with others, have shown that various species of particulates (e.g., carbon, polystyrene, poly-lactide-co-glicolide (PLGA), and liposomes) can be delivered successfully to the thoracic lymphatic system, especially mediastinal lymph nodes following intrapleural (ipl) administration (11,12). However, the impact of particle size on the lymphatic particle distribution through this route of administration has not been adequately investigated.

Although small nanometer sized colloids such as Technetium-99m Sulfer and Tin colloids are extensively applied for sentinel lymph node detection through subcutaneous administration (13), it is anticipated that relatively larger particle size suitable for intraperitoneal lymphatic targeting may be required for intrapleural lymphatic delivery due to the physiological similarity of these two body compartments. In the present study, we quantitatively examined the lymphatic uptake of ^{111}Indium labeled aminopolystyrene particles with different sizes ranging from 0.29 μm -11.2 μm administered to rats via ipl administration. The aim of the study was to identify the suitable particle size for delivery to thoracic lymph nodes via ipl administration.

MATERIAL AND METHODS

Animals

Male Sprague Dawley rats weighing about 250 g were obtained from Charles River Laboratories Inc. Rats were housed in sterilized cages and fed autoclaved food and water *ad libitum*. The Principles of Laboratory Animal Care (NIH Publication No. 86-23 revised 1985) were followed. Animal studies were conducted under a protocol approved by the Animal Care Committee of the University Health Network (Protocol No. 819.0) and in accordance with Canadian Council on Animal Care (CCAC) guidelines.

Aminopolystyrene Particles

Aminopolystyrene beads of three different sizes were purchased from Polyscience Inc. (Warrington, PA, USA). The sizes of the particles (mean ± SD) were as follows: 290 ± 4.2 nm (small), 2.18 ± 0.1 m (medium), and 11.2 ± 0.2 µm (large). The particles were stored at 4°C according to the manufacture's instructions until required for use.

^{111}Indium (^{111}In)

^{111}In-chloride ($^{111}InCl_3$) was obtained from Amersham Radiopharmacy, BC, Canada.

Determination of Radiolabeling Efficiency and Stability

We adopted a direct labeling method previously reported to radiolabel aminopolystyrene particles with ^{111}In (14). The radiolabeling efficiency of the different sized particles was first determined by dispensing, an aliquot of $^{111}InCl_3$ (0.35-0.97 MBq; MDS-Nordion, Kanata, ON) into a vial containing 0.25 mg of aminopolystyrene particles (in 4 µl) and 5 µl of 1.0 M sodium acetate buffer, pH 6.0. The mixtures were incubated at room temperature for 30 minutes. The particles were washed with dH_2O then centrifuged at 10,000 rpm for 10 minutes to separate the radiolabeled particle pellet from free ^{111}In in the supernatant. Labeling efficiency was determined by measuring the radioactivity contained in the particles using a gamma-counter (Cobra II® Series Auto-Gamma System Model 5003, Packard Instrument Company, Meriden, CT, USA) with a window of 230-270 keV set around the 245 keV photopeak of ^{111}In. Stability of the ^{111}In-aminopolystyrene particles was evaluated by incubation in normal saline or rat plasma for 1 h, 6 h, and 24 h after preparation at room temperature. Radiolabeling and stability studies were performed in triplicate.

Intrapleural Administration

Intrapleural administration was performed through trans-thoracic injection as described previously (11). Rats were anesthetized with an intramuscular injection of Ketamine /Xylazine (50 mg/kg and 10 mg/kg; CDMV Inc., Guelph, ON, Canada). The animals received a subcutaneous injection of temgesic (Buprenorphine 0.03 mg/kg; Schering-Plough.,USA) for pain relief. The animals were then placed in the right lateral decubitus position with the limbs restrained. The left chest wall was shaved and sterilized with Betadine®. A 1.5 cm transverse incision was made on the left lateral skin just below the inferior border of the scapula of the rats. A portion of the underlining fascia was dissected and a small incision was made through the external oblique muscle layer, the latissimus dorsi, to expose the serratus layers and intercostal muscles. The left lung was visible through the intercostal muscles. A blunt 25-gauge needle was inserted approximately 2 mm into the pleural space, and 4.0 mg ^{111}In-aminopolystyrene particles (7.4 MBq; 290 ± 4.2 nm, 2.18 ± 0.1 µm, and 11.2 ± 0.2 µm) in 0.5 mL normal saline was injected into the pleural cavity. The wound was closed with stainless steel wound clips. The respiration of the animal was closely monitored during the administration of the particles to prevent the complication of pneumothorax.

Biodistribution Study

The uptake of ^{111}In-aminopolystyrene particles in right and left mediastinal lymph nodes (RLN and LLN), blood, lung and pleural wash was determined in rats which received left-sided ipl administration as described above. Three groups of animals (n=4 per group) were administered 0.29 m, 2.18 µm, or 11.2 µm ^{111}In-aminopolystyrene particles (4 mg, 7.4 MBq/each) while one group of 4 rats received an ipl injection of free $^{111}InCl_3$ acetate (7.4 MBq each) as a

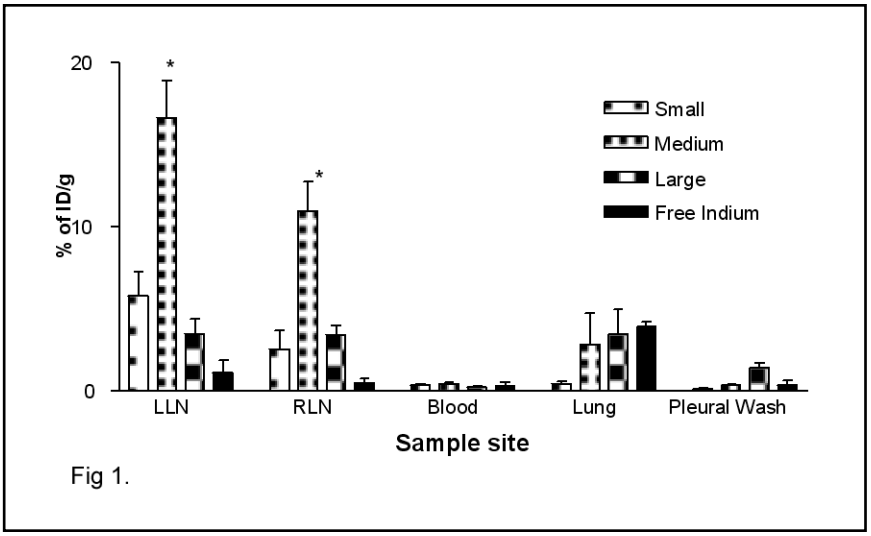

*Fig. 1. Uptake of ^{111}In-aminpolystyrene particles of different sizes in left and right mediastinal lymph nodes (LLN and RLN) and selected tissues at 24 h after intrapleural injection in rats. n=4; * p < 0.01 (ANOVA)*

control. Twenty four hours after administration, tissue samples were collected, weighed, and their radioactivity measured in a gamma-counter. The percentage of injected dose (%ID) per gram of the specimens was calculated by comparison with a standard aliquot of ^{111}In-aminopolystyrene particles of known radioactivity. The particles showing the highest lymphatic uptake at 24 h were further employed to examine the kinetics of lymphatic distribution over 6 h, 24 h, 48 h, and 72 h post ipl administration to gain insight into possible particle retention times in the regional lymph nodes.

In Vivo Imaging Studies

Additional rats were administered ^{111}In-aminopolystyrene particles (4 mg, 7.4 MBq/each) as previously described, but they were subsequently imaged at 2 h, 24 h, 48 h, and 72 h post-ipl injection. While the animals were anaesthetized with a 0.35-mL mixture of ketamine:xylazine:acepromazine (100:5:10 mg/kg), ventral whole body images were obtained using a large field-of-view gamma-camera fitted with a medium energy, general purpose collimator (ADAC Forte™, ADAC Laboratories Inc, Milpitas CA). Each static image was acquired for 1 x 10^6 counts into a 256^2 x 16 acquisition matrix using dual channels with 20% windows set symmetrically around the 171 keV and 245 keV peaks of ^{111}In.

Statistics

Values are reported as mean ± SD. Statistical analysis was performed using ANOVA to compare the percentage of injected dose per organ (%ID/g) in the mediastinal nodes and other specimens. An acceptable probability for a significant difference among means was defined as $P < 0.05$.

RESULTS

^{111}In-Labeled Aminopolystyrene Particles

Aminopolystyrene particles were directly labeled with ^{111}In acetate. The radiolabeling efficiency was 68.3 ± 2.5%, 81.9 ± 3.2%, 60.4 ± 5.8% for small, medium and large

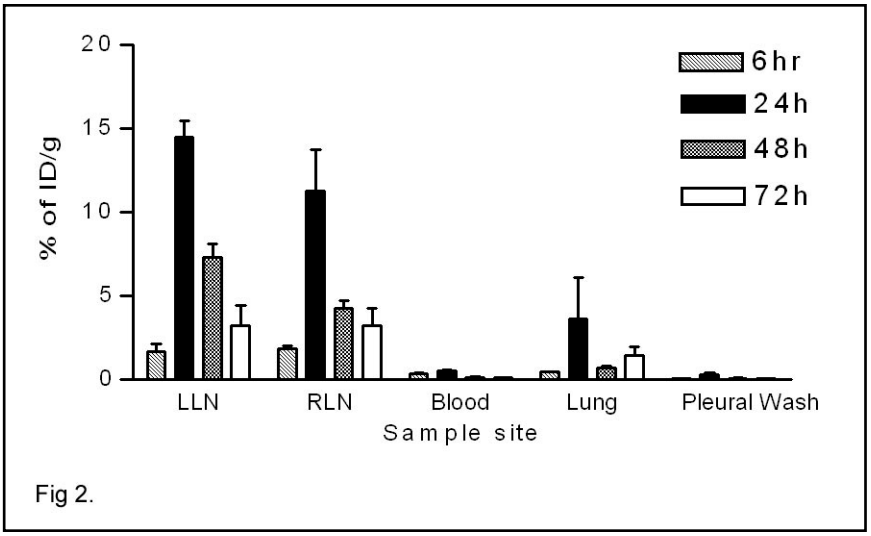

Fig. 2. Uptake of ^{111}In-aminpolystyrene particles (2.18 µm) in left and right mediastinal lymph nodes (LLN and RLN) and selected tissues in rats at different times after intrapleural injection (n=4).

(0.29 µm, 2.2 µm, 11.2 µm) size particles, respectively (n = 3).

^{111}In-labeled amino polystyrene particles were stable in normal saline and rat plasma for up to 24 h. The radioactivity of the polystyrene beads remained 78-82% and 72-76% of the original radioactivity for incubation in saline and plasma respectively.

Biodistribution

The uptake of ^{111}In-aminopolystyrene particles of different sizes in selected tissues at 24 h after ipl administration is shown in *Fig 1.* There was significantly higher lymphatic uptake to both LLN and RLN compared to other groups for the 2.18 µm polystyrene particles. For the LLN, the %ID/g values for rats administered small, medium, or large particles and the control rats administered free $^{111}InCl_3$ were 5.8 ± 2.9%, 16.6 ± 4.6%, 3.5 ± 1.8%, and 1.1 ± 1.3%, respectively. For the RLN, the %ID/g values for rats administered small, medium, or large particles and the control rats administered free $^{111}InCl_3$ were 2.6 ± 2.3%, 11.0 ± 3.6%, 3.4 ± 1.2%, 0.5 ± 0.5%, respectively (mean ± SD, n=4, $P < 0.01$). For all particles, the %ID/g values measured in the lymph nodes were higher than those of the free $^{111}InCl_3$ controls. Significant amounts of radioactivity were also detected in the contralateral mediastinal lymph nodes (RLN), while the particle distribution to the blood circulation was minimal (< 1%).

Based on these results, 2.18 µm ^{111}In-aminopolystyrene particles were selected to investigate the kinetics of lymphatic distribution over 6 h, 24 h, 48 h, and 72 h following ipl administration *(Fig. 2)*. The peak lymphatic uptake occurred 24 h after injection. Uptake in LLN and RLN at 24 h was 14.5 ± 1.5 %ID/g and 11.2 ± 4.3 %ID/g, respectively. At 72 h, there was 3.2 ± 3.2% and 2.1 ± 1.8% of the injected dose retained in the LLN and RLN, respectively. The systemic uptake into the blood and lung was of 0.1 ± 0.03% and 1.2 ± 0.7%, respectively, significantly lower than uptake in the lymphatic tissues *(Fig. 2)*.

Imaging

Scintigraphic imaging showed that rats

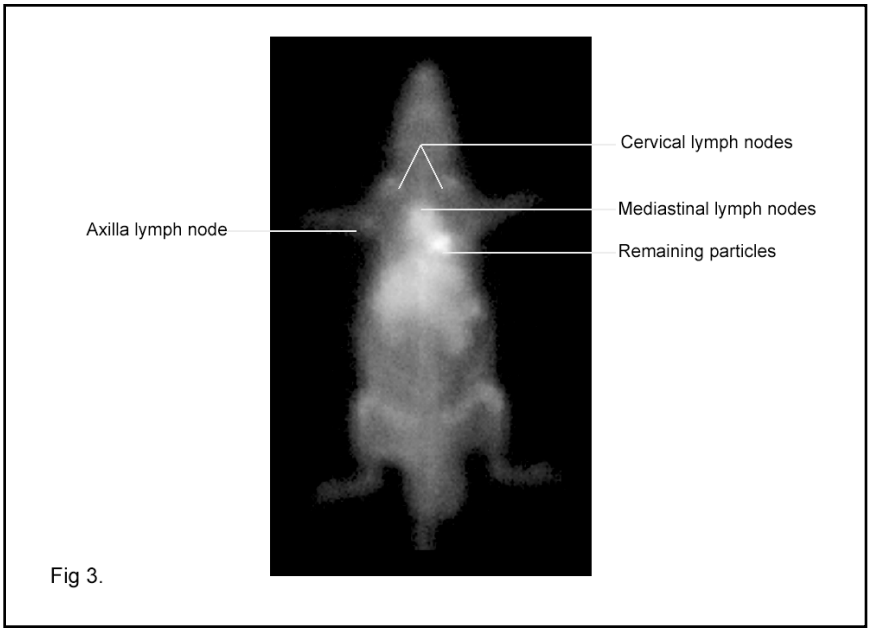

Fig. 3. Representative whole body scintigraphic image depicting the 24 h tissue distribution of radioactivity after intrapleural administration of (2.18 µm) ^{111}In-aminopolystyrene particles.

receiving 2.18 µm ^{111}In-aminopolystyrene particles appeared to exhibit higher concentrations of radioactivity in the mediastinum nodal area than those animals receiving the two other particle sizes. *Fig. 3* shows a 24-h whole body image of a rat which received an ipl injection of 2.18 µm ^{111}In-aminopolystyrene. This observation was in agreement with the greater accumulation of radioactivity in LLN and RLN for these particles in tissue distribution studies *(Fig. 1)*.

DISCUSSION

Lymphotropic delivery systems based on colloidal particulates have been employed to improve drug delivery to regional lymph nodes using various routes of administration (1). Thoracic lymph nodes, especially the mediastinal lymph nodes, are important candidates for particle-mediated drug delivery because they are involved in many pathological processes and represent a critical point for cancer and infection spread into other organs and regions of the body. Direct intralymphatic administration is technically complicated and challenging whereas interstitial administration provides low and unreliable targeting to mediastinal lymph nodes (15,16). We previously explored using the pleural space as an alternate route for delivery of particles to thoracic lymph nodes (11). The present study demonstrates that, like in many other body compartments, particulate matter represented by ^{111}In-aminopolystyrene beads, is cleared through the lymphatic system and is actively taken up into regional lymph nodes after ipl administration.

Quantitation of lymph node uptake of ^{111}In-aminopolystyrene particles by γ-scintillation counting revealed that particle size has a significant impact on delivery. A particle size of approximately 2 µm seems to favor thoracic lymph node uptake over the other two sizes (0.29 µm, 11.2 µm) tested in this study. This result is compatible with our previous findings in a qualitative study

employing carbon colloids. In that study, the lymph node uptake of carbon colloids of three different size ranges (140-240 nm, 400-600 nm, 700-1500 nm) were examined following ipl administration. The relatively large 700-1500 nm particles visually appeared to exhibit better lymph node uptake and retention than the other two sizes (11).

Since few studies have been performed using the pleural space for lymphatic delivery, it is not entirely clear what role colloid size has in lymphatic uptake using this route. The effect of particle size on intraperitoneal lymphatic delivery has been studied previously (17). For example, Hirano and Hunt administered liposome encapsulated ^{14}C-sucrose of four diameters (from 48 nm to 700 nm) into the peritoneal cavity of healthy rats. There was no size effect on the absorption of the liposomes from the peritoneal cavity into the regional lymphatics. Once in the lymphatic system, however, size became important with the smallest liposomes traversing the nodes in the lymph while the larger liposomes became trapped within the nodes. The retention of the larger particles in the lymph nodes may be explained by simple mechanical filtration and/or by phagocytosis by the cells of the reticuloendothelial system. In our previous study, the colloidal particle given intrapleurally had similar pattern of lymphatic distribution in the animals with pneumonectomy compared to those without the procedure (11). This finding indicates that in the pleural space, the lymphatic drainage of particles relies mainly on the parietal pleura. The parietal pleurae are rich in lymphatic capillaries. Many stomata open directly into the pleural space, allowing particulate matter easy access to the lymphatics. In the pleural space, mesothelial stomata with diameters ranging from 2 to 12 μm, also provide entrance points for lymphatic uptake (18,19). The selection of particle sizes for the present study took into consideration these physiological factors and the results of our previous qualitative study.

The results further show that aminopolystyrene particles can be labeled efficiently and stably with ^{111}In, providing a suitable radiotracer for the examination of particle distribution *in vivo*. Polystyrene beads have been labeled previously with fluorescein and sodium (^{125}I) iodide to investigate lymphatic distribution in other studies (20,21). Generally, quantitative measurement of the fluorescence signal is more complicated than the scintillation calibration. However, it is well known that radiolabeling of non surface modified polystyrene is difficult. NaI^{125} requires additional irradiation to facilitate the bonding of the isotope to the polystyrene particles. Nonetheless, the labeling of NaI^{125} requires additional irradiation to facilitate the bonding of the isotope to the polystyrene particles. Therefore, its application is greatly limited by the availability of irradiation. The possible mechanism of the radiolabeling may be attributable to the amino function groups appearing on the surface of the polystyrene beads which are probably able to be associated with the ^{111}In molecules. The radiaolabeling efficiency of the particles is probably more relevant to the number and the distribution of the amino groups on the surface of the particle rather than the surface area of the particle. But, it was not our intention to arbitrarily correct the ^{111}In label with respect to the amino bindings. Although the radiolabeling efficiency varied among different size particles and surface area differences are involved, the actual given dose (7.4 MBq) of radiolabeled particles used for the study is same. This simple approach provides a potential tool to study the impact of particle properties on the particle distribution *in vivo*.

This study was performed using normal, non-tumor bearing rats. The extent and kinetics of delivery of the ^{111}In-aminopolystyrene particles administered intrapleurally may change under abnormal pathological conditions such as cancer because the lymphatic channels and flow can become obstructed (22). Future studies would be preferably performed using lung cancer animal

model with nodal involvement to compare with the results observed in this work.

In summary, particle size is a critical factor for lymphatic particle distribution following ipl administration in rats. The most suitable particles to achieve the desired lymphatic distribution and retention following drainage from the pleurae appear to be those with a diameter of 2 µm. Further development of biodegradable, biocompatible particulate drug carriers is warranted in order to investigate fully the effectiveness of these delivery systems in a clinically relevant cancer model.

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