ABSTRACT

Objective: To characterize if blue vital dye and carbon solution allow surgeons and pathologists identify sentinel nodes, not impairing the pathological examination. Methods: Sixty young adult female rats were studied. Three groups of 20 animals were formed and received a blue solution containing 0.5, 1 and 6% of activated carbon, respectively. An injection of patent blue solution with activated carbon was done in the ventral aspect of the right hindpaw. Thirty minutes later, a sentinel node biopsy was performed. Sentinel nodes were submitted to conventional histological study to identify the presence of activated carbon particles within the lymph nodes, as well as their location and distribution. Results: In the group treated with 6% activated carbon solution, identification of activated carbon particles was possible in all 20 slides at 100x magnification; in the 1% group, 200x magnification was necessary. In the last group, 0.5%, in six slides was necessary to use the immersion technique (100x magnification) for correct identification; all 14 slides were adequately studied at 400x magnification. Conclusions: The identification of carbon particles in the histological study was possible in the three groups of activated carbon and patent blue solutions. However, the best solution of vital dye with the least activated carbon concentration was the 0.5% group.

Keywords: Sentinel lymph node biopsy; Models, animal; Rats; Charcoal; Coloring agents

INTRODUCTION

Sentinel lymph node biopsy started at the end of the 20th century as a procedure to detect micrometastases in lymph nodes in the draining basin(1). If there is micrometastasis in the sentinel lymph node, the patient undergoes a...
complete lymphadenectomy; whereas if it does not contain micrometastasis, lymphadenectomy is not performed. Sentinel lymph node biopsy is included in melanoma and breast cancer staging, according to the American Joint Committee on Cancer staging manual(2). Although this concept had been proposed before, the lymphatic mapping technique for sentinel lymph node biopsy of melanoma was described by Morton et al. in 2002(3). The procedure includes a preoperative lymphoscintigraphy, sentinel lymph node biopsy using lymphatic mapping with vital dye and intraoperative gamma probe detection, and histological examination of the node(3).

Lymphoscintigraphy shows drainage basin and allows the localization of the sentinel lymph node. Most of the times, the injected radiolabeled allows gamma intraoperative detection to identify the sentinel lymph node(4-5). Lymphatic mapping with vital dye simulates the lymphatic path that tumor cells could have followed from the primary lesion to the sentinel lymph node. The most utilized dyes are patent V blue and isosulfan(6).

Intraoperative gamma probe detection allows an easy localization of sentinel lymph node and a less aggressive dissection. Both the vital dye and the radiolabeled go quickly through the sentinel lymph node and the pathologist does not have any evidence of the presence of them into the lymph node. The presence of a tissue indicator that could be retained into the sentinel lymph node until pathological examination would be very useful(7-8).

Experimental studies suggest that activated carbon is retained for longer period in the node than the vital dye and allows for an easier visualization during intraoperative period and in the histological study(9-11). A previous study of our group showed that activated carbon at 6% identifies the node and lasts into the sentinel node for at least 21 days. The activated carbon forms a permanent tattoo at the injection site and high concentrations jeopardize pathological examination, it would be very interesting to determine a solution of vital dye with the lowest concentration of activated carbon that could indicate the sentinel lymph node for the surgeon and allow its identification by the pathologist. The total removal of the injection area spot by wide excision or enlargement of the margins of tumor resection is required to avoid tattoo.

OBJECTIVE
This study aims to identify an appropriate vital dye and activated carbon solution.

METHODS
Sixty young adult female rats (Rattus norvegicus: var. albinus, Rodentia Mamalia) Wistar race EPM-1 were studied, weight varying from 250 to 300 g, originated from the Central Animal Care Facility of Universidade Federal de São Paulo (UNIFESP).

Each animal was anesthetized with one intraperitoneal injection of a mixture of 125 mg of tiletamine hydrochloride and 125 mg of zolazepan (ZOLETIL® 50) at a dose of 1 mg/kg with a syringe and hypodermic needle (13 x 4.5 mm).

Activated carbon injection
Injection of 0.08 ml of patent blue solution with activated carbon was carried out in the ventral aspect of the right hindpaw of each animal. The animals were distributed into three groups of 20 animals which received blue solution containing respectively 0.5, 1 and 6% of activated carbon.

Sentinel lymph node biopsy
Thirty minutes after the injection of the solution, a skin incision from the popliteal region up to the inguinal region was made, enabling identification of a dyed popliteal node. Therefore, the sentinel lymph node was identified in the popliteal fossa near the lateral attachment of the gastrocnemius muscle and was then dissected and removed. Thirty minutes after this procedure, the animals were sacrificed with an overdose of anesthetic.

Histological study
The samples of node were sectioned in the middle of the larger diameter, placed in 10% formaldehyde for the normal technical processing. They were submitted to conventional histological study (hematoxilin-eosin method) to identify the presence of activated carbon particles within the lymph node, as well as their localization and distribution inside the node.

Ethical and human considerations
This project was analyzed and approved by the Research Ethics Committee of Universidade Federal de São Paulo (CEP 0126/06). Animal management was done following the guidelines of Colégio Brasileiro de Experimentação Animal (COBEA).

RESULTS
All sentinel lymph nodes were macroscopically identified by the activated carbon and patent blue solutions. No animal died or presented clinical disorders during the sentinel lymph node biopsy procedure or after
thirty minutes until being sacrificed. All sentinel node tissues analyzed in the three study groups presented activated carbon particles in the histological studies.

Different concentrations presented diverse difficulties characterized by the pathologists for this study: in the slides with 6% activated carbon, identification of all 20 slides was possible with a 100-time-magnification; in the 1% activated carbon group, it was necessary to magnify 200 times and all 20 slides presented easily identifiable pigmentation. In the last group, with 0.5% activated carbon, in slides of six sentinel nodes it was necessary to use the immersion technique (magnification of 1,000 times) for correct identification of the activated carbon particles; all other slides of this same group were appropriately studied with magnification of 400 times, which was enough to identify the activated carbon pigmentation in the tissues.

**DISCUSSION**

Sentinel lymph node biopsy is a minimally invasive procedure and it is considered an important advance in oncologic surgery. It selects patients with micrometastases who should undergo complete lymphadenectomy. Sentinel lymph node biopsy still presents significant false-negative results (3 to 6%)\(^{(12)}\). Therefore, although these patients present negative sentinel lymph nodes, they develop recurrence in the same lymphatic region from where the sentinel lymph node was removed.

The pathological examination of a false sentinel lymph node is an important causal factor for episodes of false negative cases\(^{(13)}\). Vital dyes (patent blue V and isosulfan) as well as radiolabeled solutions used in lymphoscintigraphy are not permanent markers; thus, they are not identified by pathologists.

A long lasting marker of sentinel lymph node that would not harm the patient and could be identified by pathologists would be of relevant diagnostic value.

This study checked if three different solutions of vital dye and activated carbon (0.5, 1, and 6%) could dye the sentinel lymph node for the surgeon and allow its identification in the pathological examination, thus identifying particles of activated carbon. The primary aim was to find the lowest concentration of activated carbon to attain those objectives.

This experiment was based on an experimental model in rats in which the sentinel lymph node of hindpaw was a popliteal lymph node\(^{(14-15)}\). The three different concentrations of activated carbon allowed identification of activated carbon particles in the sentinel node. Each carbon solution presented different difficulties for pigment visualization. In the 6% solution, all slides were easily analyzed; carbon pigment was identified in microscope with magnifications of 100 to 200 times in all sentinel lymph nodes. In the 1% solution, magnification of 400 times was needed for correct pigment identification. In the 0.5% solution, it was necessary to apply the immersion technique and a magnification of 1,000 times for studying slides of six sentinel lymph nodes; in the other 14 sentinel lymph nodes of the same group, a magnification of 400 times was enough to confirm the presence of activated carbon pigment. The slides of sentinel lymph node of rats injected with the 6% solution presented many activated carbon particles that probably made the identification of micrometastases more difficult.

Hence, our results show that the least concentrated solution (0.5%) of activated carbon and patent blue dye enabled identification of the sentinel lymph node by the surgeon; moreover, the sentinel lymph node contains enough amount of activated carbon particles to be identified by the pathologist, probably without jeopardizing the pathological identification. Total removal of the activated carbon in the injection spot by wide excision or enlargement of the margins of tumor resection is required to avoid tattoo. Another study could appropriately answer if the 0.5% solution does not produce tattoos in the primary site after wide excision.

**CONCLUSIONS**

The 0.5% group is the best among the three solutions of patent blue and activated carbon presented for finding the sentinel lymph node in rats. That was based on a lower concentration that still allows the surgeon, with only one injection, to identify the sentinel lymph node and its appropriate pathological examination. In this case, the tissue pigmentation did not compromise the slides analysis. Wide excision of the primary tumor would probably avoid tattoo at this concentration.

**REFERENCES**


