Lymphatic Drainage of Skin to a Sentinel Lymph Node in a Feline Model

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To determine the feasibility of selective lymphadenectomy, the authors developed a feline model to identify and determine the utility of mapping dyes for this purpose. Adult cats were injected intradermally with a variety of mapping substances to determine whether the anatomic site of injection had a predictable pattern of drainage to a particular lymph node. Isosulfan blue provided the optimal mapping material. Injection of isosulfan blue intradermally into the skin of the medial thigh consistently led to coloration of the central lymph node, whereas intradermal abdominal wall injections and intradermal lateral thigh injections resulted in coloration of the lateral lymph node. Intradermal injections into skin about the perineum resulted in coloration of the most medial lymph node only. The feline model proved to a useful model to examine the utility of mapping dyes and to demonstrate dermal lymphatics. The predictable pattern of drainage of the skin in this feline model supports the feasibility of selective lymphadenectomy.

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A NUMBER OF CLINICAL observations suggest that the primary route of metastasis in human melanoma is through the dermal lymphatics to the regional lymph nodes. Among these observations is the recognition that the most frequent initial site of metastatic disease is the regional lymph nodes and the occasional, although not infrequent, occurrence of dermal metastases surrounding the primary lesion (satellites) or the development of in-transit recurrent melanoma.[1,2]

Based on these clinical observations, a surgical strategy to widely encompass the primary tumor and to excise the regional lymphatics at risk for harboring metastatic disease generally has been considered standard therapy for malignant melanoma. Based on the microscopic observation of lymphatic permeation of tumor cells surrounding the primary tumor, Handley[3] advised a circular incision about the primary tumor to encompass tissue potentially involved with metastatic tumor cells. Although the concept of "wide" excision generally has been well accepted, the value of immediate lymphadenectomy in the surgical treatment of melanoma in the absence of clinically identifiable disease continues to generate considerable debate. Large, single institutional retrospective reviews have suggested that immediate lymphadenectomy results in improved survival in certain subsets of melanoma patients when compared with those who undergo therapeutic lymphadenectomy for clinically apparent disease.[4–6] Two prospective randomized trials, however, have indicated no statistically significant survival benefit for patients undergoing immediate lymphadenectomy when compared with those undergoing therapeutic lymphadenectomy.[7,8]

It is apparent that immediate lymphadenectomy can potentially benefit only those individuals with metastatic involvement of the regional lymph nodes. Microstaging of the primary lesion by both Clark's level and depth of invasion has an important correlation with the probability of lymph node metastases.[9–12] Patients with primary melanoma less than 0.76 mm in thickness rarely metastasize, whereas patients with increasingly invasive primary melanomas have an increasing incidence of node metastasis. Despite the obvious ability of microstaging to define a group of patients at either high or low risk for developing lymph node metastases, most patients with clinically uninvolved lymph nodes are not found to have microscopic disease at the time of lymphadenectomy.

The interrelationship between the skin, dermal lymphatics, and lymph nodes is poorly understood. Lymphangiographic studies have demonstrated that fine der-

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mal lymphatics coalesce to form a number of major lymphatic trunks that eventually drain to the regional lymph nodes. Despite the apparent complexity of the dermal lymphatic system, it does not seem unreasonable to anticipate that the primary lymphatic drainage of any given site of skin would be to a single, "sentinel" lymph node. Although it seems logical that metastasizing cells would spread to the geographically closest node, this is not necessarily the case.[13] If the concept of a "sentinel" lymph node is valid, then perhaps methodology could be developed that could identify that specific lymph node at risk for containing metastatic disease. This lymph node then could be excised with a selective lymphadenectomy and examined. If found to be uninvolved with metastatic tumor, the patient could be spared the risk and potential complications of complete lymphadenectomy. If the lymph node were positive, only then would a complete lymphadenectomy be potentially therapeutic.

To address this question, we sought an appropriate animal model to determine the specificity of the lymphatic drainage of the skin. In contrast to almost all rodents and mammals, in which the lymphatic drainage is to a single large lymph node, only the cat has a lymphatic anatomy similar to the multiple lymph nodes found in lymphatic basins of the neck, axilla, or groin of humans. The cat has three inguinal lymph nodes,[14] similar to the multiple lymph nodes in humans, and therefore seems ideally suited to examine the hypothesis of site-specific lymphatic drainage of the skin.

Materials and Methods

Animals

After approval of the treatment protocol by the Institutional Animal Review Committee, adult male and female cats were obtained 1 day before the planned procedure and housed overnight in the animal vivarium at UCLA. All surgical procedures were performed under general anesthesia (ketamine [10 mg/kg] and halothane). Each animal was used for evaluation of both right and left inguinal node stations, and each injection site was accounted for as a single data point. At the completion of the surgical procedures, all cats were killed by lethal injection with potassium chloride.

Dyes

A number of substances were examined for their potential utility as tracking dyes in the lymphatics. These included methylene blue (American Regent Lab, Shirley, NY), isosulfan blue (Zenith Parenterals, Rosemont, IL), and Cyalume (American Cyanamid Co., Bound Brook, NJ). All substances used were known to be nontoxic *in vivo* and were injected intradermally as provided from the supplier.

Treatment Protocol

Under general anesthesia 0.25 to 0.5 mL of the mapping dye was carefully injected intradermally, using a 25-gauge tuberculin syringe. Approximately 5 minutes later, to allow the entry of the mapping dye into the lymphatics, an oblique incision in the groin was made and the skin flap opposite the site of injection was raised sharply with a scalpel. The proximal flap was elevated in a similar fashion, taking extreme care to be superficial to the lymphatic channels to avoid dividing them. A superficial groin dissection subsequently was performed by taking the inguinal fat pad starting from the area most distal from the dye injection site and dissecting it off the fascia of the anterior abdominal wall and femoral triangle. The orientation of the specimen was preserved and the individual lymph nodes were isolated and visually characterized for presence or absence of the dye.

Results

Selection of the Optimal Mapping Dye

Three agents were studied to determine an optimal material for mapping the lymphatics. Cyalume is a fluorescent dye that allowed ready identification of the lymphatic but had substantial problems with background fluorescence because of leakage into the surrounding interstitial tissue space. For this reason Cyalume proved not to be a satisfactory lymphatic mapping agent. Methylene blue, when injected intradermally, proved to be unsatisfactory in defining the lymphatic drainage because of poor uptake in the lymphatics as well as staining of the tissue.

The most useful mapping dye proved to be isosulfan blue. Isosulfan blue, when injected intradermally, rapidly entered the lymphatics and was associated with minimal diffusion into surrounding soft tissue. Elevation of skin flaps before the injection of the isosulfan blue allowed us to visualize the rapid uptake and the progress of the dye toward the draining lymph node station. The dye was readily visualized in the lymphatic channels, as was the lymph node, which turns a light blue color as the dye diffuses through the node (Fig. 1).

Lymphatic Mapping

Per the treatment protocol, adult cats were anesthetized and 0.25 to 0.5 mL isosulfan blue was injected intradermally. An intradermal injection of the isosulfan blue was critical in achieving the results. Subcutaneous injection of the isosulfan blue resulted in rapid uptake of the dye into subcutaneous lymphatics, which subsequently enter lymph nodes in the deep groin, bypassing nodes in the superficial groin.

The lymphatic anatomy of the cat allows identification of three regional lymph nodes: a medial, a middle, and a

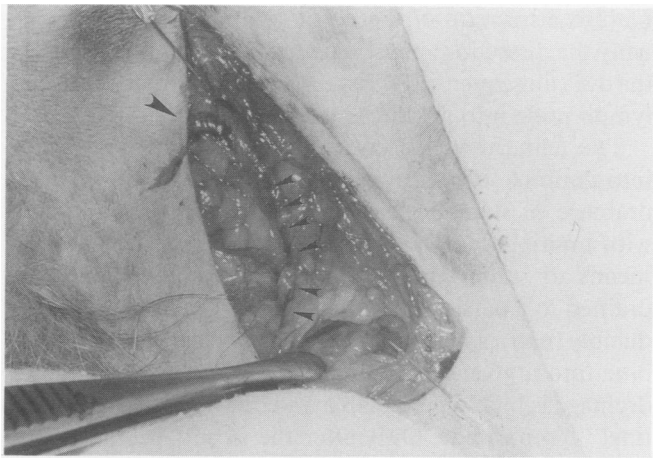

FIG. 1. Intraoperative examination of the dermal lymphatic system using isosulfan blue. The lymphatic channels are readily visualized as bright blue (*small arrows*). The lymph node (*large arrow*) is stained a pale blue that is clearly distinguishable from the adjacent lymph nodes marked by the needles. This lymph node was identified after injection of isosulfan blue intradermally in the medial thigh.

lateral node. The skin of the hind limb laterally and medially, the perineum, and the abdominal wall all were used as the sites of injection. A predictable pattern of lymphatic drainage from the various sites of injection rapidly emerged. Injection of isosulfan blue (6/6 injections) intradermally into the skin of the medial thigh uniformly resulted in blue coloration of only the middle lymph node (Fig. 2). In contrast injection of isosulfan blue intradermally into skin of the lateral thigh (10/10 injections) uniformly resulted in blue coloration of the lateral lymph node only, as did a similar injection intradermally into the skin of the anterior abdominal wall. Similarly injection intradermally into skin of the perineum (4/4 injections) resulted in blue coloration in the medial lymph node only.

Further studies were performed with the lymph nodes *in situ*. A similar elevation of skin flaps was performed and the afferent lymphatic channel was identified. Using a meticulous dissection technique, we were able to follow the dermal lymphatic channel to the primary draining lymph node. Continued observation of the *in situ* lymph nodes allowed us to follow the progression of the dye through the lymph node and sequentially into the adjacent lymph node before entering the deep lymphatic system of the groin.

Discussion

This report describes the development of a feline model to determine the feasibility of intraoperative mapping for the purpose of selective lymphadenectomy. The feline model was selected because the cat has a regional lymphatic anatomy that most closely approximates that of humans. In contrast to most rodent and mammals other than humans, who have only a single large lymph node, the cat uniformly has multiple lymph nodes in the groin. The multiple lymph nodes in the groin of the cat provide a reasonable model of the lymphatic anatomy encountered in the lymph node basins draining the skin of humans. The results achieved in this model indicate that the dye isosulfan blue (Lymphazurin®) provides a useful tool in mapping the dermal lymphatics. Of even greater importance clinically, it can be demonstrated that a particular anatomic skin site will drain to a specific lymph node.

The standard technique for visualizing lymph node architecture and determining whether the lymph node is involved with a metastatic process is lymphangiography.[13] This technique is dependent on the replacement of normal lymph node tissue by the neoplastic process that does not fill with radiopaque contrast material. Although this has been used in melanoma, it has proven not to be of use for the identification of patients who are candidates for lymphadenectomy.[15] Because lymphangiography can identify only lymph nodes in which substantial amounts of the node have been replaced by metastatic deposit, and the majority of lymph nodes that are clinically uninvolved with metastatic tumor will have metastatic tumor deposits identified only microscopically or by using sensitive immunohistochemical techniques,[16] this standard lymphangiography is of little use in this setting. For this reason the ability to map the lymphatics and to selectively identify

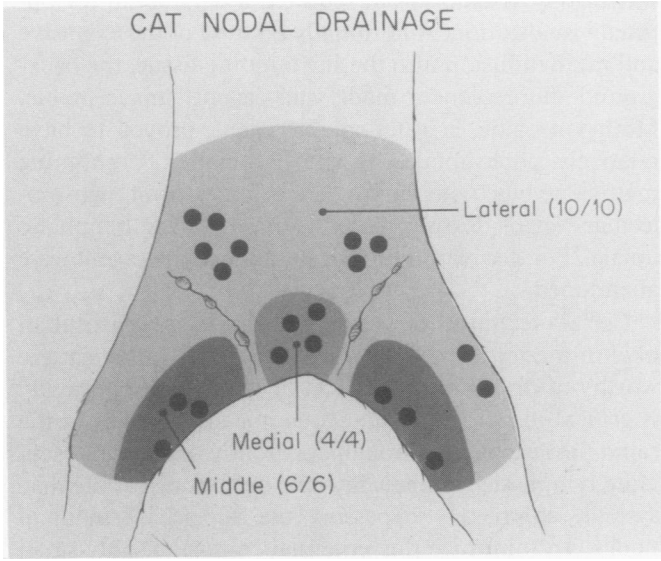

FIG. 2. Schematic representation of injection sites in the feline model. A reproducible pattern of drainage was identified by injection of isosulfan blue into the medial aspect of the thigh, which led to drainage to the middle lymph node; the lateral thigh, which led to drainage to the lateral lymph node; and the perineum and lower abdomen, which drained the medial lymph node.

the lymph node or nodes at greatest risk for containing metastatic disease appears to be critical if selective lymphadenectomy is to be clinically useful at the time of surgical excision.

Isosulfan blue is a monosodium salt of a 2,5 disulfonated triphenylmethane dye[17] that may be weakly bound to albumin and is selectively picked up by the lymphatic vessels, which then are delineated in a bright blue color, making them discernible from surrounding tissue. It has no known pharmacologic action. Isosulfan blue has been indicated as an adjunct to lymphography in humans for visualization of the lymphatic system draining from the region of injection, and comes from the manufacturer in a 1% solution. For the purpose of visualizing the fine dermal lymphatics in humans, isosulfan blue satisfactorily identified the lymphatics in approximately 97% of subjects studied.[17] It seemed to be an ideal dye for use in this model.

In this study isosulfan blue was used to identify the dermal lymphatics and to map the regional lymphatics of the cat. The dye proved to have a number of characteristics that could be useful in selective lymphadenectomy. The bright blue coloration of isosulfan blue is readily visualized and allows easy identification of the lymphatic channel. Additionally when entering the lymph node, isosulfan blue stains the node a pale blue that is readily discernible from the nonstained lymph node. In contrast the other dyes used in this study proved to be unsatisfactory and were abandoned, largely because of diffusion into the surrounding tissue and insufficient pickup by the lymphatic vessels. Cyalume as a fluorescent dye was readily visualized but required a dark room for optimal visualization. Additionally because of the extensive and rapid diffusion into the surrounding tissue, the background fluorescence made this agent unacceptable. Methylene blue, a water-soluble agent, proved to have relatively poor uptake by the lymphatics. Combining methylene blue with varying concentrations of high-molecular-weight dextran, failed to improve the lymphatic uptake. For this reason further studies with this agent were abandoned.

Certain technical aspects regarding the use of isosulfan blue in the mapping of dermal lymphatics in the cat are worthy of discussion. Injection of the dye intradermally is critical, because subcutaneous injections result in the rapid passage of dye through apparently distinct and separate lymphatic channels into the deep nodes of the iliac system, completely bypassing the superficial inguinal nodes. To minimize this potential confusion, only small volumes of dye should be injected to prevent the extravasation of the dye in the subcutaneous tissue. In defining the lymphatics, extreme caution should be used in elevating skin flaps. Furthermore it is preferable to develop skin flaps that are opposite the injection site to avoid premature disruption of these fine lymphatic channels. Finally the dissection must be performed promptly, because the dye can sequentially pass through the primary draining lymph node into an adjacent lymph node.

The feline model used in this study provides insight into a number of critical questions regarding the lymphatic drainage of skin. Because the cat is similar to humans with multiple lymph nodes in the groin, it provided the means to study whether a particular area of the skin drained to a particular lymph node. The ability to reproducibly map a single lymph node by injection of isosulfan blue into a given anatomic site suggests that lymphatic drainage is highly predictable and drains to a single "sentinel" lymph node. Only after the isosulfan blue passes through the primary draining lymph node does it begin to stain the other lymph nodes. By carefully selecting the injection site for the isosulfan blue, we could identify an area where lymph nodes that drain separate anatomic sites could be stained blue, indicating the boundary between these two areas and a "crossing over" of the lymphatics.

The results achieved by the intradermal injection of isosulfan blue in this feline model provide support for the concept of selective lymphadenectomy in those tumors of the skin at risk for lymphatic metastases, primarily cutaneous malignant melanoma. If tumor cells were to enter the dermal lymphatics and travel in a fashion similar to isosulfan blue, it would seem logical to assume that the primary lymph node draining that site would be the node at risk for developing metastatic disease. If the lymphatics of the primary melanoma could be mapped and the lymph node at risk examined, the need for complete lymphadenectomy might be avoided if this lymph node could be shown not to harbor metastatic disease. The development of this feline model to examine the lymphatic drainage of the skin supports the potential utility of selective lymphadenectomy in the surgical management of cutaneous neoplasms whose primary route of metastases is through the dermal lymphatics.

References

1. Lee YT. Loco-regional recurrent melanoma. I: natural history. Cancer Treat Rev 1980; 7:59–72.
2. Karakousis CP, Choe KJ, Holyoke ED. Biologic behavior and treatment of intransit metastasis of melanoma. Surg Gynecol Obstet 1985; 150:29–32.
3. Handley WS. The pathology of melanotic growth in relation to operative treatment (II). Lancet 1907; 1:996–1003.
4. Balch CM, Murad TMM, Soong SJ, et al. Tumor thickness as a guide to surgical management of clinical stage I melanoma patients. Cancer 1979; 43:883–888.
5. Milton GW, Shaw HM, McCarthy WH, et al. Prophylactic lymph node dissection in clinical stage I cutaneous malignant melanoma: results of surgical treatment in 1,319 patients. Br J Surg 1982; 69:108–111.
6. Reintgen DS, Cox EB, McCarty KS Jr, et al. Efficacy of elective lymph node dissection in patients with intermediate thickness primary melanoma. Ann Surg 1983; 198:379–385.

7. Sims FH, Taylor WF, Ivins JC, et al. A prospective randomized study of the efficacy of routine elective lymphadenectomy in management of malignant melanoma: preliminary results. Cancer 1978; 41:948–956.
8. Veronesi U, Adamus J Bandiera DC, et al. Delayed regional lymph node dissection in stage I melanoma of the skin of the lower extremities. Cancer 1982; 49:2420–2430.
9. Breslow A. Tumor thickness, level of invasion and node dissection in stage I cutaneous melanoma. Ann Surg 1976; 182:572–575.
10. Das Gupta TK. Results of treatment of 269 patients with primary cutaneous melanoma: a 5 year prospective study. Ann Surg 1976; 186:201–209.
11. Wanebo HJ, Woodruff J, Fortner JG. Malignant melanoma of the extremities: a clinicopathologic study using levels of invasion (microstage). Cancer 1975; 35:666–676.
12. Holmes EC, Moseley HS, Morton DL, et al. A rational approach to the surgical management of melanoma. Ann Surg 1977; 186: 481–490.
13. Kinmonth JB. The Lymphatics. Diseases, Lymphography, and Surgery. Baltimore: Williams & Wilkins, 1972.
14. Wischnitzer S. Atlas and Dissection Guide for Comparative Anatomy, 3rd edition. San Francisco: WH Freeman and Co., 1979.
15. Cox KR, Hare WSC, Bruce PT. Lymphography in melanoma: correlation of radiology with pathology. Cancer 1966; 19:637–647.
16. Cochran AJ, Wen DR, Morton DL. Occult tumor cells in the lymph nodes of patients with pathological stage I malignant melanoma. Am J Pathol 1988; 12:612–618.
17. Hirsch JI, Tisnado J, Cho SR, et al. Use of isosulfan blue for identification of lymphatic vessels: experimental and clinical evaluation. AJR 1982; 139:1061–1064.