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Tc-99m-PEG-Liposomes Target Both Adhesions and Abscesses and Their Reduction by Hyaluronate in Rats With Fecal Peritonitis

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Background. **Abdominal adhesions and abscesses are a major source of morbidity and mortality after abdominal surgery and peritonitis. Adhesions are hard to detect with standard imaging techniques. Liposomes, coated with polyethylene glycol (PEG), represent an agent developed for infection imaging. This study investigated the capacity of ^{99m}Tc-PEG-liposomes to localize early adhesion formation after peritonitis. Additionally, the value of ^{99m}Tc-PEG-liposomes for therapy evaluation of hyaluronan solution, which reduces adhesion and abscess formation in experimental peritonitis, was assessed.**

Methods. **In 24 rats, a bacterial peritonitis was induced by performing a cecal ligation and puncture procedure. The animals were treated with sodium chloride solution or 0.4% hyaluronan solution intra-abdominally. One week later, scintigraphy was performed using ^{99m}Tc-PEG-liposomes, and abnormal focal uptake in the abdomen was scored. Thereafter, autopsy was performed and adhesions and abscesses were scored.**

Results. **A significant correlation was found between the total adhesion score and the scintigraphic score ($P < 0.01$, $r = 0.65$). Treatment with hyaluronan significantly reduced the total adhesion score ($P = 0.01$). The size of abscesses significantly correlated with the scintigraphic score ($P < 0.01$, $r = 0.65$). Treatment with hyaluronan reduced the size of abscesses ($P < 0.05$).**

Conclusion. **^{99m}Tc-PEG-liposomes are able to detect early adhesions and abscesses and may be used for therapy evaluation of agents that reduce adhesions and abscesses.**

Key Words: **liposomes; adhesions; hyaluronate; rats; experimental; peritonitis.**

INTRODUCTION

Intra-abdominal adhesions and abscesses are a major source of morbidity and mortality. They are mainly caused by abdominal surgery and peritonitis. Adhesions are the foremost cause of intestinal obstruction in the Western world and account for approximately 70% of readmissions for small bowel obstruction [1]. They are responsible for 15% to 20% of cases of secondary infertility in woman and are associated with chronic abdominal and pelvic pain [2, 3]. Moreover, adhesions are associated with surgical complications during relaparotomy and a higher incidence of postoperative complications, relaparotomies, admissions to the intensive care unit, and a prolonged hospital stay [4].

Early dense peritoneal adhesion formation is frequent after peritonitis. Adhesion networks may entrap bacteria, resulting in intra-abdominal abscesses. Prevention of these networks might reduce residual abscesses after peritonitis and facilitate relaparotomy, which is regularly indicated during treatment of severe peritonitis. Computed tomography (CT) scanning and magnetic resonance imaging (MRI) are not sufficiently accurate to demonstrate early dense adhesion formation.

Liposomes, small lipid vesicles coated with polyethylene glycol (PEG), have been developed for infection imaging [5]. PEG-liposomes can be labeled with ^{99m}Tc in a rapid and efficient procedure, providing images of

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high quality at low cost and with low radiation dose [6]. When injected intravenously, the liposomes accumulate in areas with an increased vascular permeability such as infiltrates and abscesses. Experimental and clinical studies have shown excellent targeting in various models of infection and inflammation, including the cecal ligation and puncture (CLP) model [5–13]. PEG-liposomes localizing early adhesions following peritonitis have never been investigated.

The first aim of this study was to evaluate the value of ^{99m}Tc-PEG-liposomes visualizing early adhesion formation after experimental peritonitis. Second, the feasibility of ^{99m}Tc-PEG-liposomes for evaluation of intraperitoneal treatment with hyaluronate solution in peritonitis was studied. Hyaluronan-based agents are known to successfully reduce postsurgical intra-abdominal adhesions in both experimental and clinical trials [14–17], and adhesion and abscess formation in experimental peritonitis [18, 19].

MATERIALS AND METHODS

Animal Model

All experiments were carried out in accordance with the guidelines of the local Animal Welfare Committee. Twenty-four male, randomly bred Wistar rats (Harlan Nederland, Zeist, The Netherlands), weighing 270 to 310 g, were accustomed to laboratory conditions for 1 wk before experimental use. Animals were housed at 21°C with a day-night cycle of 12 h. They had free access to water and standard rodent chow (Hope Farms B.V., Woerden, The Netherlands). A bacterial peritonitis was induced by CLP, as described by Wichterman *et al.* [20]. Briefly, the animals were fasted for 12 h prior to the first operation. On d 0, rats were weighed and anaesthetized with a mixture of fluothane (Zeneca, Cheshire, United Kingdom), nitrous oxide, and oxygen. Prior to the operation, the abdomen was shaved and disinfected with 70% ethanol. *Via* a three cm midline laparotomy, the cecum was dissected and filled backwards with feces. Thereafter, the cecum was ligated just distal of the ileocecal valve, with a 3.0 polyglactin Vicryl suture (Ethicon, Norderstedt, Germany) and its antimesenterial site was punctured once with a 19-gauge needle. The abdominal wall was closed in two layers with 3.0 Vicryl suture. Immediately after operation, rats received one single dose of 6 mg/kg body weight gentamicin (Centrafarm Services B.V., Etten-Leur, The Netherlands) intramuscularly, and 0.1 mg/kg body weight buprenorphine (Temgesic; Reckitt and Colman Products Ltd., Amstelveen, The Netherlands) subcutaneously for analgesia. For resuscitation, all animals received 10 mL isotonic sodium chloride solution subcutaneously. At d 1, the abdomen was reopened under anaesthesia and peritoneal fluid was taken and collected in a BBL Port-A-Cul envelope (Becton Dickinson, Cockeysville, MD) for microbiological examination. The abdominal cavity was rinsed with 10 mL of isotonic sodium chloride solution and the ligated and perforated cecum was resected. Before closure of the abdomen, animals were randomly assigned to receive 8 mL of isotonic sodium chloride solution ($n = 12$), or 8 mL of 0.4% hyaluronate solution (Sepracoat, Genzyme Corp., Cambridge, MA), ($n = 12$) instilled throughout the whole abdominal cavity. The abdominal wall was closed in two layers as described above. Rats received 10 mL isotonic sodium chloride solution and 0.1 mg/kg body weight buprenorphine subcutaneously.

Study Design

On d 8, 7 d after resection of the cecum, rats were injected via the tail vein with 15 MBq ^{99m}Tc-PEG-liposomes. At 2, 4, and 24 h, the animals were anaesthetised with a mixture of fluothane, nitrous oxide and oxygen, and were placed prone on a single headed gamma camera equipped with a parallel-hole, low-energy collimator. Images (30,000 counts/animal) were obtained and stored in a 256 × 256 matrix (Fig. 1). The scintigraphic images were evaluated for increased uptake, and the images at 24 h appeared to show abnormal abdominal uptake most obvious. The abnormal uptake in the images at 24 h was scored by comparing the activity with other regions, as described by Schölmerich *et al.* [21]: 0 = no uptake; 1 = less than bone marrow; 2 = more than bone marrow, but less than liver; 3 = more or equal to liver.

After recording of the final image, rats were killed by carbon dioxide asphyxiation. At autopsy, macroscopic abnormalities were assessed without knowledge of the imaging results. Adhesions were scored in a blinded manner by one author (HvG) according to the method of Zühlke *et al.* [22], whereby grade 0 means no adhesions and grade IV means firm extensive adhesions that are only dissectible with

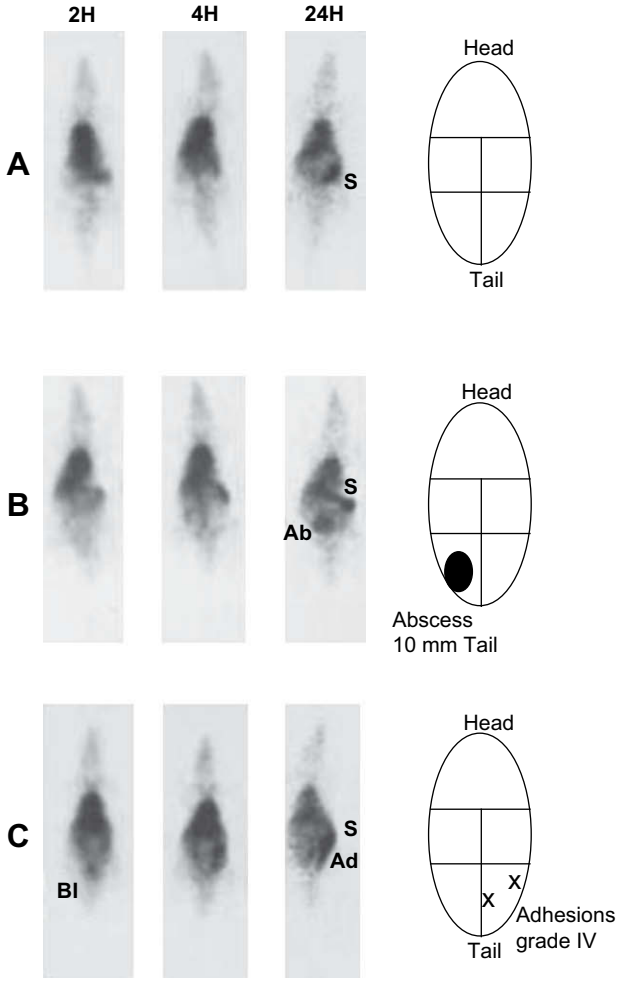

FIG. 1. Scintigraphic images of rats 2, 4 and 24 h after injection of ^{99m}Tc-PEG liposomes, and a schematic of the observations at postmortem investigation. (A) Rat without adhesions or abscesses; (B) rat with abscess; (C) rat with adhesions. S = spleen, Bl = bladder, Ab = abscess, Ad = adhesions. Note the physiologic high uptake at the spleen and bladder region. The development of adhesions or abscesses is best seen in the images 24 h after injection.