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# Mucolytic agents and mucociliary activity

*Mats Gunnarsson, Jan-Christer Hybbinette and Ulf Mercke, Lund, Sweden*

SUMMARY

*The three substances bromhexine, NA 872 (bromhexine metabolite VIII) and the $\beta_2$-receptor agonist NAB 365 (clenbuterol) and their effect on the mucociliary activity have been studied in vivo in rabbits. The substances were given parenterally in increasing dosages and 519 records were obtained from 12 rabbits. Not even at dosages in excess of recommended human dosages has any effect on the mucociliary activity been observed for any of the substances during the first 30 minutes after administration. The conclusion to be drawn is that the three substances have no effect on the mucociliary activity in rabbit in vivo, but that this does not exclude an effect on mucociliary transport or clearance.*

## INTRODUCTION

The primary task of the mucociliary system of the respiratory tract in mammals is to remove mucus together with any foreign bodies or bacteria etc. embedded in it (Hilding, 1957). The mucociliary activity is, thus, an extremely important component of the defence system of the respiratory tract. This is illustrated by the increased incidence of chronic and recurrent respiratory infections in persons with immotile cilia (Afzelius, 1979). The mucus flow is worsened by diseases such as chronic bronchitis and cystic fibrosis, where the mucus is very viscous and sticky. The driving mechanism, i.e. the cilia, is not capable of transporting this highly viscous mucus, which results in secretion stagnation. Drugs have been used to facilitate the mucus flow by increasing the strength and frequency of the ciliary movements, and by making mucus thinner and more fluid. Bromhexine belongs to the latter group of substances and was introduced on the market in the middle of the 1960s. Its mucolytic effect has been studied in animals and in clinical trials, and a decrease in sputum viscosity has been observed in patients with chronic bronchitis (Boyd and Sheppard, 1966; Engelhorn and Püschmann, 1966; Bruce and Kumar, 1968; Hamilton et al., 1970; Langlands, 1970, Harada et al., 1977). The change in viscosity is believed to be due to the severing and fragmentation of the mucopolysaccharide threads in mucus (Bruce and Kumar, 1968). Certain bromhexine metabolites, especially metabolite VIII (NA 872), have more prominent mucolytic qualities than the original substance (Merker, 1976). NA 872 has been

shown in vitro to induce an increase in production of mucus in lung specimens from rat, hamster and cat (Iravani and Melville, 1974a,b). Clenbuterol (NAB 365), a $\beta_2$-receptor agonist, has in the same animal trial been shown to have mucolytic qualities, i.e. both a viscosity decreasing effect and a volume increasing effect on mucus (Iravani and Melville, 1974 a,b). The volume increasing effect has been confirmed morphologically in an electron microscopic study by Merker (1976).

However, for all three substances bromhexine, metabolite VIII (NA 872) and clenbuterol (NAB 365) the animal experimental documentation is scanty or non-existent as regards their effect on in vivo mucociliary function. The purpose of this trial was therefore to study the in vivo effect of the three above-mentioned mucolytic substances on the mucociliary activity, by using a previously established animal model with rabbits (Hybbinette and Mercke, 1982a).

## METHOD AND MATERIAL

For the experiments, 12 healthy white male rabbits weighing 1.7–2.5 kg were used. Anesthesia and operation techniques were as previously described (Hybbinette and Mercke, 1982a). The animals were anesthetized with urethane 2 g/kg body weight i.m. and a further dose of 0.5 g/kg body weight i.v. during the surgical procedure. No further anesthesia is required during the experiments. The left internal jugular vein is catheterized for the administration of the test substance. To keep the catheter open and to compensate the animal for dehydration, a physiological saline solution is continuously supplied at 5–10 ml/hour. The left maxillary sinus is opened and the trepanation hole, 3 × 8 mm wide, is immediately covered by an antimist window, which is affixed to the bone edges with bone wax. This restores the ventilation to normal, at the same time as the mucociliary activity can be observed through the window.

The photoelectric technique previously described by Mercke et al. (1974) was used to register the mucociliary activity. A cold light beam is pointed at the fronto-superior part of the maxillary cavity and the fluctuating intensity in the light reflected by the mucociliary activity is recorded on an ink writer. Records are made continuously prior to and during the administration of the test substance, and thereafter during 30 minutes at 1–5 minute intervals. After each completed experiment, at least 30–35 minutes are allowed to pass before a new test substance is administered.

The mucociliary activity at a specific moment is calculated, both manually and by using a microcomputer, as the mean frequency during a 20 sec interval and is expressed in waves/minute. The test substances are administered intravenously in different concentrations via the catheter as standard bolus injections lasting 3 secs. Changes in the mucociliary wave frequency observed in connection with the administration of the test substance are expressed in percent of the basic muco-

ciliary wave frequency noted immediately prior to administration of the substance.

Pure physiological saline solution given in the same volume and at the same speed as the test substance served as a control, and no effect on mucociliary activity was registered.

ECG and rectal temperature are monitored continuously during the experiment, and normal body temperature (37.0–38.5 °C) is maintained by using an electric pad.

The following pharmacological substances were tested: bromhexine-hydrochloride as commercially available Bisolvon® (Boehringer Ingelheim); bromhexine metabolite VIII (NA 872; Boehringer Ingelheim); and clenbuterol (NAB 365; Boehringer Ingelheim). The last two substances were in hydrochloride form.

RESULTS

Bromhexine, given intravenously in a total of 23 experiment series (211 records), with the dosage interval 0.057–0.285 mg/kg body weight, i.e. 1–5 times the recommended human dosage, did not affect mucociliary activity during the 30 minutes following administration (Figure 1).

NA 872, given intravenously in 17 experiment series (159 records), with the dosage interval 0.23–4.7 mg/kg body weight, i.e. 1–20 times the recommended human dosage, did not affect mucociliary activity during the 30 minutes following administration (Figure 2).

NAB 365, given intravenously in 14 experiment series (149 records), with the dosage interval 0.002–0.061 mg/kg body weight. i.e. 1–30 times the recommended human dosage, did not affect mucociliary activity during the 30 minutes following administration (Figure 3).

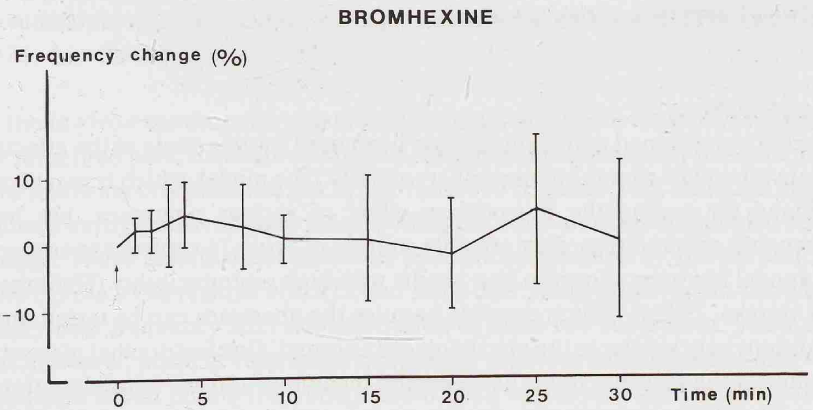


Figure 1. The effect (mean frequency change ± SD) of bromhexine 0.285 mg/kg i.v. on mucociliary activity in 6 trials (61 records).

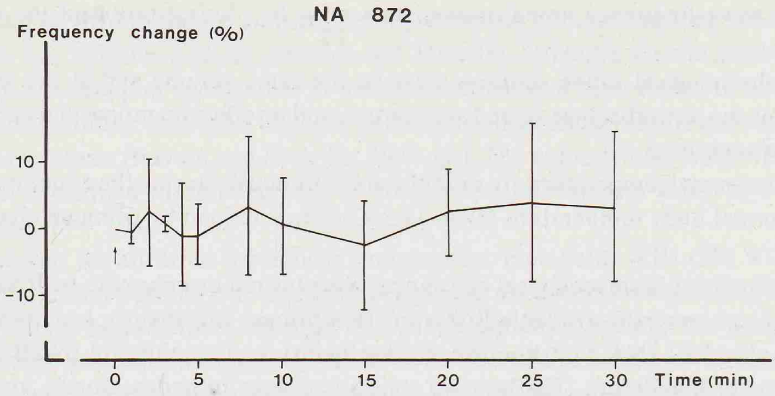


Figure 2. The effect (mean frequency change ± SD) of NA 872 0.94 mg/kg i.v. on mucociliary activity in 3 trials (33 records).

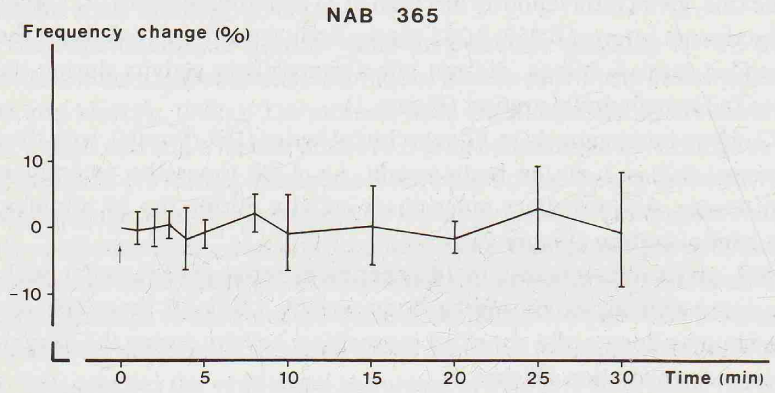


Figure 3. The effect (mean frequency change ± SD) of NAB 365 0.004 mg/kg i.v. on mucociliary activity in 3 trials (33 records).

## DISCUSSION

A recently developed animal model has been used for this study of the effects of certain mucolytic agents on mucociliary activity. The model, which is specifically designed for testing the mucociliary effect of various pharmaca, has been thoroughly checked regarding possible sources of error. In control experiments, this model has been shown to give results with high reproducibility (Hybbinette and Mercke, 1982a). This is possible because the pharmaca can be tested under conditions very similar to the physiologically normal. One feature that makes this possible is the fact that both the recording technique and the administration of the trial substance leave the examined mucous membrane unviolated. The photo-electric technique is non-invasive and requires no tracer substances and

yet it still allows immediate registration of brief and small fluctuations in the mucociliary activity during long periods.

This animal model permits administration of trial substances intra-arterially, intravenously, subcutaneously and intramuscularly, whereby the substance reaches the mucous membrane via the physiological route, i.e. through the blood (Lucas and Douglas, 1935). In the present investigation, the substances were given intravenously, i.e. as recommended for the substances in clinical use. By not having to apply the test substance directly on the mucous membrane, one does not irritate or affect the surface from which possible pharmaca effects are to be registered. It can be concluded that changes in mucociliary function, registered by using this animal model, are the effects of the administered trial substance and not of disturbances or of variations in the ciliated epithelium.

Already 50 years ago, Lucas and Douglas (1934) presented the theory that the sweeping movements of the respiratory cilia in a fluid layer of mucus (sol layer) induced transportation of a viscous layer of mucus (gel layer). This theory has, in recent years, been supported by results from electron microscopic studies (Yoneda, 1976). The Lucas-Douglas model can provide the theoretical basis for evaluating the final results of the effects ascribed to mucolytic agents, i.e. increase in mucus volume and decrease in viscosity of the gel phase of mucus. Whether these effects can only be seen when a pathologically changed mucous membrane is at hand, or if they can also be attained under normal in vivo conditions is, however, not satisfactorily proven. Favourable effects have been obtained in several studies on patients with chronic bronchitis, but the results have been based on subjective symptoms and rheological studies of expectorant sputum (Gent et al., 1969; Hamilton et al., 1970, Bach Christensen et al., 1971; Palmer, 1974; Armstrong, 1976). Specific studies on the effect of mucolytic agents on mucociliary activity and transportation, including clearance under normal and pathological circumstances, are few and have not given unambiguous results (Thomson et al., 1974; Aurnhammer et al., 1977).

In the in vivo experimental trial on normal ciliated mucous membrane from rabbit presented here, there are no signs of change in the mucociliary movement pattern following bromhexine, NA 872 or NAB 365 (Figures 1–3), although the substances were given in dosages far exceeding the equivalent recommended human dosage. These results differ distinctly from those obtained by Iravani and Melville (1974a,b) as regards NA 872 and NAB 365. They observed an increase in ciliary beat frequency after administration of these two substances. Their study was, however, made in vitro under completely different conditions and also using a method which differs from the present trial, which was done in vivo. Consequently the results are not comparable.

The absence of pharmaca effect in the present study could be due to the fact that

such an effect might not show until later than 30 minutes after administration. Considering, however, the high doses, one would expect that if the substances were to have any effect on mucociliary activity, such indications would appear at least towards the end of the test period. It is also possible that the tested pharmaca do not have any effect on normal mucous membrane and normal mucus, alternatively that their mucolytic effect on mucus is such that it does not result in a change of mucociliary activity. However, it is extremely important to point out in this connection that the absence of registered mucociliary effect does not preclude possible effects on mucociliary transportation and clearance. No conclusions regarding these two parameters can thus be drawn from the present study. A positive correlation between ciliary beat frequency (mucociliary wave movement frequency) and the rate of mucus transportation has been indicated by Blair and Woods (1969), but this correlation has not been regarded as significant (Andersen, 1971).

It has previously been shown that both salbutamol and isoprenaline have an accelerating effect on mucociliary activity in rabbits (Hybbinette and Mercke, 1982b). It may, therefore, seem surprising that NAB 365, which is a selective $\beta_2$-adrenoceptor agonist, has not had any mucociliary effect in the experiments now carried out. Different ways of administering the test substances have, however, been used in the two trials, which are thus not comparable. NAB 365 is furthermore different from both isoprenaline and salbutamol (Engelhardt, 1976), qualitatively and quantitatively, as far as the pharmacological action profile is concerned.

Kopitar et al. (1973) have shown rabbit to be the animal that most closely resembles humans regarding bromhexine clearance and metabolism. The results obtained, using the trial model described above and where the test substances, among others bromhexine, were administered parenterally as recommended, would therefore most likely be almost equivalent to the mucociliary effects expected from clinical use of these substances. Thus, no change in the mucociliary activity can be expected during the first 30 minutes after administration. However, the effect of these substances on mucociliary transportation and clearance still remains to be elucidated. Regarding NA 872 recently published facts seem to speak in favour of a positive effect on mucociliary clearance. Thus, in some very interesting reports NA 872 has been shown to stimulate the production of phosphatidylcholine, a potent surface active phospholipid in lung surfactant (Kapanci and Elemer, 1983; van Golde et al., 1983). Surfactant, which is produced in type I and II cells and probably also in Clara cells, has been found in the mucus layer right up to the bronchi (Morgenroth and Bolz, 1983). Localized to the sol layer and/or the interface between the gel and sol layer surfactant has been ascribed a "lubricating" effect either directly by facilitating the movements of the ciliae or indirectly by reducing existing adhesive forces (Reifenrath, 1983). In the present

investigation it has been shown that the mucociliary wave frequency does not change after administration of NA 872 which would support the theory that surfactant first of all reduces the adhesive forces. This in turn will facilitate the removal of mucus and help to maintain an effective mucociliary clearance (von Seefeld et al., 1983; von Seefeld et al., in press).

ZUSAMMENFASSUNG

Die Wirkung von drei mukolytischen Substanzen, Bromhexin, NA 872 (Bromhexinmetabolit VIII) und $\beta_2$-Rezeptoragonist NAB 365 (Clenbuterol), auf die mukoziliäre Aktivität in vivo wurde im Tierversuch am Kaninchen getestet. Die Wirkstoffe wurden parenteral in steigender Dosierung verabreicht. Insgesamt wurden 519 Registrierungen an 12 Kaninchen durchgeführt. Keines der Präparate zeigte einen Einfluss auf die mukoziliäre Aktivität, nicht einmal bei Applikation der mehrfachen Standarddosis. Dieser Ergebnis schliesst aber nicht aus, dass die Substanzen Einfluss auf den mukoziliären Transport oder die Clearance haben.

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U. Mercke, M.D.
Department of Oto-Rhino-Laryngology
University Hospital of Lund
S-221 85 Lund, Sweden