Intracavitary Use of Two Radiolabeled Tumor-Associated Monoclonal Antibodies

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Six patients with metastatic breast cancer and malignant pleural effusions and 13 patients with known or suspected ovarian cancer, underwent immunoscintigraphy after intracavitary (intrapleural or intraperitoneal) administration of iodine-131- (^{131}I) or indium-111- (^{111}In) labeled tumor associated monoclonal antibodies HMFG2 and H17E2. This method proved to be sensitive and specific with a true-positive result in 13 out of 14 patients with tumor and a true-negative result in five out of five patients without tumor. At any one time, 65%–80% of the whole-body radioactivity was closely associated with the cavity into which the radiolabeled antibody was administered while the radioactivity in the blood was always low, ($\sim 4 \times 10^{-3}$ of administered dose/ml of blood). Concentrations of radiolabeled antibody (per gram of tumor tissue) ranged from 0.02%–0.1% of the injected dose in intracavitary tumors, but only 0.002% in a retroperitoneal metastasis. The specificity of this approach was documented in four control patients with benign ovarian cysts and in two patients who were imaged using both specific and nonspecific radiolabeled antibody. We conclude that the intracavitary administration of ^{131}I- or ^{111}In-labeled HMFG2 and H17E2 is a favorable route of administration and offers significant advantages over previously reported intravenous administration for the localization of breast or ovarian metastases confined to the pleural or peritoneal cavities.

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Intravenous administration of radiolabeled tumor associated monoclonal antibodies for tumor localization has been used extensively, and encouraging results have been reported by many centers (*1–6*). It was found, however, in most cases that the absolute amounts of labeled antibodies reaching human tumors were small (~0.005% of the i.v. dose per gram of tumor) (*7,8*). This may explain the observed limited therapeutic success of intravenously administered radiolabeled antibodies (*9,10,11,12*).

The purpose of this study was to examine the kinetics of intracavitary administration of radiolabeled antibody in patients with pleural and peritoneal metastases from breast and ovarian cancer and patients with benign ovarian disease. Gamma camera imaging was performed to assess the diagnostic contribution of this procedure. In seven patients who underwent biopsies, we examined the radiolabeled content of tumors, benign lesions, and normal organs.

METHODS

Patients

All patients gave their informed consent. Two groups of patients were studied.

Group 1. Consisting of six patients with breast cancer and cytologically confirmed malignant pleural effusions, patients ages ranged from 42 to 67 yr with a mean of 58.6 yr.

Group 2. This group consisted of 13 patients with known or suspected ovarian cancer. Moreover, seven patients had histologically confirmed active ovarian cancer. In two patients, there was also clinical evidence of ascites. Two patients were in complete clinical remission after completion of chemotherapy, as determined by clinical examination, computed tomography (CT), and ultrasound scanning. Four patients had be-

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nign ovarian cysts as confirmed by laparotomy. Patients ages ranged from 26 to 77 yr with a mean of 57.9 yr.

Antibodies HMFG2, H17E2, and 11-4.1

Monoclonal antibody HMFG2 has been described in detail elsewhere (*13,14*). It is a mouse IgG1 immunoglobulin directed against a mucin-like molecule, normally produced by the lactating breast, but also is expressed by the majority (>95%) of breast and ovarian carcinomas (*13*).

Monclonal antibody H17E2 has been described in detail elsewhere (*4,15*). It is a mouse IgG1 immunoglobulin directed against placental alkaline phosphatase (PLAP). PLAP is normally expressed in term placenta (*16*) and is ectopically expressed by a wide range of tumors, including ovarian carcinoma (*15*).

Monoclonal antibody 11-4.1 is a mouse IgG1 immunoglobulin directed against the mouse H-2K^k antigen and does not cross react with any human tissues (*17*). It was used as a negative control.

Sterility and pyrogenity tests were carried out on samples from all batches by an independent laboratory (Safepharm Labs.).

Immunohistology

Prior to antibody guided studies, formalin fixed deparaffinized tissue sections of previously resected tumors from the patients were tested in an indirect immunoperoxidase reaction for antibody reactivity, according to a previously described method (*13*).

Iodination

Iodine-131 (^{131}I) (Amersham International, IBS30, Buckinghamshire, UK) was added to immunoglobulin (10 mg/ml), and the iodination procedure was carried out in iodogen coated tubes (*18*). Iodogen (tetrachloridiphenylglycoluril, Pierce Chemicals, UK), was dissolved in dichloromethane to make an iodogen solution and then evaporated to dryness at 20°C in sterile propylene tubes. To this tube, 1–5 mCi of ^{131}I were added together with 1–2 mg of monoclonal antibody (10 mg/ml in 0.3 *M* phosphate buffer, pH 7.4) and shaken gently. Iodination was allowed to proceed for 15 min at room temperature. The radiolabeled IgG was separated from free radioiodine by gel filtration on Sephadex-G50 column using phosphate buffer saline pH 7.4 as elution buffer. The labeling yield was determined using paper chromatography. This was always >95%.

Indium-111 Labeling

Labeling was performed as previously described (*19*). Antibody was coupled with diethylene triamine pentaacetic acid (DTPA) and labeled with indium-111 (^{111}In) (INSI, Amersham International, U.K.). Unbound ^{111}In was removed by gel filtration (Sephadex G-50).

Immunoreactivity

This was tested in a radioimmunoassay and in an enzyme linked immunosorbent assay with solid phase antigen fixed on 96 well microtiter plastic plates. Furthermore, comparison of antibody reactivity before and after radiolabeling was tested in a direct radioimmunoassay, including competition with unlabeled antibody as previously described (*7,20*).

Antibody Guided Studies

Patients receiving iodinated antibody were given potassium iodide 120 mg/day starting one day before the procedure and continuing for 10 days after injection. They were skin tested for allergy to mouse immunoglobulines prior to injection. Iodine-131- or ^{111}In-labeled HMFG2 antibody (0.5–1.0 mCi) (specific activity 5 mCi/mg) was administered intrapleurally or intraperitoneally and was washed in with 500 ml of normal saline in patients with pleural effusions or 1,500 ml of normal saline in patients with intraperitoneal lesions. The antibody was administered using a Centracath Vygon 50-cm catheter. Serous effusions, when present, were tapped to dryness prior to antibody administration.

Imaging

Gamma camera scans (model Siemens ZLC or IGE400) were taken at 0, 2, 24, 48, 72, and 96 hr after injection. Anterior and posterior views of chest and abdomen were taken with 400,000 counts/image. No blood pool or other form of image subtraction was performed. In addition to analogue imaging, digital acquisition was carried out. Regions of interest were drawn on the anterior and posterior images in order to measure serial changes. Isotope decay was corrected and values were expressed as cpm/pixel. The residual activity from the first study was almost at background level and did not interfere with the second study.

Pharmacokinetics

Blood samples were taken at 0, 2, 24, 48, 72, and 96 hr after injection; 24-hr urine collections were taken for 4 days. The total circulating activity and the urine excretion were calculated. These values were expressed as % of administered dose. The whole-body retention was calculated from the urinary excretion and with gamma camera imaging.

Resected Tissues

In seven patients undergoing laparotomy or pleural biopsy, radioactivity on normal and neoplastic resected tissues was counted and expressed as a % administered dose/g of tissue.

RESULTS

Six patients with breast cancer (ages 42–67) received ^{131}I-labeled HMFG2 intrapleurally, and successful tumor localization was achieved in all as illustrated in Figure 1.

Out of nine patients with definite evidence of ovarian cancer (ages 26–77), tumor localization was successful in seven out of eight following intraperitoneal (i.p.) administration of ^{131}I-labeled HMFG2 antibody and in one out of one following i.p. administration of ^{111}In-labeled H17E2 antibody (Fig. 2A and 2B). In one patient with retroperitoneal metastases, i.p. antibody (^{131}I-HMFG2) did not locate the tumor. In four patients with benign ovarian cysts, i.p. antibody (^{131}I-HMFG2, three cases; ^{111}In-H17E2, one case), did not locate the masses. Two patients, one with ovarian cancer and one with benign ovarian cyst received both specific (^{111}In-H17E2), and a week later, nonspecific antibody. The specific antibody located the tumor successfully and the

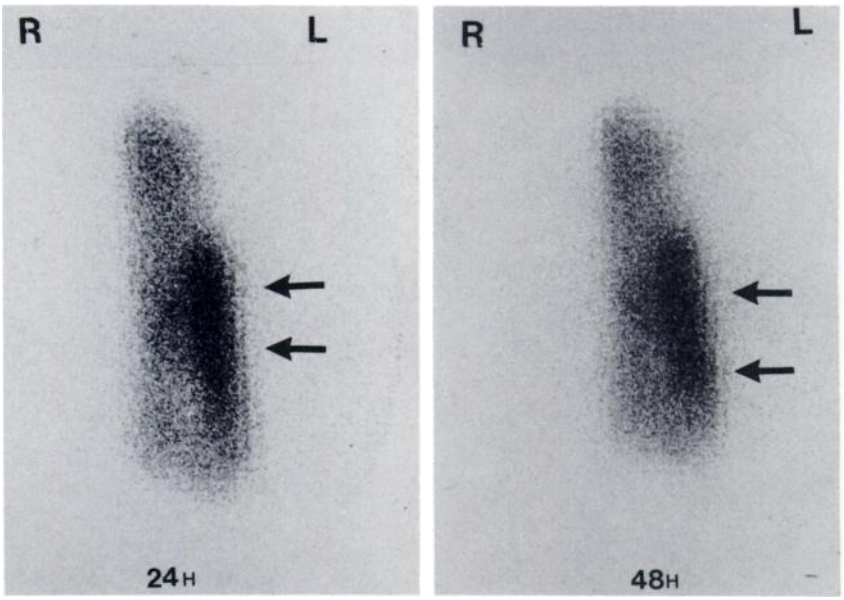

FIGURE 1
Antibody scan taken 24 hr and 48 hr after intrapleural administration of ^{131}I-labeled HMFG2 antibody in a patient with metastatic breast cancer. Arrows show areas of uptake by tumor of radiolabeled antibody.

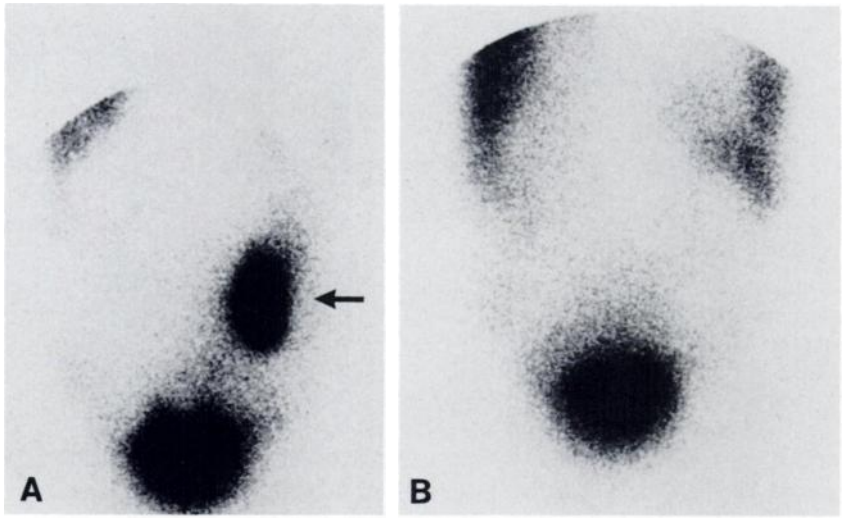

FIGURE 2
(A) Posterior view of antibody scan taken 24 hr after i.p. administration of ^{111}In-H17E2 (specific) in a patient with ovarian cancer. Note tumor localization (arrow) as well as areas such as pelvic and subdiaphragmatic regions of nonspecific antibody localization. (B) Posterior view of antibody scan taken 24 hr after i.p. administration of ^{111}In-11.4.1 (nonspecific) in the same patient as in Figure 2A. Note no tumor localization, but only nonspecific pooling of antibody in pelvic and diaphragmatic regions.

nonspecific did not (Fig. 2A and 2B) while in the case of benign cyst, neither the specific nor the nonspecific antibody located the mass (Fig. 3A and 3B). Nonspecific antibody localization can be seen in the pelvis due to gravitational pooling of antibody around the bladder (but not in the bladder) and in the subdiaphragmatic and paracolic gutters. In this study, the smallest tumor that was detected was ~1 cm in diameter.

Pharmacokinetic data for ^{131}I-labeled HMFG2 data are summarized in Figures 4A and 4B, showing results for intrapleural and i.p. administration, respectively. As can be seen, blood levels following intracavitary administration of ^{131}I-labeled antibody are low with maximum circulation radioactivity levels being reached at 48 hr after injection. Maximum circulating radioactivity ranged from 0.29 to 18.5 (mean 3.7) and 0.29 to 8.4 (mean 2.3) per ml of blood $\times 10^{-3}$% of administered dose for intrapleural and intraperitoneal antibody, respectively. The radioactivity was cleared primarily by renal excretion. Urinary clearance ranged from 1.1% to 15.7% and 2.5% to 17.9% of administered dose/24 hr urine collection for intrapleural and intraperitoneal antibody, respectively. It was of interest that in patients with definite evidence of ovarian cancer, there was more prolonged retention of radioactivity in the body, i.e., 60% retention at 24 hr and 40% at 48 hr of injected antibody as compared to patients with benign disease who had more rapid clearance of radioactivity, i.e., 40% retention at 24 hr and 20% at 48 hr. These differences were statistically significant ($p < 0.05$).

The diagnostic contribution of this approach is illustrated by the following two examples:

Patient 11: This 56-yr-old woman who originally presented with stage III (FIGO classification) ovarian cancer completed six courses of

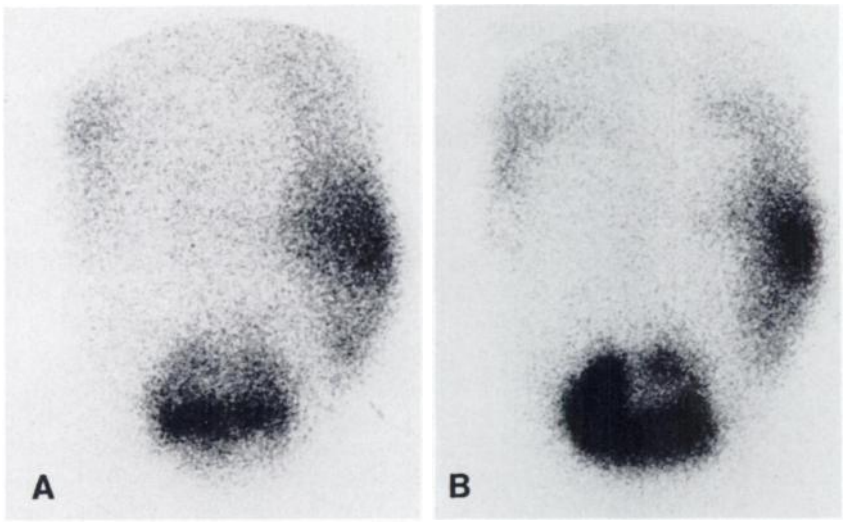

FIGURE 3
(A) Posterior view of antibody scan taken 24 hr after i.p. administration of 111In-H17E2 in a patient with a benign ovarian cyst. Note only nonspecific localization in pelvis in subdiaphragmatic regions. (B) Posterior view of antibody scan taken 24 hr after i.p. administration of ^{111}In-11.4.1 in same patient as in Figure 3A. Note again only nonspecific localization.

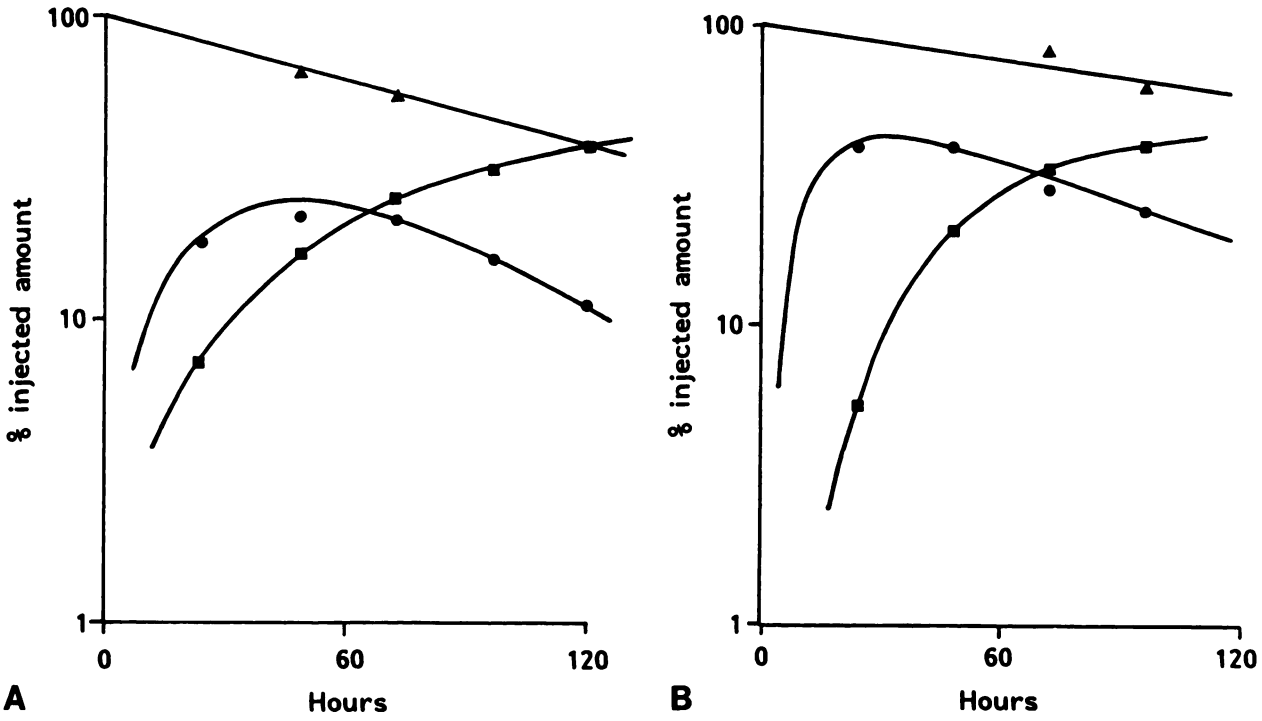

FIGURE 4
Pharmacokinetic data, i.e., whole-body clearance (▲), blood levels (●), and cumulative urinary excretion (■). (A) After intrapleural administration of ^{131}I-labeled antibody. The results represent the mean of six patients studied. (B) Pharmacokinetic data after i.p. administration of ^{131}I-labeled antibody. The results represent the mean of 11 patients studied.

chemotherapy and was thought to be in complete remission as determined by clinical examination and a negative CT scan of abdomen and pelvis. Iodine-131 HMFG2 intraperitoneal scan, however, was found to be positive with high and relatively unchanged retention of radioactivity in the abdomen (87% at 2 hr, 57% at 24 hr, and 40% at 48 hr). The patient underwent laparotomy 48 hr after antibody administration; she was found to have minimal residual disease (tumor nodules <2 cm diameter) with surgical findings corresponding to those of the antibody scan (i.e., infiltration of bowel). The radiolabel content in a sample of resected tumor tissue was found to be 0.1% of administered dose/g of tumor (Table 1).

Patient 14: This patient was suspected of ovarian cancer after a cystic mass was detected on the

TABLE 1
Radioactivity in Resected Tissue 48 hr After Intracavitary Administration of ^{131}I-HMFG2

Specimen and histology	% Uptake
Pleural biopsy of adenocarcinoma of the breast	0.02
Peritoneal ovarian adenocarcinoma	0.1
Peritoneal ovarian adenocarcinoma	0.13
Retroperitoneal ovarian adenocarcinoma	0.0019
Benign serous cyst	0.0011
Benign dermoid cyst	0.0012
Benign serous cyst	0.0016

Data reflect 48 hr tissue uptake in resected samples from seven patients who received intracavitary ^{131}I-HMFG2 expressed as % administered dose/gram of tissue. These percentages represent the maximum concentration in tissue.

left side of pelvis, behind and above the urinary bladder. An antibody study was performed. This was thought to be negative showing a rapid clearance of radioactivity between 2 hr and 24 hr after injection (79.5% at 2 hr falling to 41.7% by 24 hr). A laparotomy was performed at 48 hr after antibody injection. Laparotomy findings were negative for the presence of tumor and only free floating fluid was found in the abdomen. Wedge biopsy from a cystic ovarian tissue showed an uptake of 0.0011% of administered dose/g of tissue.

A weakness of this approach is shown by the following example:

Patient 13: A 77-yr-old woman with a known diagnosis of ovarian cancer. She relapsed with a CT scan showing a retroperitoneal cystic mass on the left side of the pelvis, connected to psoas muscle. She also had obstructive left hydronephrosis. Antibody scanning did not show localization at the known tumor site. However, high and relatively unchanged retention of radioactivity of a diffuse pattern was noted (75% at 2 hr and 57% at 24 hr), suggestive of tumor presence. A laparotomy was performed and retroperitoneal mass was excised. This was confirmed histologically to be secondary to ovarian carcinoma. The retroperitoneal tumor showed only 0.0019% administered dose/g of tissue, indicating no advantage for i.p. administration over i.v. administration.

Table 1 shows the amount of radioactivity in resected tissues (% of administered dose/g of tissue) measured at 48 hr after intracavitary administration of ^{131}I-HMFG2. The uptake of antibody by peritoneal tumor is significantly higher than the uptake by retroperitoneal tumor or by benign cysts ($p < 0.005$).

DISCUSSION

In this study, we report successful antibody-guided localization of all but one histologically or clinically detected intrapleural or intraperitoneal lesions in patients with breast or ovarian cancer confined to pleural or peritoneal cavities. Furthermore, we saw no false-positive localization in five patients, four with benign cysts and one in complete clinical remission from ovarian cancer. An important observation that proved helpful in the diagnosis was the pharmacodynamic behavior of administered antibody. Patients who showed a higher retention of antibody over time (i.e., 60% retention at 24 hr and 40% at 48 hr of injected amount) were more likely to have active disease than those who showed a more rapid clearance of antibody over time (i.e., 40% retention at 24 hr and 20% at 48 hr of injected amount). None of these patients had prior exposure to mouse immunoglobulins and did not have a human anti-mouse globulin response as previously described (*21*).

In three patients, biopsy of sites positive on antibody scanning showed pathologic involvement with higher uptake of radiolabeled antibody than previously reported with i.v. administration (*1–8*). On the other hand, in one patient with a retroperitoneal metastasis, tumor uptake of the radiolabel was low, indicating that this tumor was not successfully localized by intraperitoneally administered antibody. Only two patients were studied with ^{111}In-labeled antibody and, therefore, one cannot conclude if there is a difference in the retention values between the ^{131}I- and ^{111}In-labeled antibodies. Previous preclinical studies comparing ^{131}I- and ^{111}In-labeled antibody showed more prolonged retention times than ^{131}I-labeled antibody (Mather S, PhD thesis, 1987). In patients with active disease (whether pleural or peritoneal), 65%–80% of the radioactivity present in the body at any one time was closely associated with the cavity into which the antibody was administered while the radioactivity in the blood was always low ($<4 \times 10^{-3}$% of administered dose/ml of blood) and reaching its peak at 24 to 48 hr after intracavitary injection. Furthermore, with the use of two antibodies (specific and nonspecific), the amount of specific tumor targeting versus nonspecific antibody accumulation in a cavity was assessed (Fig. 2 and 3). The target-to-nontarget ratio at 24 hr was as high as 26:1 at its maximum, whereas previous studies using the same antibody (HMFG2) intravenously, demonstrated tumor-to-non-tumor ratios between 1.44 and 2.81 at 24 hr (*22*). These findings should provide encouragement to the therapeutic application of intracavitary radiolabeled antibodies, and in fact, promising results already have been reported (*23,24,25*). Furthermore, the potential therapeutic efficiency could be increased if pure beat emmiters with a shorter half-life such as yttrium-90 (half-life, 64 hr) were used (*10,26,27*) instead of ^{131}I (half-life, 8 days) in order to exploit the slow kinetics of antibody

transport from the intracavity compartment into the blood pool.

In conclusion, this study supports the pharmacokinetics theory of a large and exploitable concentration difference between serous cavities and the plasma (*28*). This study together with other clinical studies (*29,30*) as well as our own preclinical studies (*31*) support the notion that if any portion of a tumor can be reached by intracavitary instillation (*28*), then there is a strong rationale for the administration of radiolabeled antibodies via the intrapleural or intraperitoneal route.

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