Fluorescein-Aided Confocal Laser Endomicroscopy of the Lung

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Key Words

Alveolar imaging · Fluorescein-aided endoscopy · Pulmonary confocal laser endomicroscopy

Abstract

Background: There are only few reports about confocal laser endomicroscopy (CLE) for pulmonary imaging. In these studies, in contrast to gastrointestinal endoscopy, CLE was performed without fluorescein. **Objectives:** The aim of the present study was to evaluate the value of fluorescein usage for CLE of the lung. **Methods:** Fluorescein-aided CLE was performed in 15 consecutively recruited patients and in 4 young healthy volunteers with a miniprobe during flexible bronchoscopy. Before and after intravenous administration of fluorescein, central airways and alveolar structures were evaluated. **Results:** Fluorescein administration did not permit imaging of epithelial cells in the central airways. In the lung periphery, alveolar walls and partially macrophages could be seen in native imaging, as expected. After administration of fluorescein, alveoli were almost filled with foam in areas with normal lung tissue. The origin of this foam was shown to be artificial. Furthermore, in patients with pathologies of the lung parenchyma, dark neoplastic and inflammatory cells adjacent to the alveolar walls were identified. No relevant side effects of fluorescein administration could be

observed. **Conclusions:** Fluorescein-aided CLE of the lung appeared to be safe and well tolerated. While the lack of staining of cells in the central airways was a major limitation, it permitted analysis of the lung interstitium and alveolar space and thus emerges as a new approach for the in vivo analysis of interstitial lung diseases.

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Introduction

The field of endoscopy has witnessed many technical advances in recent years. In particular, techniques such as chromoendoscopy, high-resolution endoscopy, autofluorescence and narrow band imaging have improved the detection of suspicious lesions during endoscopy. Furthermore, the development of confocal laser endomicroscopy (CLE) has permitted imaging of mucosal surfaces at cellular resolution [1–7]. This new technique has been initially performed in the gastrointestinal tract by using dyes such as fluorescein or acriflavine. This approach has enabled endoscopists to obtain real-time in vivo images ('virtual biopsies') during ongoing endoscopy at high resolution. Numerous studies have analyzed the potential utility of this technique for the detection of pathological findings in the mucosa. In particular, it was

found that CLE permitted the detection of vessels, neoplastic and inflammatory cells in the mucosa and allowed to perform targeted biopsies with high diagnostic yield. Based on these studies, CLE is now a well-established method for in vivo imaging of inflammatory [4, 5, 7] and neoplastic diseases [1–3, 6] of the upper and lower gastrointestinal tract and the oropharyngeal area [8].

In contrast to the gastrointestinal tract, CLE was only recently applied for lung imaging in vivo [9–12]. In these pioneering studies by Thiberville and coworkers [9–12], CLE of the lung was used for native in vivo imaging of alveoli. They described detailed alveolar structures and identified elastin as the main endogenous fluorophore. In smokers, alveolar macrophages carrying autofluorescent pigments additionally permitted visualization of the alveolar content. Furthermore, they reported imaging of the central airways where elastin-containing structures were noted by native CLE [9].

In the gastrointestinal tract, fluorescein is widely used to enhance the imaging of the cellular structures during ongoing endoscopy. In contrast, so far, there is only 1 report on fluorescein-aided pulmonary CLE which focused on microimaging of the alveolar capillary network during alveoscopy [13]. The aim of the current study was to evaluate the value of additional fluorescein usage during CLE of the lung in general.

Methods

Bronchoscopy was performed in 15 consecutively recruited patients with various lung diseases (6 women, 9 men; age 64.7 ± 11.3 years) during intravenous sedoanalgesia (midazolam 3–7 mg, pethidine 50–100 mg) with a 6.0-mm video chip endoscope (Olympus BF 1T180; Olympus, Tokyo, Japan). In addition, endoscopy was performed in 4 young healthy volunteers (2 women, aged 22–24 years) after local anaesthesia, with a 5.1-mm video chip endoscope (Olympus BF Q180; Olympus). All patients were monitored via electrocardiography, pulse oxymetry and non-invasive measurement of blood pressure. Every person received at least 2 litres O₂/min via nasal probe.

Fluorescein-aided confocal endomicroscopy was performed with the commercial Cellvizio system (Mauna Kea Technologies, Paris, France). We used a 1.4-mm-diameter confocal miniprobe (AlveoFlex; Mauna Kea Technologies) which has a lateral resolution of 3.5 μ m, a field of view of 600 $\times$ 500 μ m and a penetration depth of 0–50 μ m. Before intravenous administration of fluorescein, central airways were evaluated, and then, the probe was introduced into at least 1 segment of each lobe as previously described [10] until alveolar structures could be identified. Ten millilitres of 0.25% fluorescein sodium solution (FS) were injected through a small venous catheter and immediately rinsed with 10 ml saline solution 0.9%. One minute after the injection of FS, central airway mucosa and alveoli were again evaluated at the same

Table 1. Patient characteristics

Patient No.	Age years	Sex	Indication of bronchoscopic examination
1	75	m	history of left-sided pneumonectomy due to squamous cell carcinoma of the lung
2	70	m	esophageal cancer, exclusion of fistula
3	50	f	M. Hodgkin
4	69	f	non-small cell lung cancer
5	57	m	suspected lung cancer
6	51	f	small cell lung cancer
7	75	m	unspecified interstitial lung disease
8	52	f	suspected lung cancer
9	74	m	esophageal cancer, suspected lung cancer
10	75	m	neuroendocrine cancer
11	71	f	sarcoidosis
12	60	m	non-small cell lung cancer
13	46	f	Churg-Strauss
14	64	m	systemic lupus erythematosus
15	82	m	systemic lupus erythematosus

sites and at peripheral areas where native imaging was done. This second investigation was finished within 10 min after FS administration.

The video sequences were analyzed with the included software (Cellvizio viewer, version 1.4.1; Mauna Kea Technologies). We used a grey-scaled imaging with the lower and upper level thresholds of the look-up table from 0 to 8,000 units.

This study was approved by the ethical committee of the university hospital of Erlangen, and all investigated persons gave informed consent.

Results

Fluorescence-aided CLE was performed in 15 patients and in 4 healthy volunteers. The details of the examined patients are shown in table 1. In all persons, fluorescein-aided endomicroscopy as described above could be done without complications. No side effects were observed or reported by the patients. In some individuals, contact of the CLE probe with the mucosa led to minor bleeding. In all cases, this bleeding was self-limiting and did not require any intervention.

Central Airways

Consistent with previous reports, native imaging of central airways showed only non-cellular structures in macroscopically normal areas of the mucosa (fig. 1a). After intravenous administration of fluorescein, no cellular

Fig. 1. Native CLE imaging of macroscopically normal areas in the central airways. **a** Pars membranacea left main bronchus before administration of fluorescein. **b** Pars membranacea left main bronchus after administration of fluorescein in the same patient.

No major changes were noted upon administration of fluorescein. **c** Marked irregularities of non-cellular structures in macroscopically pathological areas of the central mucosa.

Fig. 2. Mixture of bubbles and fluorescein-containing fluid in distal bronchioles. **a** Patient No. 1. **b** Patient No. 5.

structures could be visualized (fig. 1b). In macroscopically pathological areas of the mucosa, the arrangement of non-cellular structures was altered with marked irregularities of matrix arrangement (fig. 1c). However, cellular structures could not be visualized by fluorescein, even in patients with large exophytic tumors where the probe was directly placed on the tumor surface.

Peripheral Structures

As expected, native imaging of lung tissue did not show cellular structures of the alveolar walls. Then, the probe was pulled back into central airways and FS was injected as described. After re-evaluation of the central

airways, the probe was pushed back again to the periphery.

Shortly before alveolar structures could be visualized, we detected a strongly fluorescent fluid and multiple round, dark, sharply bordered, non-fluorescent structures (fig. 2). These structures showed a marked variability in size and partially conflated spontaneously.

After reaching the final position with the probe, we detected alveoli filled with these foam-like structures (fig. 3). However, there were more dark spots and less fluid. Partially, the dark spots were moving parallel with the patient's breathing. This kind of foam was also observed in almost all alveoli of healthy volunteers. After slightly

Fig. 3. Examples for the distribution of foam-like structures in the alveoles. Alveolar walls are indicated by arrows. **a** Patient No. 1, segment 8 on the right side. **b** Patient No. 4, segment 5 on the left side.

Fig. 4. Comparison of CT imaging (infiltrate marked by arrows), CLE and histology (HE staining) of the same region. **a** Patient No. 3, Hodgkin's disease; Hodgkin cells are marked by arrows. **b** Patient No. 7, unspecified ILD; inflammatory cells are marked

by arrows. CLE demonstrated marked infiltration of alveolar regions by dark neoplastic or inflammatory cells. In addition, irregularities of fiber arrangement were noted.

pulling back the probe and after some deep breaths by the volunteers, we re-inserted the probe and saw much larger spots and less fluid.

To further evaluate the origin of the observed foam-like structures, we analyzed the density values of the bubbles

in a shaken mixture of highly concentrated soap water and a solution of FS and NaCl 0.9% 1:100,000, and of bubbles which developed immediately at the tip of the probe when we pulled it out from a mixture of pure bovine surfactant (Alveofact®; Lyomark Pharma GmbH, Germany) and the

Fig. 5. Comparison of CT imaging and CLE in 2 patients suffering from lupus erythematosus: ground glass opacities as a sign of high activity (top row) and fibrotic changes (bottom row) were detected in the CT scan. In addition, severe irregularities of alveolar structures in CLE were found in both cases.

same FS solution. In both settings, we measured the mean density in the middle of 50 different bubbles and found nearly the same density values of 6.04 ± 69.6 and 2.96 ± 75.2 units, respectively. Furthermore, we analyzed the density in the middle of 100 dark spots in healthy volunteers where we found density values of 0.41 ± 112.3 units. Based on these results, we identified the dark structures as air bubbles which were encoated by FS-containing fluid, thereby creating a foam-like structure in some areas.

In contrast, in patients with infiltrations of lung tissue on CT scan, little or no bubbles were observed in the affected areas (fig. 4, 5). In some of these patients with pathologies of the lung parenchyma determined by CT scans (patients No. 3, 7, 14 and 15), differences between native CLE and fluorescein-aided CLE were noted. We found normal alveoli in patient No. 3 suffering from Hodgkin's lymphoma of the lung and destroyed alveolar walls in the other cases by native CLE in correlation to areas with pathologic CT findings. After administration of FS, we detected dark and almost round spots in areas with abnormal CT findings. These spots were localized near to the alveolar walls or distributed homogeneously in the field of view in all patients (fig. 4, 5) suggesting cellular infiltrations. This concept was proven by histological assessment of biopsies where inflammatory and neoplastic cells were noted in the lung (fig. 4).

Taken together, there was no additional information which resulted from fluorescein usage compared with native imaging, except in areas of the lung where changes on CT scan could be seen. In these cases, fluorescein-aided CLE allowed visualizing pathologic changes in the alveolar space and lung interstitium caused by inflammatory and neoplastic cells.

Discussion

The aim of the present study was to evaluate the use of fluorescein for CLE of the human lung. In our pilot study on such fluorescein-aided CLE in 19 individuals, we could demonstrate that the administration of fluorescein was safe and could easily be performed in the lung. These findings are consistent with a recent study on CLE in the gastrointestinal tract where no major adverse events were noted in a large number of patients [14]. Therefore, intravenous administration of fluorescein for CLE-based imaging of the lung appears to be a safe and feasible method.

After intravenous administration, we observed that fluorescein is secreted into the intrapulmonary fluid of the distal airways in large amounts. When pushing the probe towards the alveoli, bubbles developed in front of the tip from the airway lining liquid creating a foam-like

structure. In most cases, the visualization of the alveoli was artificially superimposed by this foam. Based on our *ex vivo* experiments with fluorescein and purified surfactant, the most likely explanation for the development of bubbles at the tip of the probe is the local detergent effect of surfactant. This hypothesis was also supported by our observation in healthy volunteers that the foam could be 'washed out' of the alveoli. Finally, in patients with inflammatory and neoplastic diseases of the lung, no local bubbles and foam-like structures were noted, consistent with the idea that cellular infiltrations in the lung suppress local surfactant levels and subsequent bubble formation during fluorescein-aided CLE.

A major limitation of fluorescein-aided CLE was the lack of staining of epithelial cells in the central airways. This observation is in contrast to the findings in CLE of the gastrointestinal tract where fluorescein results in a rapid staining of intestinal epithelial cells [4, 5, 7, 15, 16]. These differences suggest that pulmonary and gastrointestinal mucosa differ remarkably with regard to their absorption of FS. Another possible explanation is the specification of the CLE probe. In contrast to the probes used in gastrointestinal endoscopy, the AlveoFlex probe has little penetration depth which ends at the level of the basal membrane. Due to the lack of perfusion, it is possible that FS cannot reach the mucosal cells when it is administered intravenously. Furthermore, it could be shown in gastrointestinal CLE that imaging was best in a period of 8 min after FS injection [17]. Maybe a longer period would be better for CLE of the central airways but this has to be evaluated in further studies. Topical application of FS would not lead to an increased staining of epithelial cells as the dye was secreted intraluminally in a high amount. Finally, imaging of structures in central airways was also difficult due to breathing-dependent movement of the central airways that made it difficult to keep the tip of the CLE probe in a stable, specific position. However, this problem could be solved by repeated training of the examiner over time.

We evaluated FS for CLE of the lung because it is well established for staining during CLE of the gastrointestinal mucosa. Maybe other dyes are more suitable for CLE of central airways. Recently, the use of cresyl violet was reported to be an interesting alternative dye [18] as chromoendoscopy and CLE can be performed simultaneously with this substance. However, experience with topical application of cresyl violet into the lung is limited. Furthermore, topically applied methylene blue can be used for visualization of cells of central airway mucosa but a laser unit with a wave length of 660 nm is needed for this

dye [11]. The disadvantage of both methylene blue and cresyl violet is a lack of staining of lung parenchyma when it is topically applied.

Fluorescein-aided CLE provided the possibility to visualize the intra-alveolar cellular component in patients with interstitial lung disease (ILD) or other kinds of infiltrates on CT scan. A disease-related lack of surfactant in these areas could also be detected by fluorescein-aided CLE, as no local bubbles occurred during the examination. In addition, changes of the alveolar structures could be analyzed by CLE in the present pilot study. Based on these findings, ILD could become one of the major fields of application of pulmonary fluorescein-aided CLE. One might speculate that some specific pathologic patterns as seen in idiopathic pulmonary fibrosis or other types of idiopathic interstitial pneumonia could be characterized by CLE. This would lead to an accelerated diagnosis of ILD. Furthermore, CLE might reduce the need for biopsies or increase the chances for targeted biopsies in these diseases. These points need to be addressed in future controlled studies.

In conclusion, fluorescein-aided CLE of the lung appears to be a feasible and safe method. Although we found some major limitations, fluorescein-aided CLE was helpful in our pilot study to detect cellular structures in the lung periphery, and in the future, ILD might be one area of application for this emerging method of pulmonary imaging. We could clearly show that our knowledge of gastrointestinal CLE cannot be easily transferred to the lung. Further research is needed to clarify the clinical relevance of fluorescein-aided CLE in pulmonary imaging. Especially the identification of a dye for staining of central airway pathologies would be of great importance.

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