

The effect of bromhexine hydrochloride on the viscoelastic properties of mucus from the mini-pig

G.P. Martin, B.E. Loveday*, C. Marriott

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ABSTRACT: Mucus was collected daily from open-ended pouches established surgically in three mini-pigs. After a five day control period bromhexine hydrochloride (BHCl) was administered to each pig at dose levels of 0.5, 1.0 and 2.0 mg·kg⁻¹ twice daily for five days. Each study period was followed by a five day washout period, when mucus was collected but no drug given. The viscoelastic properties of each mucus sample were determined using creep compliance analysis. BHCl was shown to reduce the residual shear viscosity ($p < 0.05$) and increase the instantaneous shear compliance at all dose levels ($p < 0.005$), despite the large inherent intra- and inter-animal variation in the rheological properties of the daily samples. No change was found in the wet weight of the mucus samples throughout any of the study periods. This experimental model would appear to provide a valuable *in vivo* method of assessing the mucoregulatory potential of administered compounds.

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Pharmaceutical Sciences Research Group, Dept of Pharmacy, Brighton Polytechnic, Brighton, UK.

* ICI Pharmaceuticals, Macclesfield, UK.

Correspondence: Dr G.P. Martin, Pharmaceutical Sciences Research Group, Dept of Pharmacy, Brighton Polytechnic, Brighton, UK.

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Bromhexine hydrochloride (BHCl) is classified as an expectorant and mucolytic compound and, as such, has been reported to change the structure of bronchial secretions, increase the volume and reduce the viscosity of sputum [1]. A review of the scientific literature relating to the action of this drug suggests, however, that these claims may best be described as equivocal. For example, whereas some animal studies have shown that bromhexine increases production of respiratory tract fluid [2-4], a more recent study in the mini-pig showed a distinct reduction in the quantity of mucus produced when bromhexine was administered orally for 12 days [5]. Rheological studies have indicated that although bromhexine has little direct effect on the viscosity of sputum *in vitro* [6], it has been suggested that it may reduce the viscosity *in vivo* by "dissolving acid mucopolysaccharide fibres" [7]. Many clinical trials have been performed to assess the efficacy of bromhexine in chronic obstructive airways disease and despite the general observations of an increased sputum volume in patients [8, 9] and an improvement in symptoms such as cough and ease of expectoration [8, 10], most have been unable to show significant improvements in lung function.

The present study was devised with the aim of determining quantitatively any effect that BHCl might have on the viscoelastic properties of mucus *in vivo*. The method involved the surgical preparation of two-ended tracheal pouches in mini-pigs. Once post-operational recovery was achieved, BHCl was administered orally

and the mucus, collected from the conscious animals on a daily basis, was subjected to accurate rheological testing involving creep compliance analysis.

Methods

Surgical preparation of tracheal pouch

Three adult pure-bred mini-pigs (*Suis scrofa domestica*, Gottingen strain), weighing 25-35 kg, were anaesthetized with i.m. administered Immobilon (0.02 ml·kg⁻¹; Reckitt and Colman, Pharmaceutical Division, Hull, UK) and surgically prepared under aseptic conditions. For 24 h before and 3 days after surgery, the animals were administered 4 ml i.m. Streptopen (Glaxo Vet. Ltd, Greenford, Middlesex, UK).

The cervical trachea was exposed by a midline incision and the surrounding tissue was carefully dissected from the cartilage at two points; the first usually at the junction between the third and fourth cartilage rings below the cricoid cartilage, and the second an additional five tracheal rings caudally. The recurrent laryngeal nerves, which arise from the vagus nerve and pass cranially along the dorsolateral aspect of the trachea, were carefully separated from the airway, only at the points of dissection. The trachea was transected at these points, providing an isolated segment of five cartilage rings (2-3.5 cm) with its sheath of surrounding connective tissue and

recurrent laryngeal nerves intact. The segment was then moved gently laterally and the transected airway anastomosed end-to-end, stitching with braided polyester suture. Silastic discs reinforced with Teflon and including attached catheters were stitched to each end of the isolated segment [5].

After preparation of the pouch was complete, each catheter was exteriorized on the back of the neck of the animal. Generally the mini-pigs regained consciousness in one hour and the neck sutures were removed after seven days. Daily flushing of the pouches with sterile saline was started seven days after the operation, but at least a further two weeks was allowed to elapse before any trial was commenced.

Administration of bromhexine hydrochloride

Mucus, secreted within the pouches, was collected by flushing gently with 5 ml sterile isotonic saline. The first five days of any fifteen day study period served as a control period. BHCl (Boehringer Ingelheim Ltd, Bracknell, Berkshire, UK) was then given orally, mixed with the normal diet, twice daily for the next five days, at a dose of 0.5, 1.0 or 2.0 mg·kg⁻¹. Mucus was collected for a further five days, after withdrawal of the drug, and this was designated a washout period. Each dose of BHCl was administered randomly to each of three mini-pigs.

Collected mucus was immediately frozen and stored at -20°C until required for rheological investigation. After thawing, the mucus gel was separated from the saline supernatant by low speed centrifugation. The mucus samples were weighed and viscoelastic parameters determined using creep compliance analysis.

Creep compliance analysis

Mucus samples were placed between the cone and plate of a variable stress rheometer (Petronics Viscoelastic Analyser, Integrated Petronics, London). The samples were maintained at 25°C and a relative humidity of 100% for ten minutes, without application of torque, to allow the relaxation of stresses induced by the loading procedure. Maintaining the same environmental conditions, a small, known, constant instantaneous stress was applied to the material and the resultant strain measured by following the angular displacement of the cone [11]. Strain could be converted to compliance by dividing by the applied stress and the resultant curve analysed to produce a residual shear viscosity (η_0) and an instantaneous shear compliance (J_0) [11, 12]: the latter term being the reciprocal of elasticity.

Data representation and analysis

Four day running means of J_0 , η_0 and wet weight were calculated, throughout each fifteen day study period. The data were normalized by finding the highest value in each data set (*i.e.* a period which consisted of

45 study days per pig), expressing this as 100% and all other data points in that set as a percentage of this.

Changes in J_0 , η_0 and wet weight over each fifteen day study period were also expressed as a percentage of the mean values obtained on days 1–5. Percentage changes in these parameters on control (days 2–5), drug (days 7–10) and washout days (days 12–15) were compared for statistical difference using the two-tailed Mann-Whitney U-test [13].

Results

The administration of BHCl at all of the three dosage levels examined reduced the viscosity of secreted mucus (fig. 1). This effect was found to commence 24 h after the first dose of BHCl was administered, when the mucus which had accumulated in the intervening time was collected and subsequently subjected to rheological analysis. Concomitant with a reduction in viscosity was a decrease in elasticity of the mucus, as shown by an increase in J_0 (fig. 2). The changes in viscosity and compliance between mucus collected on days when drug was given compared with control days were significant ($p < 0.05$) for all doses of BHCl. When BHCl was withdrawn the values for both rheological parameters returned to control levels, there being no significant difference in J_0 and η_0 of mucus collected during control and washout periods.

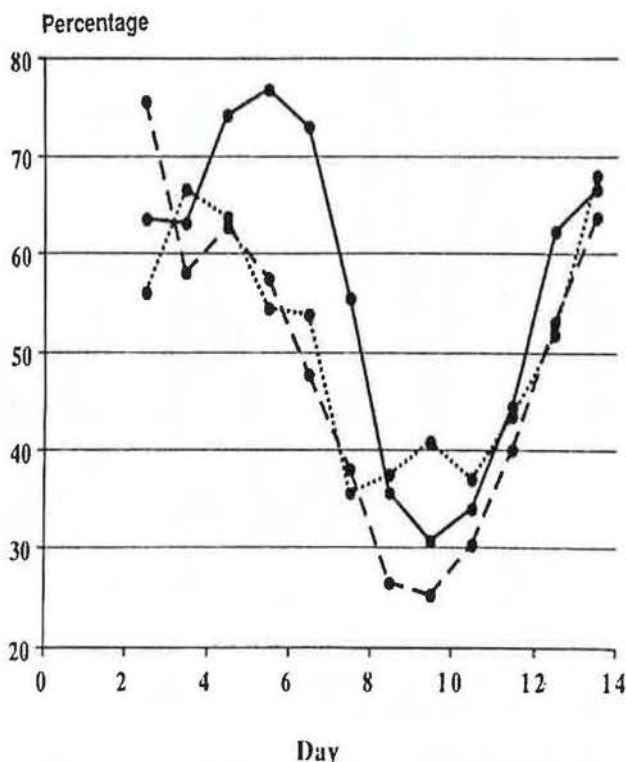


Fig. 1. — Mean percentage change in four day running means of residual shear viscosity (η_0) (normalized to highest value). —●—: 0.5 mg·kg⁻¹ BHCl;●.....: 1.0 mg·kg⁻¹ BHCl; ---●---: 2.0 mg·kg⁻¹ BHCl.

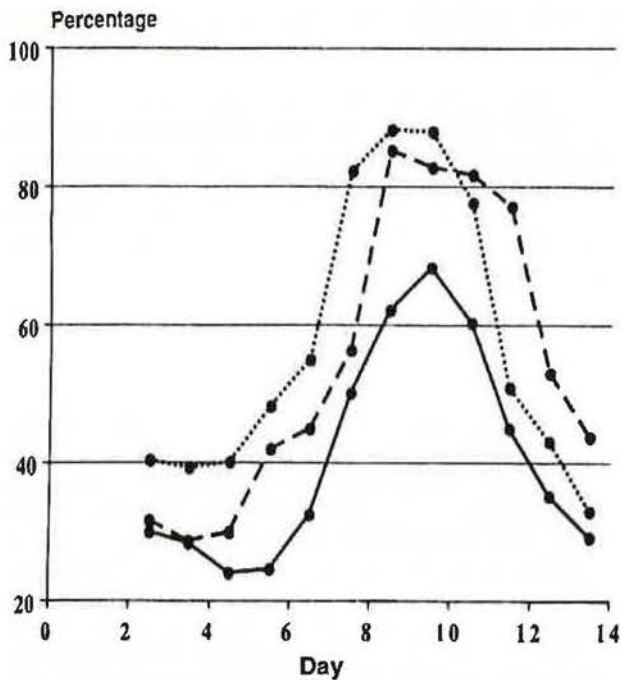


Fig. 2. - Mean percentage change in four day running means of instantaneous shear compliance (J_0) (normalized to highest value). —●— : 0.5 mg·kg⁻¹ BHCl;●..... : 0.5 mg·kg⁻¹ BHCl; ---●--- : 2.0 mg·kg⁻¹ BHCl.

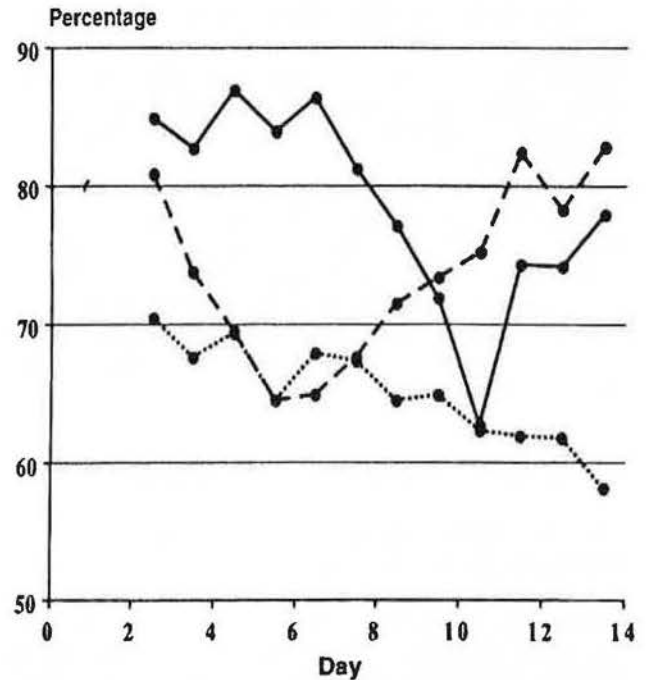


Fig. 3. - Mean percentage change in four day running means of wet weight (normalized to highest value). —●— : 0.5 mg·kg⁻¹ BHCl;●..... : 0.5 mg·kg⁻¹ BHCl; ---●--- : 2.0 mg·kg⁻¹ BHCl.

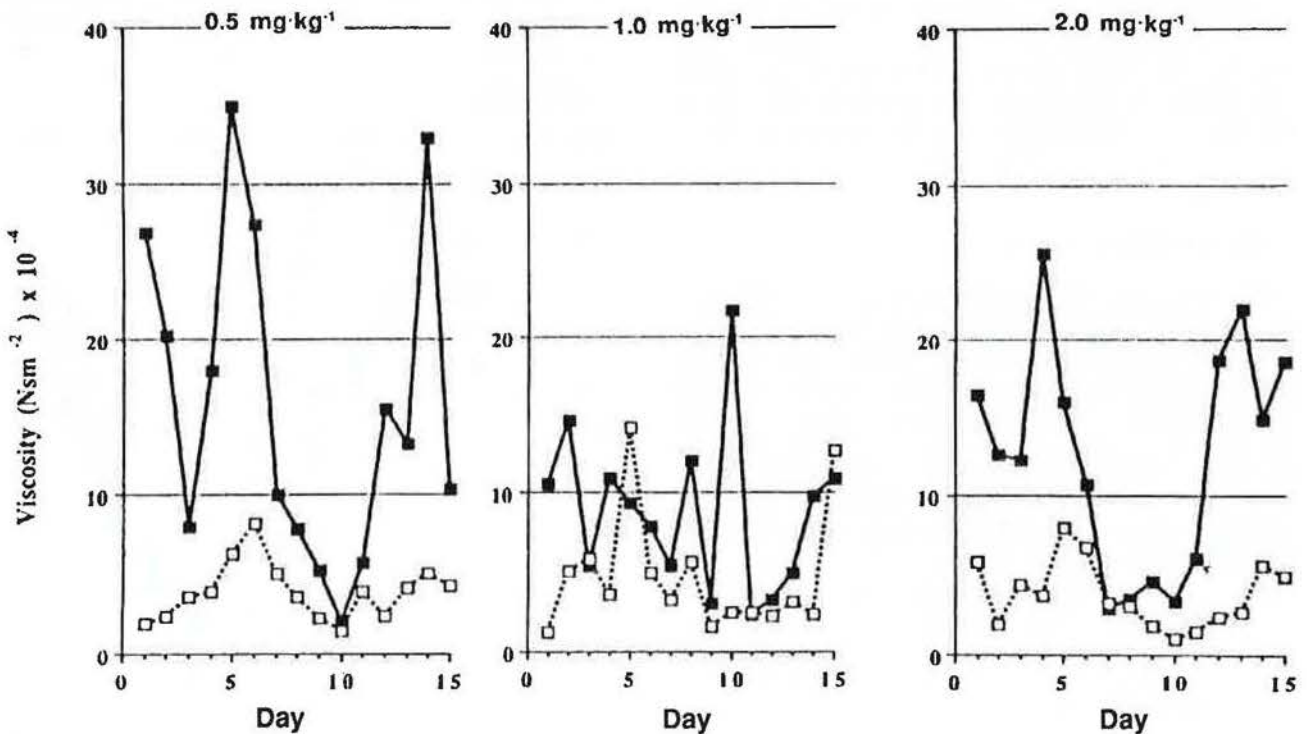


Fig. 4. Determined values for the daily residual shear viscosity (η_0) for two individual mini-pigs (—■—,□.....); BHCl (0.5; 1.0 or 2.0 mg·kg⁻¹) was administered on days 6-10.

Although there was a tendency towards a dose-response relationship for J_0 and η_0 (figs 1 and 2), differences between doses were not significant. The effect of BHCl on the wet weight of mucus collected from the pouch is shown in figure 3.

No clear trend was apparent and there was no significant difference ($p > 0.05$) in the wet weight of the daily samples collected throughout the study periods at any of the dosage levels of BHCl employed.

Discussion

Many previous studies which have aimed to investigate the mucoregulatory activity of compounds by using rheological techniques have failed because of inappropriate methodology. Firstly, since mucus is rheologically extremely complex, an appropriate rheometer [14] which enables the simultaneous determination of elastic and viscous parameters must be employed. Some putative mucolytic drugs affect elasticity, for example, without changing viscosity [6]. Secondly, inherent large intra- and inter-subject variation in rheological properties of mucus secretions occur routinely on a daily basis, even without any drug intervention. The daily rheological parameters of mucus collected from a single tracheal pouch in this study also fluctuated considerably. The crude values for η_0 for mucus samples collected from two pigs throughout each 15 day study period is shown in figure 4. Since the drug was first administered on day 6, immediately after collection of the daily mucus sample, and continued to day 10, any effects on mucus would be expected between days 7 and 11. The values of η_0 varied within control and washout periods by a factor of four, as did values of J_0 . Although careful examination of the data does show a general trend towards the viscosity being lower (during most drug treatment periods) than in control and washout periods, the use of four day running means to represent the data enables any true underlying trend to become apparent (fig. 1). Inter-pig variation in rheological properties was even greater than intra-pig variation, η_0 values (and J_0 values) in control and washout periods differing by up to ten times (fig. 4). Accordingly, the data was normalized as described above, before taking the mean values of the three animals. Despite this manipulation to obtain a clarified representation of the data, so that any trends can be visualized more readily (figs 1-3), statistical analysis was carried out using the original data. The inherent intra- and inter-variation in rheological properties of mucus indicated in figure 4 demonstrates the difficulty in determining mucolytic activity.

Some previous studies have depended upon determination of the rheological characteristics of mucus secretions prior to administration and then re-examined the characteristics after treatment, usually on only one occasion [15]. In effect, only a two point determination is carried out in such studies and the large inherent variation in the flow properties of mucus is likely to mask any induced changes. It is only by administering the drug over a prolonged period of time to a number of animals and monitoring the rheological properties on a daily basis, as in this study, that any trends will become apparent.

The intra- and inter-animal variation in the rheological properties of secreted mucus, as manifested by this investigation, occurred under relatively controlled environmental conditions. Similar variations occur in patients, especially those with bronchial disease [16]. Tracheobronchial infection, different ambient environments and the coadministration of other drugs [17] are

all factors which are likely to exacerbate rheological variations.

The oral administration of BHCl at all of the three dosage levels examined, was shown to significantly decrease ($p < 0.05$) both the elasticity and viscosity of secreted mucus. The mode of action of the drug cannot be defined from this study. It has been shown in a number of studies that BHCl has no *in vitro* mucolytic activity [6, 15, 18]. Although a distinct reduction in the quantity of mucus produced by a tracheal pouch after BHCl administration ($0.4 \text{ mg} \cdot \text{kg}^{-1}$ per day) for 12 days has been reported [5], no significant change was detected in this study. It is likely that the effects found on the rheological properties of mucus are attributable to the drug affecting synthesis and/or release of the glycoprotein fraction. One problem with the use of tracheal pouches is the necessity to use saline to remove the native secreted mucus. This process requires the subsequent separation of the saline and mucus employing low speed centrifugation, which may affect the rheological properties of the mucus sample. Recent work has suggested, however, that BHCl may exert a similar activity on mucus within the cervix, allowing the transmission of bacteria from vagina to uterus *via* a compromised cervical mucus plug [19]. In the latter study, no isolation of *in situ* secreted mucus was necessary. This observation also suggested that the effects of BHCl may not be limited to action upon respiratory tract secretions.

The experimental model consisting of a surgically prepared tracheal pouch in the mini-pig, combined with viscoelastic measurement of the rheological properties of mucus secreted therein, provides a valuable and powerful *in vivo* method of assessing mucoregulatory properties of administered compounds. Candidate drugs, however, have to be administered for prolonged periods in a number of animals and the rheological properties determined on a daily basis, because of the large inherent intra- and inter-animal variation.

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Les effets de l'hydrochloride de bromhexine sur les propriétés visco-élastiques du mucus du mini-pig. G.P. Martin, B.E. Loveday, C. Marriott.

RÉSUMÉ: L'on a prélevé quotidiennement du mucus dans des trachéales à terminaison ouverte, créées chirurgicalement, chez 3 "mini-pigs". Après une période de contrôle de 5 jours, l'hydrochloride de bromhexine (BHCl) a été administré à chaque cochon aux doses de 0.5, 1.0 et 2.0 mg·kg⁻¹ deux fois par jour pendant cinq jours. Chaque période d'étude était suivie d'une période de washout de cinq jours pendant laquelle le mucus a été prélevé, mais aucune drogue n'a été administrée. Les propriétés visco-élastiques de chaque échantillon de mucus ont été déterminées par analyse de la compliance au glissement. BHCl réduit la viscosité résiduelle de cisaillement ($p < 0.05$) et augmente la compliance instantanée de cisaillement à toutes les doses ($p < 0.005$), malgré les grandes variations inhérentes, intra- et inter- animaux dans les propriétés rhéologiques des échantillons de mucus collectés quotidiennement. Aucune modification n'a été trouvée dans le poids sec des échantillons de mucus pendant toute la période d'étude. Ce modèle expérimental pourrait fournir une méthode *in vivo* valable pour l'appréciation du potentiel muco-régulateur des composants administrés. *Eur Respir J.*, 1990, 3, 392-396.