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Ultrastructural study of immotile cilia syndrome

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SUMMARY

Recently immotile cilia syndrome has gained the interest of a number of investigators from the aspects of the physiology and pathology of the ciliary movement. This is because microstructural abnormalities of the dynein arms in the cilia of the respiratory mucosal epithelium and in the flagella of sperm tails have been identified in this syndrome.

The present study was designed to find a simple clinical method for detecting patients having this syndrome, and was conducted to elucidate the clinical significance and etiology of this syndrome.

In order to detect patients with immotile cilia syndrome, 72 patients with one of more conditions such as sinusitis, bronchiectasis, situs inversus and sterility were examined using a ciliary function test and electron microscope observation of the nasal cilia.

Seven of the examined patients were diagnosed as having immotile cilia syndrome on the basis of the presence of the characteristic ultrastructural patterns of the nasal cilia, i.e., disorders of the dynein arms.

The possibility of positive test results increases greatly as the degree of the complications increases, especially in the case of a combination of chronic inflammation of the respiratory tract with situs inversus and sterility.

As the electron microscope findings of this syndrome, Afzelius (1979) has reported defects of the dynein arms, spoke head and central sheath. Beyond these microstructural abnormalities, I have identified abnormal attachment of the dynein arms as a new parameter in the electron microscopic diagnosis of immotile cilia syndrome.

INTRODUCTION

Today congenital anomalies of the cilia of the respiratory mucosal epithelium are thought of as a cause of chronic airway disease, and electron microscopy observations on cilia in patients with airway disease have revealed that axonemes containing ATP-ase called dynein arms are missing in those cilia. This was first reported in 1977 by Eliasson et al. as immotile cilia syndrome (I.C.S.). Since then, we have developed an easy-to-use, systematic method of diagnosing the disease, striven to devise an effective method for its early discovery, defined its clinical

implications, and elucidated its etiology (Watanabe, 1983). Besides these morphological abnormalities of cilia, dynein arms are often observed to stick to abnormal positions on microtubules. This has also been proved to be a cause of lowered ATP-ase activity and has been considered to serve as a basis for diagnosing I.C.S.

SUBJECTS AND RESEARCH METHOD

Subjects: Of a series of outpatients who sought medical advice at our hospital during the period between October 1980 and December 1982, 72 patients with one or more of I.C.S.'s component diseases, i.e., sinusitis, bronchiectasis, sterility and situs inversus, were selected for examination.

Research method:

1. Mucociliary clearance test.
   The saccharin method, developed by Anderson (1974), was used in measuring the mucociliary transit time (M.T.T.). A 20% saccharin granule, measuring 2.5 × 1.0 mm and weighing 5 mg, was placed on the middle meatus side of the inferior turbinate, and the time until the subject sensed a sweet flavor as measured. The value thus obtained was taken as M.T.T. The control group consisted of 30 adult patients with normal nasal mucosa devoid of inflammatory symptoms.
2. Micro-morphological examination.
   To observe the ciliary movement of epithelial cells, epithelial tissue was scraped off the surface of the nasal mucosa on the middle nasal meatus side of the inferior turbinate in 33 patients who had shown prolongation of M.T.T. The control group consisted of healthy subjects and patients with sinobronchitis.
   Specimens were collected, immediately fixed with 2.5% glutaraldehyde, washed with a phosphate buffer solution, and fixed with a 1% osmium acid solution. After dehydration with alcohol, they were embedded in Epon 812 and sliced into ultrathin sections by an ultra microtome (Porter-Blum, Model MT-2B). After double-staining with uranium acetate and lead hydroxide, the specimens were examined with an electron microscope (Hitachi-HU-12A).
3. Clinical trials.
   The clinical trials we conducted included rhinoscopy, X-ray of the nose (both projection Water's position and anterior posterior), chest X-ray (simple and bronchography).

RESULTS

The mucociliary transit time (M.T.T.) for the 30-patient control group ranged from 6.5 to 23 minutes, averaging 14.2 ± 4.0 min. Of the series of 72 outpatients with one or more of I.C.S.'s component diseases (among them 57 cases of sinus-

itis, 11 cases of bronchiectasis, 11 cases of situs inversus, and 26 cases of sterility), 33 patients showed M.T.T. in excess of 30 minutes. Among this over-30 minute-M.T.T. prolongation group, 7 patients (2 males and 5 females) were found to have I.C.S. In other words, I.C.S. was found in 3 out of 4 cases of Kartagener syndrome (i.e., simultaneous sinusitis, bronchiectasis and situs inversus), 1 out of 31 cases of sinusitis alone, 1 out of 3 cases of concomitant sinusitis, bronchiectasis and sterility, 1 out of 12 cases of concomitant sinusitis and sterility, and 1 case of concomitant sinusitis, sterility and situs inversus (Table 1). An electron microscopy examination of the 7 I.C.S. cases showed absence of inner dynein arms in all the cases, but 2 of them were suspected of absence of outer dynein arms. Radial spokes and central sheaths were missing in only 1 case. Also, there were directional abnormalities in the basal foot. A further look at the dynein arms under the electron microscope revealed that in 6 cases they were stuck to an abnormal position on the microtubule.

In contrast, in the control subjects with sinobronchitis, two pairs of dynein arms were recognized, and the direction of the basal foot was uniform (Figure 1).

Figure 2 represents an electron microscopy picture of a cilium in a patient with I.C.S. The cilium is surrounded by a double basement membrane. In the center of this membrane is a pair of central microtubules (C.M.T.), which is surrounded by 9 pairs of peripheral microtubules (P.M.T.) However, the dynein arms, which, as

Table 1. Mucociliary transit times (M.T.T.) in the patient with sinusitis, bronchiectasis, sterility or situs inversus (S.I.).

	case	M.T.T. ~ 30	~ 60	~min
sinusitis	31	20	2	9●
bronchiectasis	4	2	1	1
sterility	3		1	2●
S.I.	4			4●●●
sterility	12	6	2	4●
S.I.	1			1●
S.I.	2		1	1
sterility	11	7	3	1
S.I.	4	4		
total	72	39	10	23●●●●●●●

● : the number of patients in immotile cilia syndrome

can be seen in Figure 1, should project from the A-subfibers to B-subfibers of P.M.T., are missing. Even residual arms are found in sites different from the normal binding sites of microtubules (arrow).

Figure 3 shows and electron micrograph of 3 cilia in a patient with I.C.S. All these cilia are enclosed by cell membranes, but the arrangement of P.M.T. is irregular, and there are some abnormal dynein arms. In other words, almost all structures considered to be inner arms are missing. It seems that the irrugelar arrangement

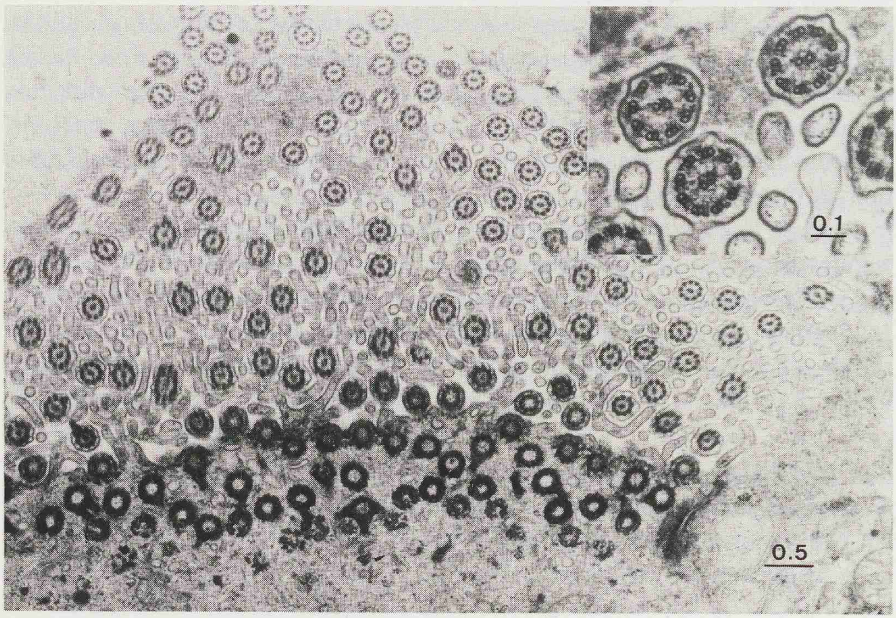

Figure 1. Ciliary epithelium of nasal mucosa from a patient with sinobronchitis. Two pairs of dynein arms are recognized, and the direction of the basal foots are uniform.

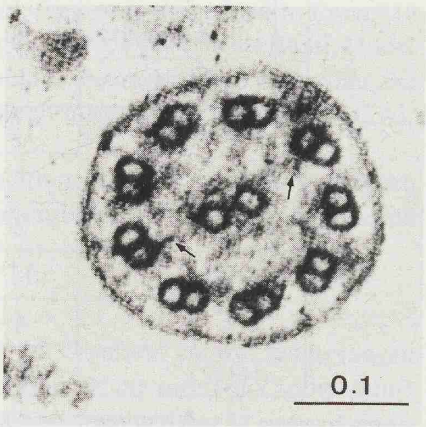

Figure 2. Cross-section of ciliary shaft (in the patient with immotile cilia syndrome). Dynein arms are missing, even residural arms are found in sites different from the normal binding sites of microtubules (arrow).

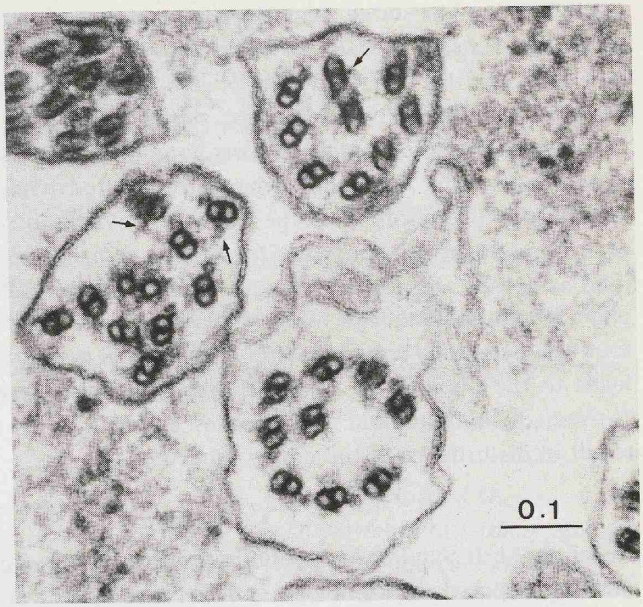

Figure 3.
Cross-section of 3 ciliary shafts (in the patient with immotile cilia syndrome). Peripheral microtubules are irregularly arranged, and the disorders of dynein arms are observed. Also abnormal binding of the dynein arms are seen at many sites (arrow).

of microtubules is due to the abnormality of the dynein arms. In addition, the central sheath is obscure, and because of the abnormal P.M.T. arrangement, no radial spokes are to be found.
Also, abnormal attachment of the dynein arms are seen at many sites (arrow).

DISCUSSION

Afzelius et al. (1979) were the first to report that hindrance of ciliary movement due to the absence of dynein arms is chief cause of I.C.S., and they also reported that I.C.S. is due to absence of the spoke head and central sheath.
In the course of the above studies, we found that dynein arms were absent in 7 cases, that is, either inner arms or outer arms or both were non-existent. In one of the 7 cases, both radial spokes and the central sheath were absent. This case was considered to be fairly advanced. In addition, careful electron microscopy observations of the 7 cases revealed that in 6 cases the dynein arms' binding sites were abnormal. This gave us the suggestion that the dynein arms, earlier considered absent, were actually present at incorrect binding sites on the microtubules.
Hyans et al. (1974) reported that dynein arms were bound pair by pair with microtubules at intervals of about 25 nm longitudinally, thus suggesting that microtubules have special sites for binding with dynein arms. Gibbons (1963) and Shimizu (1975) reported that dynein arms, once isolated with Tris EDTA buffer, combine again with the A tubes of microtubules when these isolated arms are added to the microtubules in the presence of Mg.

Also, Shimizu has stated that when the number of dynein arms added in the above experiment is small, only one pair of arms combines wth the original A tube sites, and that when the number is increased they stick to other A tube sites and even to B tubes. These facts suggest that dynein arms possibly bind to abnormal microtubule sites in actual disease conditions. He has further reported that in experiments with Ciliata Perty, when once-isolated dynein arms are bound to the original sites of microtubules, the ATP-ase activity is increased. This implies that when dynein arms are bound to sites other than the normal binding sites of microtubules or they are isolated from microtubules, the ATP-ase activity should decline. In our patients with I.C.S., dynein arms were in part missing or were not found at the normal binding microtubule sites. It is speculated that this condition leads to the disturbance in the sliding phenomenon which is seen to occur between dynein arms and microtubules, and in ATP-ase activity. These changes result in disturbance of the ciliary movement.

RÉSUMÉ

Récemment, le syndrome de cilia immotiles intéresse plusieurs investigateurs pour les aspects de la physiologie et de la pathologie de mouvement ciliaire. C'est parce que chez ce syndrome on est arrivé à identifier des anomalies microstructurales des bras dyneins dans les cilia de l'épithélium muqueux respiratoire et dans les flagella de queues de spermatide.

Cette étude présente a ètè désignée afin de trouver une simple méthode clinique pour détecter des patients ayant ce syndrome, et d'élucider sa significance clinique et son étiologie.

En vue de détecter des patients porteurs du syndrome de cilia immobilites, 72 malades mâles avec une ou plus de deux conditions, telles sinusite paranasale, bronchiectasie, situs inversus, et stérilite, ont été examinés selon un test de fonction ciliaire et des observations par la microscope electronique pour les cilia nasaux.

Sept des patients étudiés ont été diagnostiqués d'avoir le syndrome de cilia immotiles sur la base de la présence des modelès ultrastructuraux caractéristiques des cilia nasaux, i.e. désordres des bras dyneins.

La possibilité du test positif s'augmente grandement en fonction de la sévérité des complications, surtout dans le cas d'une inflammation chronique de la voie respiratoire associée avec le situs inversus et la sterilité.

Utilisant la microscope électronique chez ce syndrome, Afzelius a observé des défauts des bras dyneins, la spoke tête, et le fourreau central. Outre ces anomalies microstructurales, l'auteur a constaté une attache anomale des bras dyneins comme un nouveau parametre dans la diagnose selon la microscope électronique du syndrome de cilia immotiles.

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