

LYMPH NODAL VITAL STAINING WITH NEWER CARBON PARTICLE SUSPENSIONS COMPARED WITH INDIA INK: EXPERIMENTAL AND CLINICAL OBSERVATIONS

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ABSTRACT

CH40 and CH1500AA are newly prepared carbon suspensions which were examined as vital staining dyes for their usefulness in visualizing lymphatics at operation and to blacken lymph nodes. In mice, these carbon suspensions at 0.001ml/g of body weight and India ink were injected subcutaneously into the footpad of the right hindpaw. Regional lymph nodes were visualized and were examined stereomicroscopically to determine how intensely these nodes blackened with carbon suspensions. Compared with India ink, CH40 and CH1500AA blackened the regional lymph nodes much faster and more vividly (1-8 min. after subcutaneous injection). As analyzed by centrifugal particle size distribution, CH40 and CH1500AA are narrowly distributed with a small particle size (150 and 167nm, respectively, in mean diameter). By contrast, India ink is comprised of widely distributed and relatively large particles in suspension (mean diameter—254nm). In 10 patients undergoing radical gastrectomy for treatment of stomach cancer, CH40 blackened 69% of regional lymph nodes with metastases (38 of 55) and 76% of those nodes without metastases (387 of 512).

In operations for cancer, the malignant tissues of both the primary and the metastatic lesions in the regional lymph

nodes are best excised completely. However, the lymph nodes and draining lymphatics are typically hidden in the adjacent fat, and it is often technically difficult to identify regional lymph nodes and draining lymphatics in a blood-stained operative field.

We have developed two new types of carbon suspensions, namely CH40 and CH1500AA. After interstitial injections, these suspensions are readily absorbed into regional lymphatics and stream along with the lymph flow to blacken regional lymph nodes (1,2). In other words, both the draining lymphatics and adjacent lymph nodes are clearly defined using these new carbon suspensions, particularly during operation (3,4). Nonetheless, experience with this technique is still limited (5) and accordingly we now detail our updated experience including carbon particle size distribution and the ability to define lymph nodes in the intact host.

MATERIALS AND METHODS

Preparation of carbon particle suspension

Two types of new carbon particle suspensions (2,6), namely CH40 and CH1500AA, were prepared.

Carbon 40 (50mg/ml) (Mitsubishi Chemicals Co., Ltd., Tokyo, Japan) contains very small carbon particles (21nm) as determined by scanning electron microscopy

and was combined with 20mg/ml of polyvinylpyrrolidone (PVP, K-30 Nakarai Chemicals Co., Ltd., Kyoto, Japan) (40,000 daltons) mixed in saline and kneaded with three rollers to turn the carbon into a suspension (CH40).

Using a similar procedure, CH1500AA was prepared. 50mg/ml of 20nm-sized #1500AA carbon (Mitsubishi) was combined with 20mg/ml of PVP and saline. For purposes of comparison, India ink, another carbon suspension, was also tested. India ink was composed of 60mg/ml of amorphous carbon black (Mitsubishi) (particle size not definable), 50mg/ml of glue (Ita-nikawa, Morikawa-Shohten Co., Ltd., Kyoto, Japan) and 50mg/ml of CaCl₂ in water.

These three carbon suspensions (CH40, CH1500AA, and India ink) were sealed in glass tubes and sterilized at 120°C for 10 min.

Size of the carbon particles in suspension

CH40, CH1500AA, and India ink were measured for particle size distribution in suspension by use of centrifugal analysis (Horiba CAPA 500, Horiba Instrument Co., Ltd., Kyoto, Japan).

EXPERIMENTAL DATA

Lymph node staining in mice

120 Shimizu mice (Hamamatsu, Japan) (CDF1 strain, female, 5 weeks old, 20g body weight) were divided into three equal groups of 40 mice. Each carbon suspension (CH40, CH1500AA, and India ink) at 0.001ml/g of body weight (equal to 0.02ml/mouse) was injected subcutaneously into the footpad of the right hindpaw with a 30 gauge needle and microsyringe over 4 seconds.

One, 2, 4, and 8 min. after injection, 10 mice in each subgroup were killed by neck-breaking and immediately thereafter the regional lymph nodal areas were exposed to determine color change (blackness). These nodes included the popliteal, inguinal-iliac, and para-aortic (right renal arterial node) as the first, second, and third level nodal basins, respectively.

Using stereomicroscopy, these respective nodal areas were examined for black staining and color change was coded as follows: 0—no color change; 0.5—lymph node sinus was gray or partially black; 1.0—the entire lymph node or the marginal sinus was stained intensely black.

The lymph node data in each mouse was totaled (i.e., for 10 mice) at each time interval after injection (i.e., 1, 2, 4, 8 min.).

Clinical experience

Ten patients with gastric cancer received preoperatively CH40 to facilitate detection and removal of regional lymph nodes during laparotomy. Two to 7 days before operation, each patient received an injection of CH40 (2-3ml) via a gastrofiberscope (type Q-10, Olympus Optical Co., Ltd., Tokyo, Japan) using a needle injector into two sites of the gastric wall adjacent to the primary tumor. Thereafter, each patient underwent gastrectomy with regional nodal dissection using the blackened lymph nodes as a guide. From the resected specimen, the lymph nodes were classified macroscopically into blackened, and non-blackened nodes, and then examined for metastases by histology (hematoxylin-eosin stain).

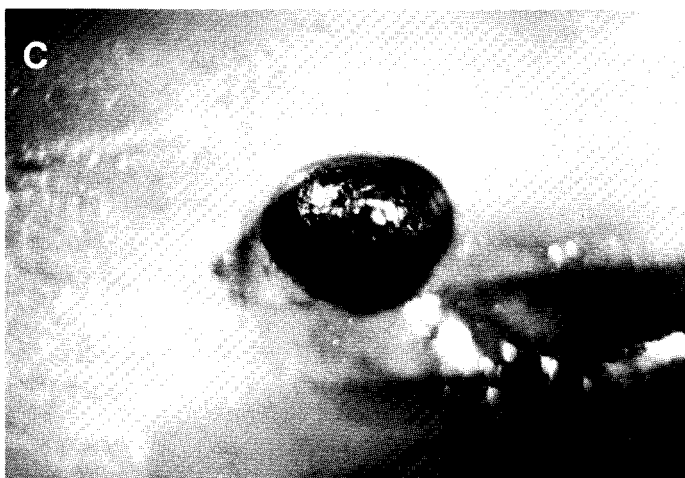
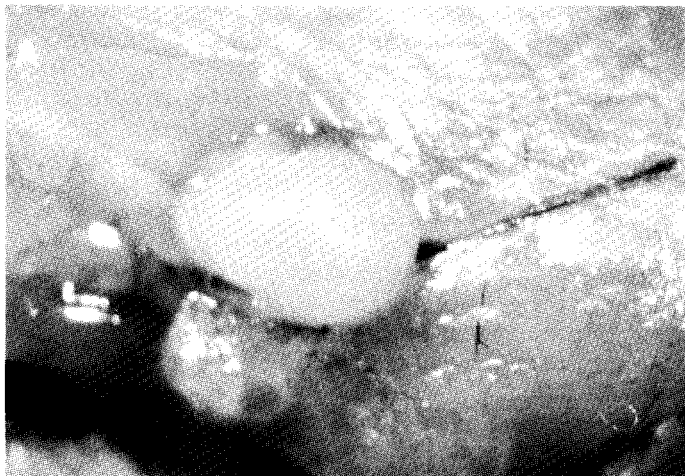
RESULTS

The size of the particles in the carbon suspension for each agent is shown in (Fig. 1). In CH40, the particles were uniformly less than 700nm in size and the mean was 150nm (Fig. 1A). In CH1500AA, particles were less than 1000nm (93%) and the mean was 167nm (Fig. 1B). India ink, on the other hand, 21% of the particles were from 1000 to 2000nm and the mean was 254nm (Fig. 1C).

Fig. 2 demonstrates the appearance of mouse lymph nodes using the scoring system outlined in the Methods.

Table 1 summarizes results of regional lymph node staining in mice. The popliteal lymph nodes were stained black completely within 2 min. after injection by CH40 and nearly completely by CH1500AA; in

*Fig. 1. Stereomicroscopic view of blackened lymph nodes. The degree of blackness was arbitrarily divided into three categories. **A**—0 or no change in color; **B**—0.5 whether the lymph node is gray or the marginal sinus is partially blackened. **C**—1.0 where the entire node or sinus is deeply blackened.*



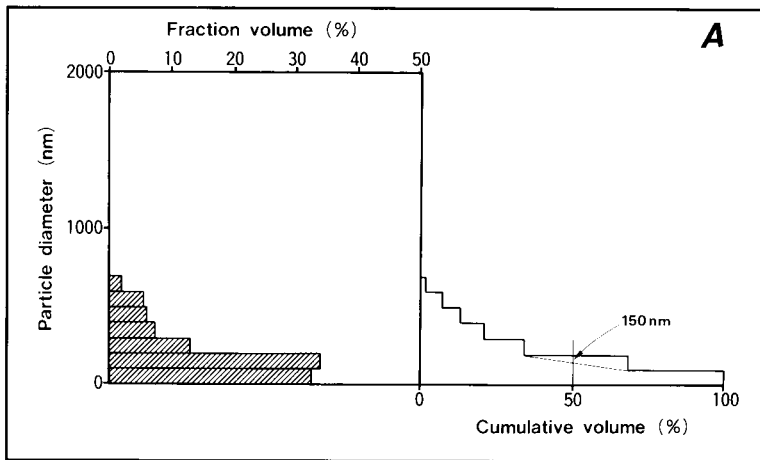


Fig. 2. Particle size in each carbon suspension. **A**—CH40: the particle size was less than 700nm with a mean of 150nm. **B**—CH1500AA: 93% of the particles were less than 1000nm with a mean of 167nm. **C**—India ink: 21% of the particles were from 1000 to 2000nm in size; the mean was 254nm.

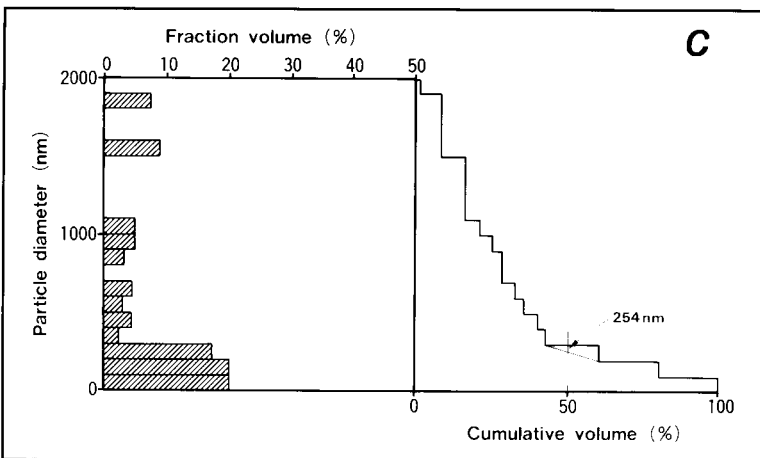
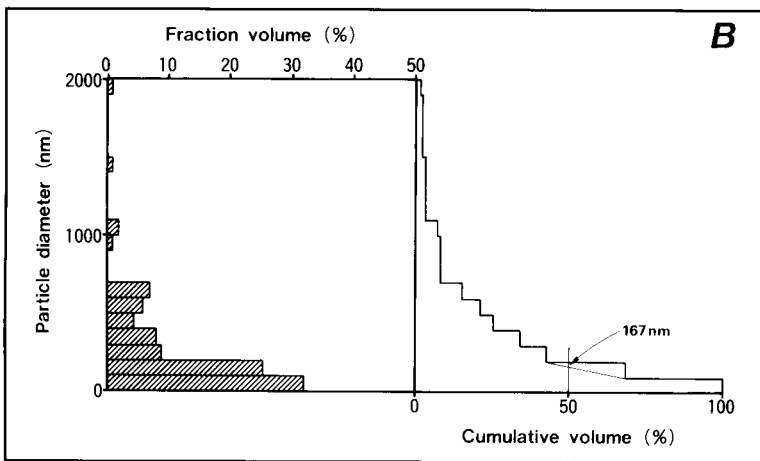


TABLE 1
Staining Intensity of Mouse Lymph Nodes by Carbon Suspension

Carbon Suspension	Mean Particle Size (nm)	Total Score (10 mice)											
		Popliteal node				Iliac node				Para-aortic node			
		Post-injection (min)				Post-injection (min)				Post-injection (min)			
		1	2	4	8	1	2	4	8	1	2	4	8
CH40	150	9	10	10	10	6	6.5	7	8.5	2	3.5	5	6.5
CH1500AA	167	7	9	10	10	5	5.5	6.5	8	1	3	5	6.5
India ink	254	0	3	3.5	5	0	1	2	3	0	0.5	1.5	2.5

contrast, these nodes were blackened poorly by India ink. Similarly at the iliac and para-aortic node, CH40 and CH1500AA stained the lymph nodes much more intensely than India ink up to 8 min. after injection. Carbon particles were usually found in macrophages in the marginal sinus soon after administration and in the germinal centers much later. Despite homogeneous coloring of the node, detection of carbon by microscopy often required considerable macrophage phagocytosis as the mean individual particle size was extremely small.

Table 2 summarizes the staining characteristics of the regional nodes after preoperative intragastric injection of CH40 removed during radical gastric resection. In patients the total number of lymph nodes removed was 567 of which 512 were without tumor metastases and of which 387 or 76% were stained with carbon particles. 55 nodes were positive for metastases and of these 38 or 69% were blackened with carbon. Activated carbon adhered to the cancer cell surface more commonly of poorly differentiated or signet ring cell carcinoma cells and rarely to well differentiated adenocarcinoma cells.

DISCUSSION

The newly prepared carbon suspensions

CH40 and CH1500AA, have been used in experimental lymphology (4,7), during operation (3,8), and with antineoplastic chemotherapy (9,10) to better delineate lymph flow pathways and nodal uptake by drugs. However, basic data on particle size distribution, which influences the absorption into lymphatics (11) has only briefly been described.

Our mice experiments demonstrate that CH40 and CH1500AA are narrowly distributed small particle carbon suspensions as compared with commonly used India ink, and after tissue injection they blacken the regional lymph nodes much faster and more intensely than India ink. These data suggest that these newer carbon suspensions are absorbed into lymph capillaries and transported to regional lymph nodes more rapidly because of their smaller particle size.

Clinically, the carbon suspension CH40 stained paraaortic lymph nodes both with and without tumor metastasis. Three or more days after injection of the carbon suspension into the stomach wall of dogs previously (1) and in patients as described here, the regional intraabdominal lymph nodes were still vividly colored black and readily identified at laparotomy.

Carbon suspensions, including larger size carbon particles, have been previously

TABLE 2
Blackened Lymph Nodes
With (+) and Without (-)
Metastases After Radical Gastric
Resection in 10 Patients

#	Nodes (-)	Nodes (+)	Total Nodes
All nodes	512	55	567
Blackened nodes	387	38	425
Non-stained nodes	125	17	142

examined for local or systemic toxicity (12,13) including histology of regional lymph nodes and other organs (1,12). These studies, in brief, substantiate that local injection of these carbon suspensions (i.e., CH40) promote no notable systemic side effects nor necrosis or severe inflammation of regional lymph nodes even when the suspension is combined with anticancer drugs.

Because these newer carbon suspension readily and vividly visualize lymphatics and regional lymph nodes even with metastases and are free of notable toxicity, they may prove useful during operation to identify draining lymph node basins more completely.

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