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Influence of Chinoin-170, a Novel Antitussive, on the Mucociliary Activity in Respiratory Airways of Rats, Rabbits, Guinea-pigs and Man

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Abstract—Chinoin-170 (Ch-170; 3,7-dihydro-1,3-dimethyl-7-[(5-methyl-1,2,4-oxadiazol-3-yl)methyl]1H-purine-2,6-dione) is a new antitussive with bronchodilating activity. Its effects on the ciliary beating frequency (CBF) and mucociliary clearance were studied. In tracheal explants of rats, Ch-170 dose-dependently at concentrations 2 and 5 mg mL^{-1} depressed CBF by 24 and 33%, respectively. In human mucosal explants, however, no effects were seen at concentrations up to 5 mg mL^{-1}. In anaesthetized guinea-pigs, an intravenous 50 mg kg^{-1} dose of Ch-170 caused no changes, and 100 mg kg^{-1} increased the CBF by 15%. Intravenous Ch-170 dose-dependently increased by 93 (50 mg kg^{-1}), 179 (70 mg kg^{-1}) and 253% (100 mg kg^{-1}) the tracheobronchial mucociliary clearance in rabbits. The effect, studied using ^{99m}Tc-labelled red blood cells as a marker, was of similar quantity to that brought about by administering 16, 25 and 40 mg kg^{-1} doses of bromhexine. It is concluded that unlike many older antitussives, Ch-170 in-vitro only slightly decreases the CBF in rats and has no adverse effects on the CBF in human mucosal explants at concentrations up to 5 mg mL^{-1}. In-vivo, Ch-170 does not significantly alter the CBF in guinea-pigs, but dose-dependently increases the mucociliary clearance in rabbits. The increase is most probably a result of changes in the production and the properties of respiratory mucus.

The 1,2,4-oxadiazole ring, characteristic of prenoxdiazine, the original chinoin non-narcotic antitussive drug (Harsányi et al 1966; Tardos & Erdély 1966) has been combined with the methylxanthine moiety to obtain a new substance, chinoin-170 (Ch-170; Fig. 1). This compound has a marked antitussive activity measured in various tests (Korbonits et al 1984, 1987; Nógrádi 1985; Minker et al 1985). It also has a bronchodilating effect: it antagonizes the bronchoconstrictor effect of histamine, acetylcholine and 5-hydroxytryptamine and also increases the respiratory volume depressed by codeine or dextromethorphan (Minker et al 1990).

Various antitussive compounds, such as codeine (Melville & Iravani 1975; Karttunen et al 1991) and dextromethorphan (Karttunen et al 1990) impair ciliary function, thereby disturbing the important defence mechanism of the respiratory system, the mucociliary clearance. The aim of the present experiments was to investigate whether Ch-170 influences the mucociliary clearance and affects the ciliary function in respiratory airways. Preliminary results have been reported by Joki et al (1990).

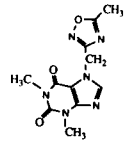

FIG. 1. Chemical structure of chinoin-170.

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Materials and Methods

Drug effects on ciliary activity: mucosal specimens

Pieces (minimum measurements approx. 5 × 5 mm) of mucosa were excised during intra- or extranasal antrostomy operations in patients with chronic maxillary sinusitis (n = 24) and from sphenoidal sinus (n = 1 for Ch-170 2·0 mg mL^{-1}) during transsphenoidal hypophysectomy. Explants were transported to the measurement laboratory in a container filled with aerated Locke–Ringer solution at room temperature (20°C), within 30 min of the excision.

The mucosal explant was attached, epithelial surface outwards, to a slightly convex piece of silicone rubber with four needles pierced through the corners of the explants. The explant was then placed into a measurement chamber below the microscope, and after this the explant was not mechanically touched. The explant was kept warm (37 ± 0·1°C) from beneath by circulating water, and moist from above with a flow (2 L min^{-1}) of heated (37 ± 1°C), humid (r.h. > 95%) air.

Animals

Male albino Wistar rats (280 g; 5 in each group) and female Dunkin-Hartley guinea-pigs (750 g; 5–6 in each group) were used. They were kept in laboratory conditions (temperature 20 ± 1°C, r.h. 55–75%, lights on from 0700 to 2100 h) with food and water freely available.

Rats were killed with a blow on the head, the neck region was opened and trachea from the second to the sixth tracheal cartilage below the larynx was excised. The tissue explant was prepared for the study as described above. Guinea-pigs were anaesthetized with a combination of 7·5 mg kg^{-1} midazolam, 0·473 mg kg^{-1} fentanyl and 15 mg kg^{-1}

fluanisone, intraperitoneally. The ventral neck area was dissected, vena jugularis was cannulated, and the trachea carefully liberated from surrounding tissues. Trachea was incised from the 2nd to the 6th cartilage caudally from the larynx, and gently elevated from below by a slightly convex steel support to prevent the respiratory movements from shaking the measurement area. The animal was placed on an electrically heated blanket, and the dissected area was covered with a plastic shroud, into which warm (37 ± 1°C), humid air (r.h. >95%) was blown continuously (2 L min^{-1}).

Measurement of ciliary activity

Ciliary beating frequency (CBF) was measured using a modification, as described in Karttunen et al (1990), of the photoelectric method published by Mercke et al (1974).

In the in-vivo study, CBF was measured before the drug administration, and 8, 16, 24, 32, 40, 48, 56 and 64 min after it. In the in-vitro study, mucosal explants were kept in the measurement chamber, immersed in drug-free Locke–Ringer solution, for 10 min. The CBF was measured (pretreatment value), after which the explant was immersed in drug solution. Control explants were kept in drug-free solution. The CBF was measured 10, 20, 40 and 60 min (human mucosal explants) and 10, 20, 40, 60, 80, 100 and 120 min after the measurement of the pretreatment value.

Drug administration

Ch-170 was diluted in Locke–Ringer solution: NaCl 9 mg mL^{-1}, KCl 0·42 mg mL^{-1}, $NaHCO_3$ 0·5 mg mL^{-1}, $CaCl_2$ 0·11 mg mL^{-1}, glucose 1·0 mg mL^{-1}; pH was adjusted between 6·8–7·4 with 0·1 M HCl. In the in-vivo experiments, drug solution was injected into the vena jugularis interna immediately after measuring the pretreatment values of CBF; injection took one minute. The liquid volume was 1 mL kg^{-1}; controls received the respective amount of drug-free Locke–Ringer.

In in-vitro studies, Locke–Ringer solutions containing 0 (controls), 0·4, 1·0, 2·0 and 5·0 mg mL^{-1} of Ch-170 were used. The solution was carefully pipetted onto the bottom of the measurement chamber (not directly on the explant), until the liquid covered the explant totally. Just before the measurement of the CBF, the liquid was removed by suction. After measurement, which lasted approximately one minute, the chamber was again filled with the solution. Each explant was used only for the measurement of one drug concentration.

Analysis of results

CBF was calculated from the undistorted sections of the recording. In the in-vitro study, the significance of the differences (Ch-170-treated vs controls) at each measuring time was analysed (SPSS/PC+, version 3·1, SPSS Inc., Illinois, USA) using analysis of variance and, in case of differences, Student's two-tailed *t*-test. For a more sensitive analysis, in both the in-vitro and in-vivo studies the differences between time zero values and values after drug administration (changes in CBF during the course of experiment) were analysed using a paired *t*-test. Differences with values of $P < 0.05$ were judged as statistically significant.

Mucociliary clearance: animals

New Zealand rabbits of both sexes, 2800–3000 g, were anaesthetized with 25 mg kg^{-1} intravenous pentobarbitone and the trachea and jugular vein cannulated.

Measurement of mucociliary clearance

The details of the methods are described elsewhere (Vastag et al 1985; Achterrath-Tuckerman et al 1992). Essentially, ^{99m}Tc-labelled homologous blood cell suspension was administered by inhalation. The cell suspension was prepared just before its application according to the method of Hamilton & Alderson (1977) and had a radioactivity of 250–270 MBq mL^{-1}. A sample of the cell suspension (0.3 mL) was nebulized with 12 L air which was blown into a 4 L reservoir equipped with a liquid trap. The aerosol was inhaled from the reservoir with the aid of a respirator (Medicor B 200, Budapest, Hungary). Three times 3-min inhalation periods were applied with 2-min intervals.

Drug administration

The test substances were administered intravenously (2% Ch-170 and 0·2% bromhexine solution) 30 min before the inhalation of labelled red blood cells dissolved in water. The control group received physiological salt solution.

Analysis of results

After the last inhalation of the nebulized cell suspension, the radioactivity was measured with a gamma-camera (GAMMA MBglgO, Medicor, Budapest, Hungary) over the closed chest over a period of 60 min. One hundred and twenty pick-ups were made on each side of the lungs. The measured data were stored on-line, and evaluated later by computer (MB 9101, Medicor, Budapest, Hungary). Time-activity curves were generated and fitted to the measured points. The biological half-life of the marker in the airways was calculated, assuming first order elimination of labelled blood cells ($t_{\frac{1}{2}eff}$). The mucociliary clearance was expressed as percentage of inhaled red-cells eliminated in one hour. Statistical analysis was carried out by Student's *t*-test.

Results

In rat tracheal mucosa, Ch-170 in-vitro slightly, but dose-dependently decreased CBF (Fig. 2). The CBF of the tissue explants used for studying 5 mg mL^{-1} concentration was 13% ($P < 0.05$) lower than that of the control explants before the drug administration. This does not explain the differences between controls and Ch-170-treated tissue explants; when the CBF values after immersion were compared with respective pretreatment values, the decrease in the CBF was already significant after 10 min Ch-170 exposure, but no decrease was seen in control explants.

In human mucosal explants, Ch-170 at concentrations 0·4–5 mg mL^{-1} did not cause any changes in the CBF (Table 1). In anaesthetized guinea-pigs, an intravenous 50 mg kg^{-1} dose of Ch-170 caused no changes in the CBF. A dose of 100 mg kg^{-1} increased the CBF (by 15%; $P < 0.05$ compared with the pretreatment value), but this was statistically significant only 8 min after drug administration. When compared with the control group, no differences were found (Table 2).

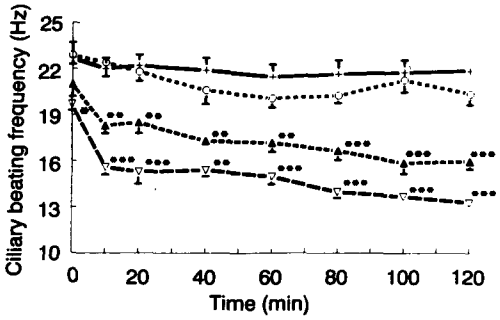

FIG. 2. Effect of chinoin-170 (0 (+), 1 (○), 2 (▲) and 5 (▽) mg mL^{-1}) on the ciliary beating frequency in rat tracheal mucosa in-vitro. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared with controls.

Table 1. Effect of chinoin-170 on the ciliary beating frequency of human nasal mucosa in-vitro.

	Concn (mg mL^{-1})				
	0	0·4	1·0	2·0	5·0
Before administration:					
	16·0±1·1	18·5±1·8	19·2±1·5	19·8±1·4	20·2±0·9
After administration:					
10 min	16·5±1·0	17·1±6·9	16·6±1·5	19·2±1·0	20·3±0·7
20 min	15·7±1·5	17·6±1·7	20·2±1·4	18·5±1·1	19·7±1·3
40 min	15·6±1·6	15·3±3·3	19·7±1·2	18·6±1·0	22·0±0·4
60 min	14·9±1·5	19·4±3·3	21·2±1·3	18·1±1·7	21·9±0·8

Table 2. Effect of intravenous chinoin-170 on the in-vivo ciliary beating frequency of tracheal mucosa in anaesthetized guinea-pigs.

	Dose (mg kg^{-1})		
	0	50	100
Before administration:			
	19·1±1·2	17·6±1·0	16·0±0·8
After administration:			
8 min	17·6±1·0	16·8±1·1	18·4±1·2*
16 min	18·3±0·9	17·5±1·3	17·5±0·6
24 min	18·3±0·8	17·4±1·3	17·6±0·5
32 min	18·2±0·8	16·7±0·9	17·0±0·9
40 min	17·9±1·2	16·6±0·8	17·6±0·4
48 min	17·6±1·0	16·6±0·8	17·6±0·4
56 min	15·9±0·7	16·2±1·0	16·8±0·7
64 min	16·7±0·7	16·7±1·5	16·1±0·9

Controls vs drug-treated animals: no statistically significant differences by analysis of variance. Values after drug administration vs pretreatment values: $*P<0.02$ (paired t-test).

Intravenous Ch-170 at doses of 50, 70 and 100 mg kg^{-1} dose-dependently increased the mucociliary clearance in rabbits. A similar increase in mucociliary clearance of Tc-labelled red blood cells was also seen after intravenous administration of bromhexine (16, 25 and 40 mg kg^{-1}; Table 3).

Table 3. *Tracheobronchial mucociliary clearance in rabbits after intravenous treatment with chinoin-170 and bromhexine.*

Treatment	n	Dose (mg kg^{-1})	Clearance (% h^{-1})	Activity ratio
Controls	10		6·82±1·54	
Chinoin-170	5	50	13·19±4·15*	1·93
	5	70	19·00±4·51**	2·78
	5	100	24·08±6·88**	3·53
Bromhexine	5	16	8·95±2·24	1·23
	5	25	13·54±5·90*	1·90
	5	40	21·64±4·71**	3·17

$*P<0.01$, $**P<0.001$ compared with controls. ^{99m}Tc-labelled red blood cells were used as a marker.

Discussion

Mucociliary clearance, the major defence mechanism of respiratory airways, is significantly impaired by chronic bronchitis (Dirksen et al 1987). Ciliary beating activity is also lower than normal in asthma (Messina et al 1991). Patients with bronchitis and asthma are among those most frequently using antitussive drugs. Traditional opiate antitussives, such as codeine, as well as synthetic opioid agonists, such as dextromethorphan, exert a depressing action on respiratory cilia (Karttunen et al 1990, 1991).

Methylxanthines and some, but not all (Isawa et al 1990) β-adrenergic agonists have been shown to increase ciliary motility and mucociliary clearance (Ahrens et al 1990; Matthys et al 1983; Sanderson & Dirksen 1989; Wanner 1986; Weich et al 1988). Ch-170 is structurally related to theophylline, and in addition to its antitussive activity, also has bronchodilator action. Therefore, it was of interest to study if this combination of pharmacological activities would produce an antitussive drug without ciliodepressive properties.

Our results from in-vitro experiments with rat tissues show slight ciliodepression by Ch-170, but only after very high concentrations. An interesting feature is that the decrease in the CBF remains stable up to almost 2 h during which the tissue explant was kept immersed in 5 mg mL^{-1} concentration of drug. After immersing rat tracheal explants in 5 mg mL^{-1} vadocaine, a new antitussive, the CBF was decreased by approximately 40% after 10 min and was completely inhibited after 20 min of exposure (Karttunen et al 1990).

In human mucosal samples, Ch-170 caused no changes in the CBF. The result demonstrates the differences in drug effects between man and animals. In guinea-pig, which is in most cases the most sensitive laboratory animal to the effects of drugs affecting the respiratory system (Friebel 1969), intravenous Ch-170 at high doses caused no marked changes in ciliary activity. On the contrary, a trend towards ciliostimulation was seen. The higher dose (100 mg kg^{-1}) consistently produced CBF values higher than those measured before drug administration, but the difference was statistically significant only after 8 min.

Radioisotopic methods have been used for measuring mucociliary transport in man (Kärjä et al 1982). The method applied in the present study shows the real clearance of the labelled blood cells. One of the control animals was killed by a blow on the neck on each experimental day, and the blood radioactivity was measured. The average radioactivity was 0·082 kBq mL^{-1} in blood and 436 kBq in the lungs. The labelled particles were eliminated in only one direction, by the flow of respiratory mucus propelled by ciliary movements. Bromhexine has been shown to increase the mucociliary clearance (Pavia et al 1983). Ch-170, at doses which cause antitussive action (Minker et al 1985), promotes the emptying of the foreign particles from the airways in a manner comparable with that of bromhexine. The 100 mg kg^{-1}

intravenous dose of Ch-170 produced an increase in the mucociliary clearance as great as that attained with a 40 mg kg^{-1} intravenous dose of bromhexine.

β-Adrenergic agonists have been proposed to increase mucociliary clearance, at least partly by enhancing the rate of mucus protection (Pavia et al 1987; Miyata et al 1987). The mucociliary clearance was dose-dependently increased by Ch-170 in rabbits, although Ch-170 had no or only minor effects on the CBF in rat and human tissues and in guinea-pigs. It seems that the main effect of Ch-170 is focused not on the CBF, but on the properties of respiratory mucus. Our evidence on this is indirect: we did not measure either the amount or the properties (e.g. viscosity) of the mucus.

Ch-170 lacks affinity for opiate receptors in the central nervous system (Minker et al 1990), which suggests that it lacks the sedative and other untoward effects common to opioid antitussives. Especially in long-term use, the ciliostatic action may be a significant adverse effect, increasing susceptibility to infections. Our results suggest that Ch-170, at antitussive concentrations, lacks ciliostatic action, which is a common side-effect of many older antitussives. Clinical studies are needed for evaluating whether Ch-170 is also devoid of ciliodepressive effects in man in-vivo, and whether the Ch-170-induced increase in mucociliary clearance seen in rabbits is also brought about in man to a therapeutically significant extent.

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