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ORIGINAL ARTICLE

Detection of airway ischaemic damage after lung transplantation by using autofluorescence imaging bronchoscopy

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Abstract

OBJECTIVES: Airway complications related to ischaemia are a major cause of morbidity after lung transplantation. Early detection of airway ischaemia and optimal management of the anastomotic site could reduce the risk of airway complications. Autofluorescence imaging (AFI) bronchoscopy has been increasingly recognized as an effective technique for detecting abnormal mucosal thickening. The aim of this study was to investigate whether AFI bronchoscopy can facilitate the detection of airway ischaemic damage in lung transplant patients.

METHODS: Twenty Landrace pigs were used to create a tracheal autotransplantation model. A four-ring length of trachea was excised and implanted orthotopically. The tracheal autograft was observed on postoperative days 0, 2, 4 and 7 with AFI bronchoscopy. The extent and origin of graft autofluorescence were examined using histology and measured according to fluorescence intensity.

RESULTS: The lesions on the tracheal autografts appeared as bright green fluorescence on AFI bronchoscopy. On confocal fluorescence microscopy, high-intensity green fluorescence was observed in the elastin fibre layer of the submucosa. The fluorescence intensity of elastin was significantly higher in the graft showing fluorescence than the graft that did not show fluorescence and that at the control site.

CONCLUSIONS: Bright green fluorescence was seen in an elastin fibre layer in the submucosa, which was likely a result of epithelial sloughing. There is a close relationship between the bright green fluorescence pattern observed using AFI bronchoscopy and airway ischaemic damage. We conclude that AFI bronchoscopy may detect airway ischaemic damage after lung transplantation.

Keywords: Airway ischaemia • Lung transplantation • Autofluorescence imaging

INTRODUCTION

Airway complications related to ischaemia remain a significant cause of morbidity and mortality after lung transplantation. Airway ischaemia causes not only anastomotic dehiscence or ulceration but also stenosis in the late postoperative period. Therefore, early detection of ischaemic changes may be important to initiate prompt management of the anastomotic site, which may reduce the risk of late bronchial stenosis.

Advances in optical technology have contributed significantly to medical science, particularly endoscopy. Autofluorescence is the natural emission of light by biological tissues. The bronchial wall contains autofluorescent substances, such as flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NADH), collagen and elastin [1]. Autofluorescence imaging (AFI) is based on the detection of natural tissue fluorescence emitted by fluorophores.

AFI bronchoscopy visualizes the intensity and spectral contrasts of the autofluorescence of healthy bronchial mucosa and neoplastic or early cancerous lesions. AFI bronchoscopy has been increasingly recognized as an effective technique to detect abnormal mucosal thickening, which is difficult to recognize using standard bronchoscopy [2–4]. We speculated that AFI bronchoscopy could detect mucosal change or damage related to bronchial ischaemia.

The aim of this study was to investigate whether AFI bronchoscopy can facilitate the detection of airway ischaemic damage at anastomotic sites of lung transplant patients.

MATERIALS AND METHODS

Autofluorescence imaging bronchoscopy system

An AFI bronchoscopy (EVIS LUCERA SPECTRUM, Olympus BF-F260 autofluorescence bronchovideoscope, Olympus Medical Systems Co.) displays enhanced images of normal and thickened mucosa by irradiating excitation light (390–470 nm) to observe autofluorescence emitted from fluorescent substances and green light (540–560 nm), which is absorbed by circulating haemoglobin. A charge coupled device (CCD) captures the light and divides the autofluorescence input signals into the G channel and reflects signals into the B and R channels. Autofluorescence input signals

were amplified by the processor and transmitted to the G channel because of the low autofluorescence intensity.

A pseudocolour image is reconstructed such that areas of high autofluorescence intensity appear light green and those of low autofluorescence intensity appear magenta. In general, on AFI bronchoscopy, normal mucosa appears light green and abnormal mucosa appears magenta, such as in hyperplasias and malignant lesions. On AFI bronchoscopy, blood vessels appear dark green [5].

Experimental study and animal care

Twenty Landrace pigs (weight, 28–32 kg) were premedicated with intramuscular ketamine (7–9 mg/kg) and atropine sulphate (0.5 mg). The animals were intubated and artificially ventilated using halothane inhalation with 100% oxygen at a tidal volume of 10 ml/kg and a respiratory rate of 15 breaths/min. Antibiotics (cefazolin 1 g) were administered intravenously at the time of bronchoscopy.

The animals used in this study received humane care in compliance with *The Principles of Laboratory Animal Care* (formulated by the National Society for Medical Research) and *The Guide for the Care and Use of Laboratory Animals* (prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health; NIH publication no. 86-23, revised 1996). The study protocol was approved by the Animal Care and Use Committee of Okayama University.

Airway ischaemic damage model

We performed tracheal autotransplantation in our model of airway ischaemic damage (n = 20). The trachea was exposed via a median cervical incision. The tracheal fascia and small vessels were dissected away to disrupt blood flow. A four-ring length of trachea was excised and reimplanted orthotopically to interrupt blood flow to the tracheal wall. The tracheal anastomosis was performed with 4-0 Polydioxanone (PDS) II running sutures.

Evaluation of the anastomotic site

Under general anaesthesia (100% oxygen and 1.0% halothane), we evaluated the tracheal anastomotic site using AFI bronchoscopy on postoperative days 0, 2, 4 and 7. Tracheal samples were obtained and evaluated to determine the extent and origin of autofluorescence at the times when any changes were observed on AFI bronchoscopy.

Histology

The entire trachea (n = 10), including normal trachea, was excised when abnormal changes were seen on AFI bronchoscopy. The tracheal tissue samples from postoperative days 2, 4 and 7 were fixed in 10% buffered formalin and stained with haematoxylin and eosin and Elastica van Gieson stain.

The autograft sample was compared with normal trachea from the same sample (control) by using conventional microscopy. Fluorophores were detected using fluorescence microscopy and a WIB filter. A WIB filter is included within the range of AFI excitation and emission light wavelengths (WIB filter excitation 450–490 nm, emission 515–565 nm).

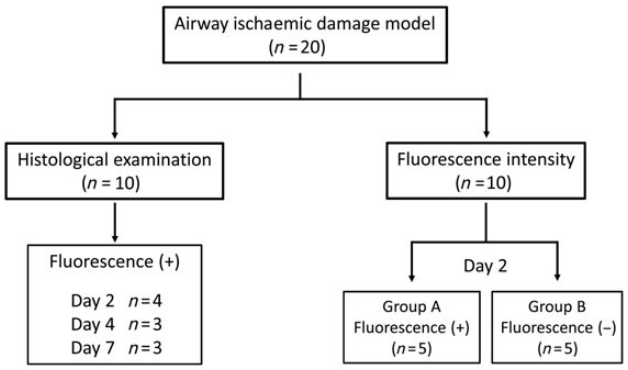

Figure 1: Division of samples into histological examination and measuring fluorescence intensity.

Fluorescence intensity

The samples (n = 10) obtained on postoperative day 2 were used to measure the fluorescence intensity, as shown in Fig. 1. Tracheal samples obtained on postoperative day 2 were divided into Groups A (n = 5) and B (n = 5) based on the presence or absence of bright green fluorescence, respectively. Full-thickness 6-mm tissue slices were obtained using a hollow punch. The samples were randomly chosen from five areas of each control and autograft site. The fluorescence intensity of emitted light from elastin was measured by a FlexStation® 3 Benchtop Multi-Mode Microplate Reader (Molecular Devices). It is known that elastin, when irradiated with excitation light of 410 nm, emits light with a wavelength of 440 nm [1].

Statistical tests were performed using Statcel version 3 (OMS Publishing, Inc., Japan) on a personal computer (PC)-compatible computer running Windows 7. The results are presented as the mean ± standard deviation (SD). Statistical comparisons were conducted using a paired t-test. A P-value of <0.05 was considered statistically significant.

RESULTS

Bronchoscopic findings on Autofluorescence imaging bronchoscopy

The reimplanted trachea looked pale after the operation. There was no change on AFI bronchoscopy on postoperative days 0 and 1. However, the autograft started to show bright green fluorescence on postoperative day 2 (Fig. 2 and Video 1). On postoperative days 2, 4 and 7, bright green fluorescence was detected in 9 (60%), 3 (20%) and 3 (20%) of the 15 pigs, respectively.

Five pigs were sacrificed on postoperative day 2 before the appearance of green fluorescence because tracheal samples without fluorescence were taken to measure fluorescence intensity.

When evaluating the cut surface of the autograft and the control trachea, most layers appeared magenta. However, a part of the submucosa showed a bright green fluorescent band (Fig. 3).

Histological findings

The autograft was found to have full-thickness necrosis and epithelial sloughing. Infiltration of inflammatory cells and microabscess formation were observed in the submucosa. Fluorescence microscopy revealed that green fluorescence was widely observed in the elastin fibre layer of the submucosa (Fig. 4).

The control trachea had a healthy epithelium and elastic fibre layer. Green fluorescence was similarly observed using fluorescence microscopy.

Fluorescence intensity

The results of fluorescence intensity are shown in Fig. 5. In Group A, there was a statistically significant difference in the fluorescence intensity of elastin between the control site and the autograft site (control: 452.9 ± 63.10; autograft: 1499.1 ± 984.54, $P = 0.033$). In Group B, there were no statistically significant differences between the control site and the autograft site (control: 435.9 ± 93.59; autograft: 371.9 ± 54.86, $P = 0.938$).

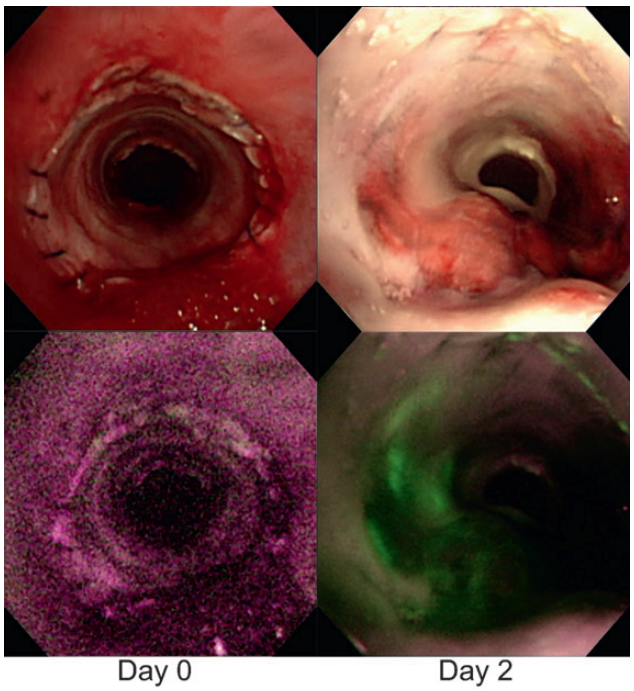

Figure 2: Bronchoscopic findings of the anastomotic site of a pig autograft trachea. Top: white-light image. Bottom: AFI mode image.

DISCUSSION

Airway complications are a significant cause of morbidity and mortality after lung transplantation [6–10]. Airway complications such as dehiscence, necrosis, stenosis, malacia and endobronchial infections are largely attributed to ischaemia in the early post-transplant period. The bronchial blood supply to the donor bronchus is dependent upon collateral retrograde blood flow from the pulmonary artery, which is poorly oxygenated for 2–4 weeks after lung transplantation. This poor oxygenation is because the donor bronchial arteries are interrupted during the harvest [8, 11, 12]. The anastomotic sites and distal bronchus are susceptible to ischaemia until revascularization from the recipient's arteries is developed. Therefore, early detection of ischaemic changes and optimal management of the anastomotic site could reduce the risk of airway complications. However, it is difficult to determine the extent of mucosal damage using only white-light imaging, because in this imaging, the donor bronchus appears pale and oedematous owing to the poor blood supply from the pulmonary artery or ischaemia reperfusion injury in the early post-transplant period.

It has been reported in the oncology literature that AFI bronchoscopy is more useful for detecting abnormal mucosal thickening than white-light bronchoscopy [2, 13]. In contrast, none has applied AFI bronchoscopy to excavated lesions such as mucosal defects caused by airway ischaemia. This study provides a new approach to the detection of airway ischaemia using image-enhanced endoscopy. In our model of airway ischaemic damage, bright green fluorescence was observed at the autograft tracheal segment after 2 postoperative days.

The fact that abnormal mucosal thickening appears magenta is well known, but no study has reported bright green fluorescence for ischaemic lesions. Fluorescence microscopy revealed high-intensity fluorescence in the elastin fibre layer of the submucosa. The histological findings support several reports of high-intensity fluorescence from elastin fibres in the submucosa [14, 15]. The result of the fluorescence intensity test (Fig. 5) indicates that the light emitted from elastin was easily transmitted in the autograft as bright green fluorescence, with an equivalent transmission of light in the autograft without fluorescence at the control site. This result suggests that the main source of autofluorescence in the ischaemic lesion was elastin, and airway ischaemic damage led to epithelial sloughing, which exposed the layer of elastin fibre in the submucosa.

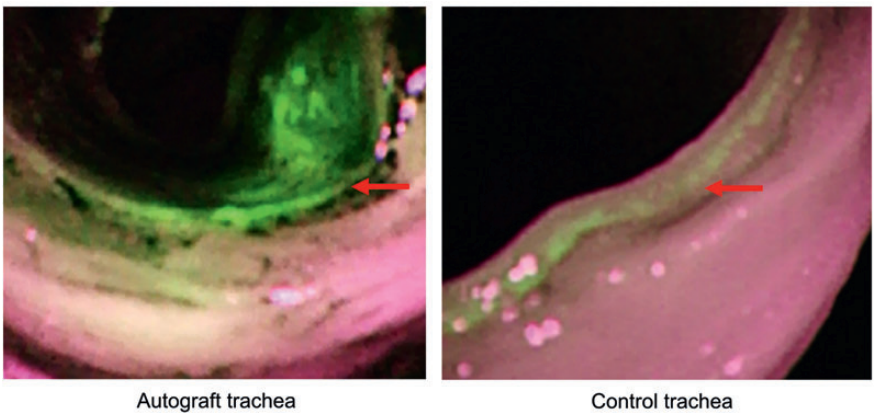

Figure 3: Images of the cut surfaces of the autograft and control trachea (fresh samples) on AFI bronchoscopy.

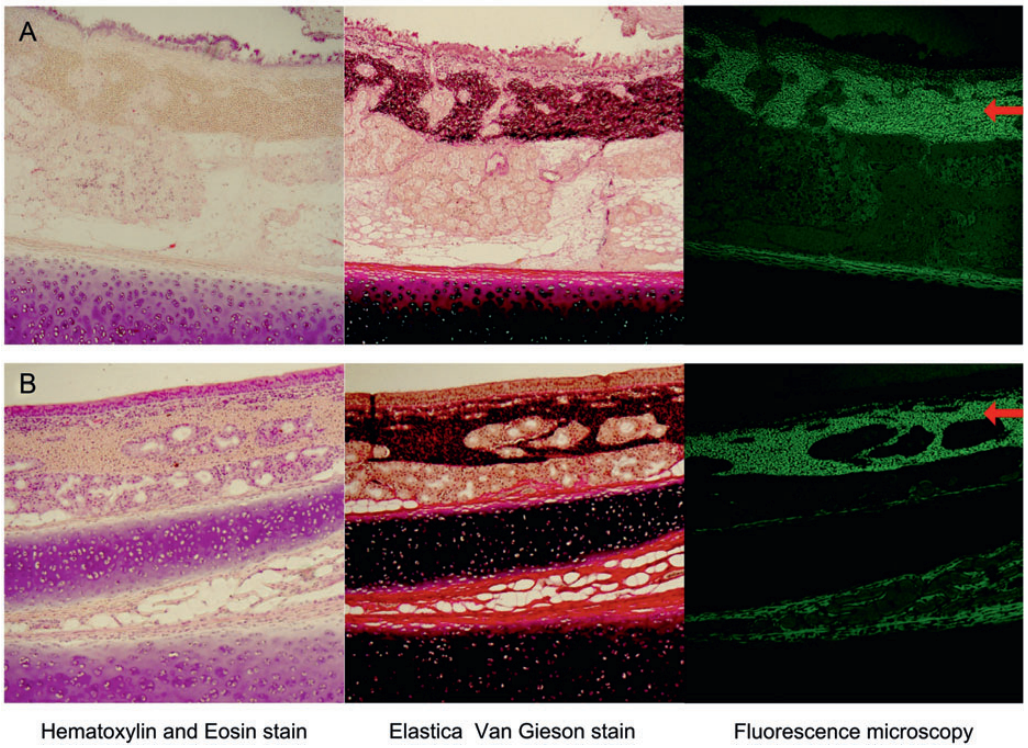

Figure 4: Histological findings of the autograft tracheal segment. (**A**) Autograft trachea. (**B**) Control trachea. The autograft developed full-thickness necrosis and epithelial sloughing. Bright green fluorescence was widely observed in the elastin fibre layer (arrowhead) of the submucosa of the autograft and control trachea using fluorescence microscopy.

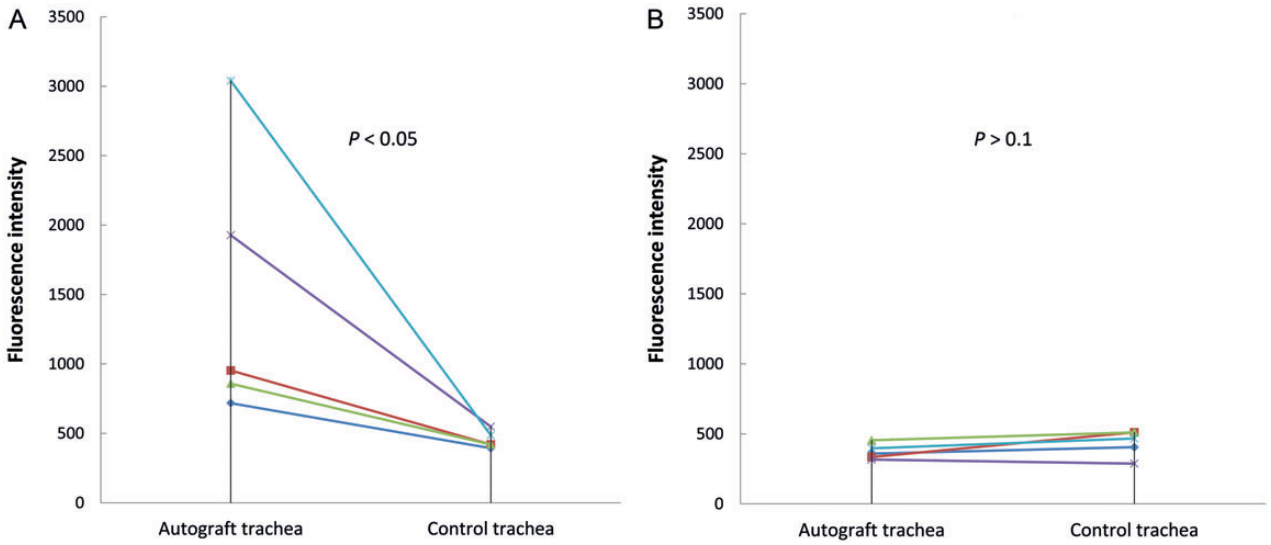

Figure 5: The fluorescence intensity of elastin in the group showing bright green fluorescence (**A**) and the group without fluorescence (**B**) on postoperative day 2.

It is still unclear as to why green fluorescence was brighter in autograft trachea than normal trachea, as seen in Fig. 3. We hypothesized that the elastin denatured by necrosis or heat emits a stronger autofluorescence, because fluorescence enhancement was observed in the airway stump cut using an electric scalpel.

The bronchial wall has fluorophores such as FAD, NADH and collagen in addition to elastin. However, it is not clear which of these substances is responsible for autofluorescence. In the current study, bright green fluorescence was also observed in the autograft after death. Moreover, fluorescence from collagen (Type 1 collagen distributed widely in living tissues, and Type 2 collagen present in cartilage) was not observed by AFI bronchoscopy (Fig. 3) or fluorescence microscopy (Fig. 4). Therefore, collagen and unstable fluorophores, such as FAD or NADH, are not the main fluorescence sources in ischaemic lesions.

Our technique with AFI bronchoscopy may offer further opportunity for research. Our findings indicate that AFI could be used for assessing bronchial healing after lung transplantation in clinical

situations. It may take several days to clarify ischaemic mucosal changes using standard bronchoscopy; however, with AFI bronchoscopy, abnormal fluorescence in the early period may clarify mucosal damage. Furthermore, bright green fluorescence on AFI bronchoscopy indicates epithelial slough that could be important for deciding the course of treatment, such as administration of prostaglandin E1 to accelerate the regeneration of epithelium.

CONCLUSIONS

There seems to be a close relationship between bright green fluorescence patterns on AFI bronchoscopy and epithelial sloughing from airway ischaemic damage. AFI bronchoscopy may detect latent airway ischaemic damage that is difficult to recognize using white-light imaging. This may allow for early treatment of the anastomotic site and prevent stricture formation.

ACKNOWLEDGEMENTS

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Conflict of interest: none declared.

SUPPLEMENTARY MATERIAL

Supplementary material (Video 1) is available at EJCTS online.

Video 1: Findings of the anastomotic site of a pig autograft trachea on AFI bronchoscopy. Bright green fluorescence was widely observed from the anastomotic site on postoperative day 2.

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