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Effect of s-carboxymethylcysteine on ciliary activity in chronic sinusitis*

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SUMMARY

This study was designed to investigate the possible pharmacological effect of S-carboxymethylcysteine (S-CMC) on the ciliary activity, using an in vitro experimental system after removing mucus. Ciliary activity from healthy rabbit maxillary sinus and from healthy human nasal mucosa demonstrated no significant change in RPM! 1640 containing S-CMC. On the other hand, the effect of S-CMC on the reduced ciliary activity from patients with chronic sinusitis was quite varied among the cases examined. S-CMC demonstrated no stimulatory effect on the beating activity of cilia that have a baseline activity of less than 400 beats/min. However, S-CMC was able to enhance the beating activity of cilia that demonstrated a baseline activity of more than 400 beats/min. S-CMC at 0.5% induced a larger ciliostimulatory effect than 0.05% S-CMC. In conclusion, our study has clearly demonstrated that S-CMC could directly enhance ciliary activity of chronic sinusitis in the absence of significant organic change of ciliated cells.

Key words: chronic sinusitis, ciliary activity, s-carboymethylcysteine

INTRODUCTION

S-carboxymethylcysteine (S-CMC) is available in Japan and the United Kingdom, and in Japan, particularly, is widely prescribed for chronic sinusitis and bronchitis, and otitis media with effusion (Kumazawa and Ushiro, 1987). This mucolytic drug is a derivative of acetylcysteine, which is able to split the disulphide bonds of the long-chain glycoproteins of the mucus layer, thereby reducing the latter's viscocity. S-CMC is also able to produce cellular hyperplasia (Huyen et al., 1966). We previously reported that S-CMC could alleviate to some extent SO_i-induced ciliary depression in the sinus of the rabbit (Ohashi et al., 1985). The mechanism was considered to be primarily due to S-CMC-induced cellular hyperplasia. However, there is a possibility that **S-CMC** might possess an ability to directly enhance ciliary activity. This study was designed to investigate possible pharmacological effect of **S-CMC** on the ciliary activity *in vitro.*

MATERIALS AND METHODS

Effect of S-CMC on sinus ciliary activity in rabbits

Five healthy rabbits (average weight: 2 kg) were used. They were painlessly decapitated and two mucosa! samples (50-70 μm thick) from both maxillary sinuses were obtained from each animal. The mucosa! samples were carefully rinsed in RPMI 1640 solution (Moore et al., 1967; Ohashi et al., 1986) to remove the mucus. Each mucosa! sample was placed in a sealed chamber containing 3 ml of **RPMI** 1640 solution. Thus, a total of 10 cultures were prepared. First, the baseline ciliary activity (beats/min) in the most active cell of each culture was determined at an ambient temperature of 30 °C, using the direct and quantitative photoelectrical method of Ohashi and Nakai (1983a). After determining the baseline ciliary activity, the **RPMI** 1640 solution in each chamber was decanted. Five cultures were immediately superfused with RPMI 1640 containing either 0.5% or 0.05% **S-CMC.** At intervals of 5, 10, 15, 20, 30, 40, 50, and 60 min after sampling, the ciliary activity of cells, of which the baseline ciliary activity was determined, was measured at 30 °C according to the same photoelectrical method.

Effect of S-CMC on ciliary activity from healthy volunteers

Two healthy volunteers entered in this trial with prior informed consent. Of each of them, five mucosal samples from the inferior turbinate (50-70 μm thick) were obtained by gently scraping with a curette. Subsequent steps were the same as described for the rabbit sinus samples.

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Effect of S-CMC on mucosal ciliary activity from patients with chronic sinusitis

The subjects studied were chosen from among patients who operated on by the Caldwell-Luc method during 6 months (January to June, 1990) because of chronic maxillary sinusitis that had resisted conservative treatment for more than one year. Seven patients entered this trial with prior informed consent. The mucosal samples of maxillary sinus taken at operation were used for the present study. The mucosal samples were immersed in **RPMI** 1640 solution with a minimum of delay to avoid artificial damage.

Each mucosal sample excised at operation was cut into 15 pieces. Then, each small piece was sliced to a thickness of 50-70 μm with a minute knife for optical surgery, and each slice was enclosed in a chamber containing RPMI 1640. Thus, a total number of 15 mucosal cultures were prepared. The next steps were identical to the ones described above. In this study the significant difference after replacement was statistically accepted at $p < 0.05$ using the Student's t-test.

RESULTS

Effect of S-CMC on sinus ciliary activity in rabbits

Ciliary activity from rabbit maxillary sinus demonstrated no significant change **in RPMI** 1640 containing 0.5% **S-CMC,** nor in RPMI containing 0.05% **S-CMC** (Figures 1 and 2).

Effect of S-CMC on ciliary activity from healthy volunteers

Ciliary activity from healthy volunteers demonstrated no significant change in **RPMI** 1640 containing 0.5% **S-CMC,** nor in **RPMI** containing 0.05% **S-CMC** (Figures 3 and 4).

Effect of S-CMC on mucosal ciliary activity from patients with chronic sinusitis

Ciliary activity of case 1 (Figure 5) was extremely poor. The baseline ciliary activity of this group was less than 400 beats/min. The ciliary activity did not demonstrate any significant change in **RPMI** 1640 up to 60 min. The ciliary activity showed no significant change at any time examined in RPMI 1640 containing 0.5% S-CMC, nor in RPMI 1640 containing 0.05% S-CMC.

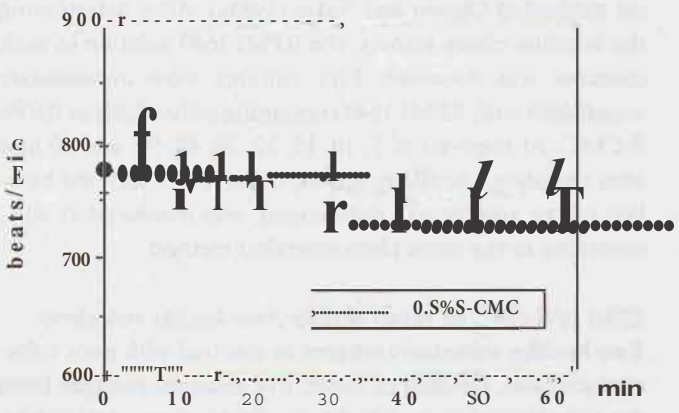

Figure 1. Ciliary activity from healthy rabbit maxillary sinus in RPMI 1640 containing 0.5% S-CMC. No significant change is observed at any time examined.

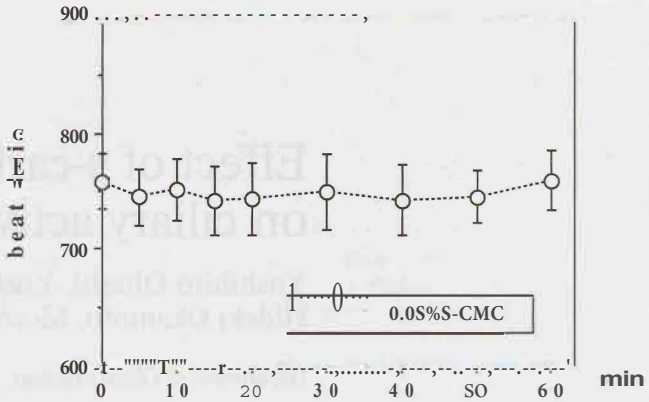

Figure 2. Ciliary activity from healthy rabbit maxillary sinus in RPMI 1640 containing 0.05% S-CMC. No significant change is observed at any time examined.

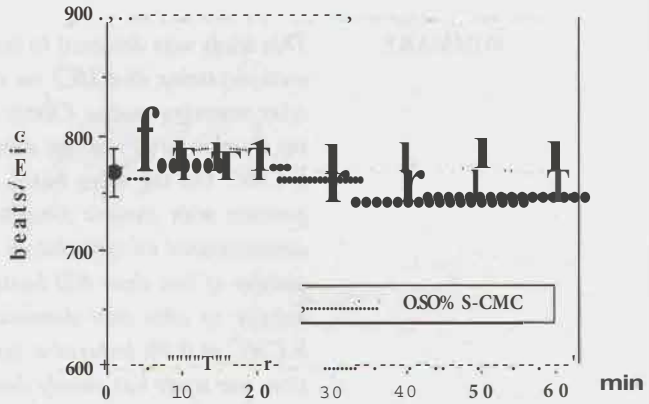

Figure 3. Ciliary activity from healthy human nasal mucosa in RPMI 1640 containing 0.5% S-CMC. No significant change is observed at any time examined.

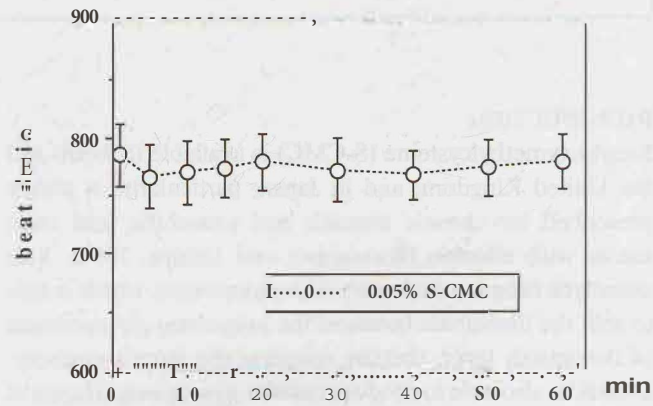

Figure 4. Ciliary activity from healthy human nasal mucosa in RPMI 1640 containing 0.05% S-CMC. No significant change is observed at any time examined.

Ciliary activity of case 2 (Figure 6) was extremely poor; baseline activity was less than 400 beats/min. No significant change of the ciliary activity was observed in RPMI 1640 alone up to 60 min. No significant change was observed, either, in RPMI 1640 containing 0.5% S-CMC or in RPMI 1640 containing 0.05% S-CMC.

The baseline ciliary activity of case 3 (Figure 7) was between 400 and 550 beats/min. The ciliary activity did not change significantly in **RPMI** 1640 during a 60-min observation. On the other hand, the ciliary activity was significantly increased as

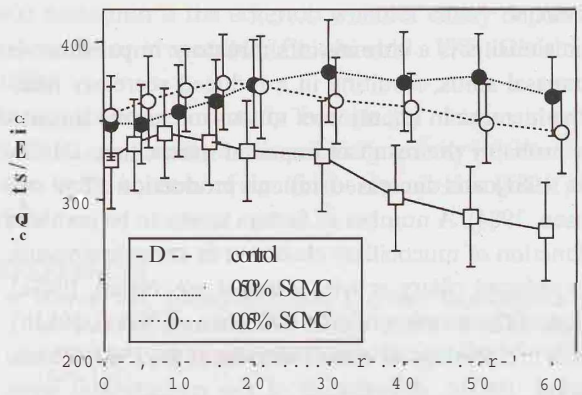

Figure 5. Change of ciliary activity from the maxillary sinus of case 1. The baseline ciliary activity is less than 400 beats/min. No significant change is observed in RPMI alone, RPMI 1640 containing 0.5% S-CMC, nor RPMI 1640 containing 0.05% S-CMC.

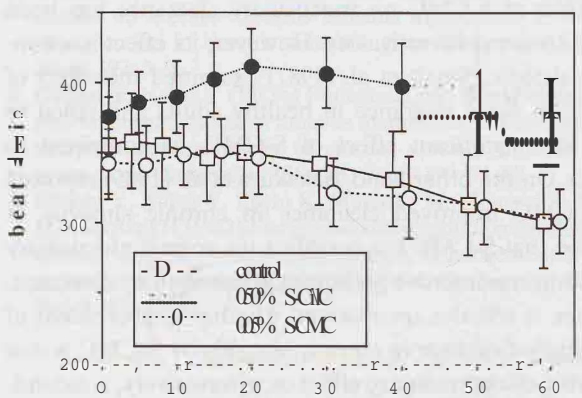

Figure 6. Change of ciliary activity from the maxillary sinus of case 2. The baseline. ciliary activity is between 300 and 400 beats/min. No significant change is observed in RPMI alone, RPMI 1640 containing 0.5% S-CMC, nor RPMI 1640 containing 0.05% S-CMC.

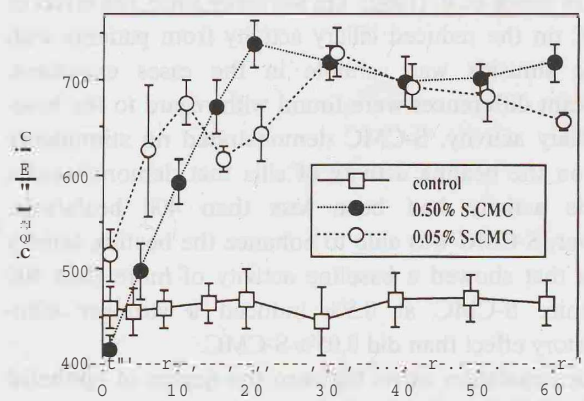

Figure 7. Change of ciliary activity from the maxillary sinus of case 3. The baseline ciliary activity is between 400 and 550 beats/min. No significant change in ciliary activity is observed in RPMI. However, significant increase in ciliary activity is observed at 5 and 10 min in RPMI 1640 containing 0.5% and 0.05% S-CMC, respectively.

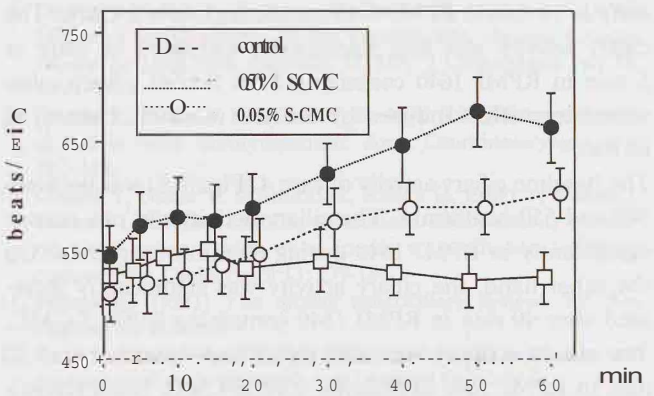

Figure 8. Change of ciliary activity from the maxillary sinus of case 4. The baseline ciliary activity is between 500 and 550 beats/min. No significant change in ciliary activity is observed in RPMI. On the other hand, significant increase in ciliary activity is observed at 30 and 40 min in RPMI 1640 containing 0.5% and 0.05% S-CMC, respectively.

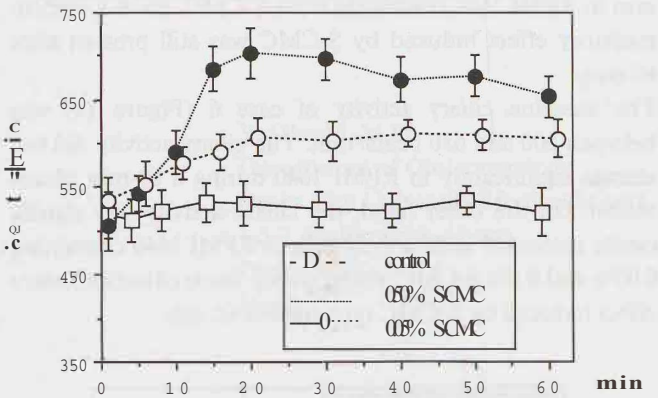

Figure 9. Change of ciliary activity from the maxillary sinus of case 5. The baseline ciliary activity is between 500 and 550 beats/min. No significant change in ciliary activity is observed in RPMI alone. On the other hand, significant increase in ciliary activity is observed as early as 10 and 20 min in RPMI 1640 containing 0.5% and 0.05% S-CMC, respectively.

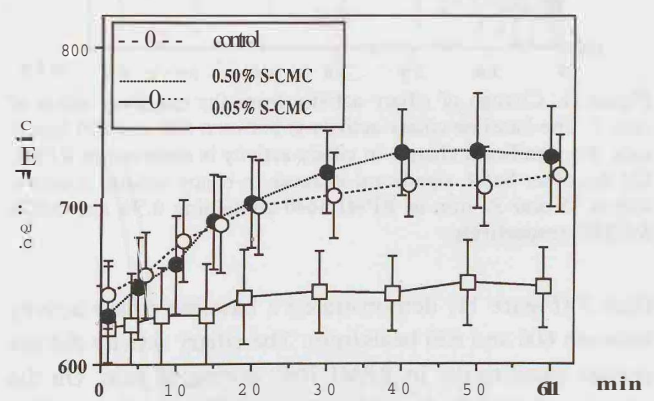

Figure 10. Change of ciliary activity from the maxillary sinus of case 6. The baseline ciliary activity is between 600 and 650 beats/min. No significant change in ciliary activity is observed in RPMI. On the other hand, significant increase in ciliary activity is observed at 20 and 30 min in RPMI 1640 containing 0.5% and 0.05% S-CMC, respectively.

early as 10 min in RPMI 1640 containing 0.05% S-CMC. The ciliary activity was also significantly increased as early as 5 min in RPMI 1640 containing 0.5% S-CMC. Such ciliostimulatory effect induced by S-CMC was still observed at 60min.

The baseline ciliary activity of case 4 (Figure 8) was between 500 and 550 beats/min. The ciliary activity did not chal)ge significantly in RPMI 1640 during 60-min observation. On the other hand, the ciliary activity was significantly increased after 40 min in RPMI 1640 containing 0.05% S-CMC. The ciliary actiivity was also significantly increased at 30 min in RPMI 1640 containing 0.5% S-CMC. Such ciliostimulatory effect induced by S-CMC was kept after 60 min.

The baseline ciliary activity of case 5 (Figure 9) was between 500 and 550 beats/min. The ciliary activity did not change significantly in RPMI 1640 during the 60-min observation period. On the other hand, an increased ciliary activity was observed at 20 min in RPMI 1640 containing 0.05% S-CMC. An increased ciliary activity was also observed as early as 10 min in RPMI 1640 containing 0.5% S-CMC. Such a ciliosti.-mulatory effect induced by S-CMC was still present after 60 min.

The baseline ciliary activity of case 6 (Figure 10) was between 600 and 650 beats/min. The ciliary activity did not change significantly in RPMI 1640 during a 60-min observation. On the other hand, the ciliary activity was significantly increased at 20 and 30 min in RPMI 1640 containing 0.05% and 0.5% S-CMC, respectively. Such ciliostimulatory effect induced by S-CMC remained at 60 min.

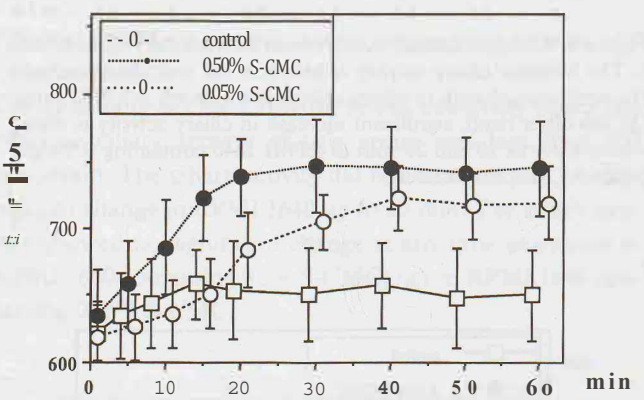

Figure 11. Change of ciliary activity from the maxillary sinus of case 7. The baseline ciliary activity is between 600 and 650 beats/min. No significant change in ciliary activity is observed in RPMI. On the other hand, significant increase in ciliary activity is observed at 15 and 30 min in RPMI 1640 containing 0.5% and 0.05% S-CMC, respectively.

Case 7 (Figure 11) demonstrated a baseline ciliary activity between 600 and 650 beats/min. The ciliary activity did not change significantly in RPMI 1640 during 60 min. On the other hand, the ciliary activity was significantly increased at 15 and 30 min in RPMI 1640 containing 0.05% and 0.5% S-CMC, respectively. Such ciliostimulatory effect induced by **S-CMC** was also present at 60 min.

DISCUSSION

Chronic sinusitis is a chronic inflammatory impairment in the paranasal sinus, resulting in a reduced secretory function. The increase in retantion of mucus in chronic sinusitis is most probably the result of impaired mucociliary activity (Phipps, 1981) and increased mucus production (Tos and Mogensen, 1984). A number of factors seems to be involved in dysfunction of mucociliary clearance in chronic sinusitis, such as reduced ciliary activity (Ohashi and Nakai, 1983a), reduction of the number of cilia (Ohashi and Nakai, 1983b), abnormal morphology of cilia (Takasaka et al., 1980; Ohashi and Nakai, 1983b), disturbance of the metachronal wave (Saito and Tsubokawa, 1991), and pathological rheology of the mucus (Majima et al., 1986). Improvement of either of these factors could improve mucociliary clearance in chronic sinusitis, thus leading to clinical alleviation of the affection. Indeed, drugs with a known ciliostimulatory effect are able to bring about clinical improvement in patients with chronic sinusitis (Ohashi et al., 1983).

The effect of S-CMC on mucociliary clearance has been studied by some investigators. However, its effect is a controversial topic. Smelt et al. (1987) examined the effect of S-CMC on nasal clearance in healthy adults and failed to reveal any significant effect of S-CMC, as compared to placebo. On the other hand, Sakakura et al. (1985) reported that S-CMC improved clearance in chronic sinusitis. It might be that S-CMC has no effect on normal mucociliary system but can improve pathological mucociliary clearance. However, it remains unanswered whether improvement of mucociliary clearance in chronic sinusitis by **S-CMC** is due to a direct ciliostimulatory effect or, alternatively, a secondary effect due to changes in mucus rheology.

We studied the direct effect of S-CMC on the beating frequency of cilia, using an *in vitro* experimental system after removing mucus. First, we assessed the effect of S-CMC on the beating activity of cilia from the maxillary sinus in healthy rabbits and from the inferior turbinate in healthy humans. We failed to find any effect of S-CMC on normal ciliary activity. This result is in perfect agreement with the report of Smelt et al. (1987). On the other hand, the effect of S-CMC on the reduced ciliary activity from patients with chronic sinusitis was varaible in the cases examined. Significant differences were found with regard to the baseline ciliary activity. S-CMC demonstrated no stimulatory effect on the beating activity of cilia that demonstrated a baseline activity had been less than 400 beats/min. However, **S-CMC** was able to enhance the beating activity of cilia that showed a baseline activity of more than 400 beats/min. **S-CMC** at 0.5% induced a stronger ciliostimulatory effect than did 0.05% **S-CMC.**

A close correlation exists between the degree of epithelial pathology and the ciliary activity in chronic sinusitis (Ohashi and Nakai, 1983b). Irreversible organic changes take place in sinus mucosa with a ciliary activity of 400 beats/min or less (Ohashi and Nakai, 1983b). A ciliary beating frequency of

400 beats/min is the criterion whether ciliary depression is reversible or irreversible (Ohashi et al., 1983; Ohashi et al., 1988).

In conclusion, our study has clearly demonstrated that S-CMC can directly enhance ciliary activity in chronic sinusitis, in the absence of significant organic changes of ciliated cells.

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