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# Ambroxol Improves the Broncho-spasmolytic Activity of Clenbuterol in the Guinea-Pig

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## Abstract

The effects of ambroxol on the spasmolytic action of clenbuterol were investigated on acetylcholine-induced bronchospasm in guinea-pigs.

Ambroxol (50 mg kg$^{-1}$ day$^{-1}$) or vehicle was administered orally for 14 days. Approximately 45 min after the final dose on day 14, the animals were anaesthetized and the spasmolytic effects of clenbuterol (3, 6 or 12 μg kg$^{-1}$ injected intravenously) were determined by use of acetylcholine (40 μg kg$^{-1}$, i.v.)-induced bronchoconstriction. For both vehicle- and ambroxol-treated animals, a positive linear relationship was observed between the log-dose of clenbuterol and the percent inhibition of bronchospasm. The calculated ED25 of clenbuterol (i.e. the dose producing 25% inhibition of the acetylcholine-induced bronchospasm) was 3·98 μg kg$^{-1}$ (3·29 to 4·82 μg kg$^{-1}$, 95% confidence interval) in the presence of ambroxol and 5·81 μg kg$^{-1}$ (4·98 to 6·79 μg kg$^{-1}$) in the absence of ambroxol. The linear regressions with or without ambroxol differed from each other ($P < 0·001$) but ran parallel (covariance analysis), enabling us to calculate a relative potency, the value of which was 1·46 (1·16 to 1·84).

These results demonstrate that the spasmolytic activity of clenbuterol is significantly improved in animals pretreated with ambroxol.

In the treatment of respiratory diseases where bronchoconstriction is associated with impaired mucus clearance (for example spastic bronchitis, bronchitic emphysema or bronchial asthma), bronchodilators such as $\beta_2$-adrenoceptor agonists are often used in conjunction with mucoregulators (expectorants, secretolytics or mucolytics) such as ambroxol, bromhexine or *N*-acetylcysteine. It is unknown whether mucoregulators have only complementary effects or if they can improve the spasmolytic activity of the $\beta_2$-agonists.

Ambroxol is often co-prescribed with spasmolytic agents. A fixed combination with the $\beta_2$-adrenoceptor agonist clenbuterol (Engelhardt 1976) is even commercially available (Spasmo-Mucosolvan).

In addition to its effects on mucus production and clearance (Püschmann & Engelhorn 1978), ambroxol has other pharmacodynamic properties including phospholipase $A_2$ inhibition (Heath & Jacobson 1985), antioxidant activity (Winsel 1992) and a 'carrier function' to the lung, i.e. it increases the bronchopulmonary concentrations of antibiotics (Wiemeyer 1981; Fraschini et al 1987; Gené et al 1987). Because activation of phospholipase $A_2$ (Taki et al 1986; Nijkamp 1993) and oxygen free-radical generation (Kramer et al 1987; Nijkamp 1993) reduce the number of $\beta$-adrenoceptor binding sites in the lung, ambroxol could facilitate the spasmolytic activity of clenbuterol by a sparing action on the $\beta_2$-receptor population. As a result of its carrier function ambroxol might, furthermore, also facilitate the distribution of clenbuterol to the lung. The aim of this study was, therefore, to test whether ambroxol affects the spasmolytic action of clenbuterol in a model of acetylcholine-induced bronchospasm in the guinea-pig.

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## Materials and Methods

### *Animals*

Hartley male guinea-pigs, approximately 500 g at the beginning of the study, were used. They were housed individually on sawdust bedding. Standard laboratory diet and drinking water were freely available.

### *Drugs*

Clenbuterol (N-AB 365 Cl, batch 833032) and ambroxol (N-A 872 Cl, batch 630467) were supplied by Dr Karl Thomae GmbH (Biberach an der Riss, D-88397 Germany). Acetylcholine chloride (reference A6625, batch 65F-0357) was purchased from Sigma (France). Clenbuterol and acetylcholine were administered intravenously in isotonic saline solution (0·9% NaCl). Ambroxol was administered in a suspension with 0·5% aqueous hydroxyethylcellulose solution (Natrosol 250 MX, Aquahloh, Düsseldorf, Germany).

### *Spasmolytic activity of clenbuterol*

Animals were randomly allocated to eight different study groups of 20 animals each; homogeneity of groups was validated by the criterion of body weight on the day of the study. Four groups were pretreated with ambroxol 50 mg kg$^{-1}$ day$^{-1}$ orally for 14 days while the other four groups received the vehicle (hydroxyethylcellulose). Ambroxol and its vehicle were administered via an oesophageal tube in a volume of 1 mL kg$^{-1}$. Animals were then treated with either the clen-

buterol vehicle (0·9% NaCl) or with clenbuterol in doses of 3, 6 or 12 $\mu g\,kg^{-1}$ intravenously (i.v.), administered in a volume of 1 $mL\,kg^{-1}$, approximately 45 min after the last ambroxol or vehicle treatment on day 14.

Animals were placed on a water-only fast the day before the final experiment. On the study day (day 14), animals were anaesthetized with sodium pentobarbital (35 $mg\,kg^{-1}$, i.p., in a volume of 1 $mL\,kg^{-1}$). The trachea and a jugular vein were cannulated and the animals were artificially ventilated with an air flow of approximately 1 mL/100 g at 54 cycles $min^{-1}$. Total pulmonary resistance was measured continuously using a Statham P23 Xl pressure transducer fitted to a side-branch of the tracheal cannula. The temperature of the animal was kept between 37 and 38·5°C. Maximum pulmonary resistance was measured at a time when the air out-flow of the ventilation circuit was clamped for approximately 4 to 8 cycles. Ventilatory flow was then adjusted to obtain a baseline pulmonary resistance between 5 and 10% of the measured maximum pulmonary resistance. Bronchial response to acetylcholine (40 $\mu g\,kg^{-1}$, i.v.) was measured at 5-min intervals until the response stabilized. Clenbuterol or vehicle was administered intravenously 5 min after reference acetylcholine-induced bronchoconstriction was obtained (and approximately 45 min after the last oral administration of ambroxol or vehicle). A further injection of 40 $\mu g\,kg^{-1}$ acetylcholine was given 5 min after the administration of clenbuterol or vehicle. The spasmolytic effects of clenbuterol were expressed as percent inhibition of the reference bronchoconstriction induced by acetylcholine.

Data were expressed as mean ± standard error (s.e.). Effects of pretreatment with ambroxol on the spasmolytic effects of clenbuterol were assessed using covariance analysis. ED25 (the dose producing 25% inhibition of the acetylcholine-induced bronchospasm) and relative potency, i.e. the ratio ED25 without ambroxol/ED25 with ambroxol, was calculated from regression curves.

## Results

In the vehicle-pretreated groups, acetylcholine-induced bronchospasm was reduced by 13·3 ± 1·8 (mean ± s.e.), 22·5 ± 2·6 and 41·1 ± 3·2% by 3, 6 and 12 $\mu g\,kg^{-1}$ clenbuterol, respectively. In groups pretreated with ambroxol, bronchoconstriction was reduced by 18·8 ± 2·5, 32·7 ± 2·1 and 49·5 ± 4·1% by the same doses of clenbuterol. The ED25 of clenbuterol was 3·98 $\mu g\,kg^{-1}$ (3·29 to 4·82 $\mu g\,kg^{-1}$, 95% confidence interval) in the presence of ambroxol and 5·81 $\mu g\,kg^{-1}$ (4·98 to 6·79 $\mu g\,kg^{-1}$) in the absence of the compound. For both groups, a positive linear regression could be demonstrated between the logarithm of the dose of clenbuterol and the percentage inhibition of the bronchospasm. The linear regressions with or without ambroxol (Fig. 1) differed from each other ($P < 0·001$) but ran parallel (covariance analysis), enabling us to calculate a 'relative potency'. The relative potency, i.e. the ratio ED25 without ambroxol/ED25 with ambroxol, was 1·46 (1·16 to 1·84, Table 1).

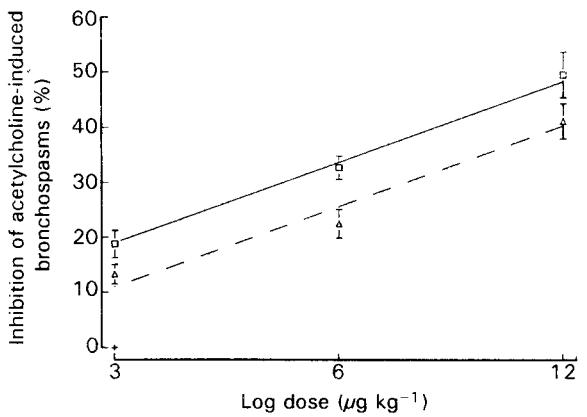


FIG. 1. Linear regression plots of the spasmolytic effects of clenbuterol in animals pretreated for 14 days with either ambroxol (50 $mg\,kg^{-1}\,day^{-1}$, p.o., □) or vehicle (0·5% hydroxyethylcellulose, 1 $mL\,kg^{-1}\,day^{-1}$, p.o., △). Data are presented as means ± s.e., n=20 per group.

## Discussion

By use of the model of acetylcholine-induced bronchospasm in the guinea-pig, we have demonstrated that ambroxol significantly improves the spasmolytic activity of the $\beta_2$-adrenoceptor agonist clenbuterol. The mechanism of action of this effect is not yet known.

Ambroxol has previously been shown to inhibit phospholipase $A_2$ ($PLA_2$) and activation of $PLA_2$ is known to reduce the number of $\beta$-adrenoceptor binding sites in the lungs (Taki et al 1986). $PLA_2$ is strongly activated during inflammatory and allergic reactions (Loesberg et al 1989) and during chronic $\beta$-adrenoceptor stimulation (Suzuki et al 1987). Inhibitors of $PLA_2$ activity, such as mepacrine and tetracaine, have been shown to block the development of isoprenaline-induced desensitization of cAMP production and the decrease in the number of $\beta$-adrenoceptors (Mallorga et al 1990). One might postulate that ambroxol, as a result of its inhibitory effects on $PLA_2$ (Heath & Jacobson 1985), could protect the $\beta$-adrenoceptor population of the lungs and therefore improve the spasmolytic activity of $\beta_2$-agonists. One might compare such a mechanism with the effect of anti-inflammatory steroids which also up-regulate the $\beta$-adrenoceptor population (Nijkamp 1993), most likely by inhibition of $PLA_2$ activity (Blackwell et al 1978). This mechanism could be of particular relevance during pathological conditions when the lungs are inflamed and $\beta_2$-adrenoceptor agonists are administered chronically leading to a 'desensitization' (Verberne & Kerrebijn 1993). It might also play a role in our experimental conditions for it has been shown that lung tissues have a basal $PLA_2$ activity which can be further stimulated by the trauma occurring during the surgical procedure (Blackwell et al 1978).

Ambroxol also has antioxidant properties (Winsel 1992). $\beta$-Adrenoceptor density is decreased both in-vitro and in-vivo in the lungs of rats after incubation with oxygen-radical-generating systems (Kramer et al 1986) and the response of rat airway smooth muscle to $\beta_2$-agonists is reduced after incubation with hydrogen peroxide (Kramer et al 1986). An inverse relationship between the amount of superoxide production by alveolar macrophages and $\beta$-adrenoceptor function has, moreover, been demonstrated in the guinea-pig trachea (Loesberg et al 1989). As a result of its antioxidant activity, ambroxol might have a sparing effect on the $\beta_2$-adrenoceptor population which might lead to improved activity of $\beta_2$-agonists.

Table 1. Calculated ED25 and potency coefficient of clenbuterol in animals pretreated for 14 days with ambroxol (50 mg kg$^{-1}$ day$^{-1}$ p.o.) or vehicle (0·5% hydroxyethylcellulose, 1 mL kg$^{-1}$ day$^{-1}$ p.o.).

| | Calculated | 95% Confidence interval |
|---|---|---|
| Dose (μg kg$^{-1}$) producing 25% inhibition of acetylcholine (40 μg kg$^{-1}$ i.v.)-induced bronchospasm with ambroxol | 3·98 | 3·29–4·82 |
| Dose (μg kg$^{-1}$) producing 25% inhibition of acetylcholine (40 μg kg$^{-1}$ i.v.)-induced bronchospasm without ambroxol | 5·81 | 4·98–6·79 |
| Relative potency | 1·46 | 1·16–1·84 |

Relative potency is the ratio ED25 without ambroxol/ED25 with ambroxol.

Ambroxol also differs from other mucoregulators in its pharmacokinetic profile. Selective distribution to the lung has been demonstrated, the drug reaches the pulmonary tissue in concentrations that are at least 16 times higher than blood levels. This ratio between ambroxol concentrations in pulmonary parenchyma and in blood is maintained for up to 12 h after the end of drug administration (Mezzetti et al 1990). Although the mechanism of this pulmonary tropism is unknown, the drug might preferentially distribute to lung tissue because of its chemical characteristics (amphiphilic structure) or by binding to a pulmonary constituent.

It is not known whether this specific distribution to the lung also explains a carrier function of the compound. Ambroxol has been shown to increase the bronchopulmonary concentrations of various antibiotics such as ampicillin, erythromycin, amoxycillin and cefuroxime (Wiemeyer 1981; Fraschini et al 1987; Gené et al 1987). One might postulate that as a result of a 'carrier' function, ambroxol can also facilitate the distribution of clenbuterol to the lungs and consequently improve its spasmolytic activity.

In conclusion, ambroxol improves the spasmolytic activity of clenbuterol on acetylcholine-induced bronchospasm in the guinea-pig. This effect is of particular relevance in pathological conditions where the response to β-adrenoceptor stimulation is impaired by inflammatory processes or chronic administration of β-adrenoreceptor agonists, or both.

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