Effects of Induction Therapy on Wound Healing at Bronchial Anastomosis Sites in Rats

Objectives: Preoperative chemotherapy is frequently used for advanced lung cancer. As a valid alternative to pneumonectomy, bronchoplasty has the advantage of enabling lung parenchyma function to be preserved. The effects of antineoplastic agents on healing bronchial anastomosis remain unclear. We studied the effects of preoperative chemotherapy on wound healing in bronchial anastomoses and clarified causes of wound healing impairment in rats. Methods: In experiment I, at 3 days before surgery, rats were injected with cyclophosphamide, doxorubicin, and vincristine (CAV group) or cisplatin and etoposide (PVP treated rats). In experiment II, at 48 hrs before surgery, rats were treated with rabbit antirat macrophage serum and antirat monocyte chemoattractant protein-1 antibody to inhibit macrophage infiltration. On days 3, 5, and 7 after bronchus anastomosis, wound healing was assessed by examining bursting strength and hydroxyproline tissue content. Results: CAV-treated rats showed significant impaired wound healing, marked severe leucopenia, and reduced macrophage infiltration. The PVP group showed no significant changes. In experiment II, rats exhibited inhibited macrophage infiltration, which is associated with significantly impaired of wound healing. Conclusions: Our study suggests that induction chemotherapy, associated with leukopenia in the early phase of wound healing, increases the risk of bronchial anastomosis leakage. Postoperative macrophage depletion is one of the most important causes of impaired wound healing. (Jpn J Thorac Cardiovasc Surg 2003; 51: 217-224)

Key words: wound healing, MCP-1, rat, bronchus, macrophage, induction therapy, lung cancer

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Lung cancer is most effectively treated by tumor resection. Antineoplastic agents are being increasingly used in preoperative induction therapy to treat advanced lung cancers to prevent micrometastasis and to increase curative resection progress.1 Lung tumors may be resected via bronchoplasty as a valid alternative to pneumonectomy, preserving lung parenchyma function. Although these procedures are generally accepted as curative and safe in lung cancer, surgery are associated with frequently fatal anastomotic complications.2 Surges are thus generally cautious in using antineoplastic agents in healing bronchial anastomosis.

Antineoplastic agents are known to impair wound healing in skin3 and intestinal anastomoses,4 but why this impairment occurs has not been well studied. Using an experimental rat model, we studied the effects of preoperative antineoplastic protocols on wound healing at bronchial anastomoses. We found that, wound healing was only impaired by agents having leukopenia as a side effect, with macrophages infiltrating the anastomotic wounds of treated rats only negligible.5 Macrophages are known to produce several cytokines that stimulate fibroblasts, so we speculated that antineoplastic agents that depleted macrophages impaired wound healing.

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healing at bronchial anastomoses. Using monoclonal and polyclonal antibodies, we studied the effects of macrophage depletion on anastomotic wound healing in rats.

**Subjects and Methods**

**Animals.** To assess wound healing, we used 90 male Wistar rats (Charles River, Japan) weighing between 280 and 300 g: 54 to study the effects of antineoplastic protocols (experiment I) and 36 to study the effects of macrophage depletion (experiment II). An additional 60 rats were used to determine the 10% lethal drug dose (LD$_{10}$). All rats were provided with standard diet and water ad libitum. Animal experiments were conducted in accordance with the Guidelines of the Animal Care and Use Committee of Nagasaki University. These procedures conformed to the guidelines established by the National Institutes of Health and published in Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23).

**Drug administration in experiment I.** Rats were divided into 3, 18-animal groups of control (group I) and treated rats. The 2 treatment protocols were used: group II, combining cyclophosphamide, doxorubicin, and vincristine (CAV); and group III, combining of cisplatin and etoposide (PVP). CAV is the most leukocytotoxic of lung cancer induction therapy protocols, whereas PVP is only mildly leukocytotoxic. Using CAV and PVP, we created rat models of inhibited wound healing.

In both CAV and PVP groups, cyclophosphamide (92 mg/kg), doxorubicin (3.68 mg/kg) and vincristine (0.5 ml/body) were injected intraperitoneally (iv) twice daily for 3 days before surgery. The 2 treatment protocols were used: group II, combining cyclophosphamide, doxorubicin, and vincristine (CAV); and group III, combining of cisplatin and etoposide (PVP). CAV is the most leukocytotoxic of lung cancer induction therapy protocols, whereas PVP is only mildly leukocytotoxic. Using CAV and PVP, we created rat models of inhibited wound healing.

**Treatment in experiment II.** Rats were divided into control (group IV) and treated (group V) groups. Controls were treated with nonspecific rabbit serum (0.5 ml/body) and nonspecific mouse IgG (1 mg/kg). Macrophage infiltration was inhibited in group V by injection of rabbit antirat macrophage serum (AMS, 0.5 ml/body) intraperitoneally (ip) 48 hrs before operation and 1 and 4 days after operation. Antirat mono-cyte chemoattractant protein-1 monoclonal antibody (MCP-1 mAb, clone C4, 1 mg/kg) was injected via the tail vein (iv) on the day of operation and daily thereafter. Blood was sampled from 6 rats from each of the control and treated groups. To assess monocyte depletion at operation, a 0.5-ml blood sample was obtained 48 hrs after each injection.

**Surgical procedures.** Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium 30 mg/kg and ketalar sodium 20 mg/kg, then intubated and ventilated using a respirator (SN-480-7, Shimano, Japan) at a rate of 10 ml/kg at 90 respirations/min. A 4 cm incision was made and thoracotomy was done through the 4th intercostal space. A left main stem bronchial transection and end-to-end anastomosis was then conducted under surgical microscopy using an 8-0 polypropylene suture (Ethicon, NJ, USA). Cartilaginous and membranous portions of the bronchi were anastomosed using 6 continuous and 4 interrupted sutures. The same surgery was conducted in controls, but without preoperative chemotherapy.

**Generation of antibodies.** Potent rabbit AMS (Inter-Cell Technologies, Inc., Hopewell) was prepared in rabbits by immunization with thiglycolate-elicited rat peritoneal monocytes. Serum was confirmed to be cytotoxic for rat monocytes and a few T lymphocytes. Antirat MCP-1 mAb (C4), an IgG class mouse antibody, produced by hybridoma. Hybridoma cells were injected intraperitoneally into Pristane-primed nude Balb/c mice. MAb was grown in ascites fluid and purified over a protein A affinity column (Pharmacia, Piscataway, NJ, USA), and used at a concentration of 1 mg in 1 ml PBS.

**Determination of antibody treatment efficacy.** The efficacy of treatment with AMS was assessed by determining depletion of absolute monocytes in peripheral blood and anastomotic wounds. Peripheral monocytes were analyzed with monoclonal antibody by flow cytometric analysis. MAb used in this study included fluorescein isothiocyanate (FITC)-conjugated antimouse mAb (Ed1 mAb, Serotec, Oxford, UK). The surface immunofluorescence of individual cells was determined using whole-blood labeling. Briefly, whole blood (100/μl) was incubated for 60 min on ice in the dark with 10 μl of FITC conjugated mAbs. Erythrocytes were subsequently lysed by 10-min incubation with FACS lysing solution (Becton Dickinson, NJ, USA). Cells were suspended in 1.0 ml of 0.5% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). Counts of positively stained cells were computed as percentages of total leukocytes, using...