ORIGINAL ARTICLE

Guaifenesin enhances the analgesic potency of ibuprofen, nimesulide and celecoxib in mice

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Abstract

OBJECTIVES: Previously, we found that guaifenesin enhances analgesia induced by paracetamol. The aim of the present study was to determine whether guaifenesin is able to also increase analgesic activity in the non-steroid anti-inflammatory drugs ibuprofen, nimesulide and celecoxib. In addition we investigated the influence of guaifenesin on plasma levels of nimesulide.

METHODS: A model of visceral pain consisting of intraperitoneal injection of acetic acid (writhing test) was used. Levels of nimesulide in plasma were measured by HPLC. All drugs were given orally and tested in mice.

RESULTS: Guaifenesin alone did not produce any antinociceptive effect. Simultaneous administration of guaifenesin (200 mg/kg) and subanalgesic doses of ibuprofen (10 and 30 mg/kg), nimesulide (10 and 20 mg/kg) or celecoxib (1 and 5 mg/kg) resulted in a significant antinociceptive effects. The plasma levels of nimesulide were significantly higher in combination with guaifenesin at 30, 60 and 90 min after oral administration in comparison to nimesulide monotherapy.

CONCLUSION: The present results suggest that guaifenesin might enhance the analgesic activity of various non-steroidal anti-inflammatory drugs.

1. INTRODUCTION

Guaifenesin has been used worldwide as an expectorant in numerous non-prescription over-the-counter (OTC) preparations for many years (Dicpinigaitis & Gayle, 2003; Rubin, 2007; Smith *et al.,* 2008). It also has muscle relaxant and sedative properties (Carter, 1966; Gorski & Kuchler, 1971; Maier, 1980b; Haga *et al.,* 2000). Guaifenesin is also used in a veterinary anesthesiology as well (Matthews *et al.,* 1997; Taylor *et al.,* 2008).

Guaifenesin has been found to significantly enhance the analgesic activity of paracetamol in a model of visceral pain consisting of intraperitoneal injection of acetic acid (writhing test) in mice (Dolezal & Krsiak, 2002; Krsiak & Tomasikova, 1979; Kršiak *et al.,* 1980a). A fixed combination of guaifenesin with paracetamol and caffeine (Ataralgin) has been extensively used as an OTC analgesic in the Czech and Slovak Republics since 1981, and it is still widely used.

We wondered whether guaifenesin is able to increase analgesic activity also in the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen, nimesulide and celecoxib. These three NSAIDs differ in their effects on cyclooxygenase-1 (COX-1)

and cyclooxygenase-2 (COX-2): ibuprofen is a nonselective COX-1 and COX-2 inhibitor while nimesulide is COX-2 preferential and celecoxib is a COX-2 selective inhibitor. They also have different adverse effects profiles.

Guaifenesin was reported to increase absorption of paracetamol in a clinical pharmacokinetic study (Perlik *et al.,* 1988). Therefore, we also investigated the influence of guaifenesin on plasma levels of nimesulide.

2. MATERIALS AND METHODS

2.1. Animals

Male NMRI mice weighing between 24 and 30 g bred in VUFB Konarovice, Czech Republic, were used in all experiments. The animals were housed under standard laboratory conditions (12 hrs light/12 hrs dark), and had a free access to food and water. Food was withheld for 24 hrs prior to starting the experiment, although, free access to water was continued. The animals were adapted to the laboratory environment for at least 1 h before being used; all relevant ethical standards and guidelines were carefully followed. The duration of the experiments was as short as possible, the number of animals involved in each experimental group was kept to a minimum, and the animals were sacrificed, immediately after the recording period had finished, with an anesthetic overdose. All experiments were carried out during the light phase.

The experimental protocol for each procedure was approved by the Committee for protection of laboratory animals of the 3rd Faculty of Medicine, Charles University.

2.2. Acetic acid writhing

Mice were randomly assigned to treatment groups (9 animals per each experimental group) and administered the vehicle or the evaluated substance. The test of abdominal writhing, as previously described in a variety of papers, was employed in this study (Collier *et al.,* 1968; Hendershot & Forsaith, 1959; Millan, 1994). During the test, the total number of writhes (i.e. abdominal constrictions followed by dorsiflexion and stretching of the hind limbs) in the 20 minutes following the intraperitoneal (i.p.) administration of acetic acid solution (0.7%, 0.1 ml/10 g) were counted by an observer who was blinded to the treatment protocol. Antinociception was quantified as the total number of writhes and expressed as percent inhibition for each dose using the following formula (Hiramatsu *et al.,* 2001):

$$\textit{Inhibition of writhes (\%)} = [(\textit{control responses} - \textit{test responses})/\textit{control responses}] \times 100$$

2.3. Drugs

Celecoxib was provided by Pfizer Ltd., all the others drugs used in the experiment were obtained as a gift from the Zentiva, a.s. The following drugs were used for the acetic acid writhing test: ibuprofen (10 and 30 mg/kg), nimesulide (10 and 20 mg/kg), celecoxib (1 and 5 mg/kg), guaifenesin (200 mg/kg) and saline. All the drugs were dissolved in sterile water and administered orally in a volume of 0.10 ml/10 g of body weight 30 minutes before intraperitoneal administration of the dilute acetic acid solution.

2.4. Measurement of nimesulide plasma levels

In order to investigate the mechanism by which guaifenesin might potentiate the analgesic efficacy of nonsteroidal anti-inflammatory drugs, we determined a plasma profile for nimesulide, administered either alone at dose 10 mg/kg, p.o. or in combination with guaifenesin 200 mg/kg, p.o. Measured intervals were 5, 10, 15, 20, 30, 45, 60, and 90 minutes after administration. Blood samples were obtained after mice decapitation (9 mice per each group and interval).

2.4.1. Chemicals

Acetonitrile and methanol were of HPLC gradient grade and came from Merck (Germany), ortho-phosphoric acid, 50%, p.a. for HPLC and potassium hydroxide p.a. were purchased from Fluka.

2.4.2. Chromatography equipment

The chromatographic system (Agilent 1100) consisted of a binary pump, a micro vacuum degasser, a thermostatted autosampler, a thermostatted column compartment and a diode array detector (all from Agilent, Germany). The HPLC instrument was controlled by Agilent software (Chemstation, v. 9.1). The chromatographic column was a Zorbax Eclipse XBD-C8, 5μm, 4.6 × 150 mm (Agilent) equipped with a Luna C18, 4.0 × 2 mm guard column (Phenomenex, USA).

The mobile phase consisted of acetonitrile – 10 $mmol^{-1}$ orthophosphoric acid, adjusted with KOH to pH = 7.5 (before the addition of acetonitrile), 1:1 (v:/v). The flow rate was 0.5 mL/min, the column was thermostatted at 20 °C. The detection was performed at 404 nm.

2.4.3. Standard & sample preparation

Stock solutions of nimesulide were prepared by dissolving approximately 10 mg in 25 mL of methanol. The nimesulide solutions of known concentrations were further added to drug-free plasma in volumes up to 2% of the plasma volume. All solutions were stored at −20 °C and protected from light.

A slightly modified version of the Ptacek et al. method was employed (Ptacek *et al.,* 2001). 800 μL of methanol was added to 200 μL of plasma in Eppendorf vials. The vials were vortexed for 20 s and centrifuged for 5 min at 3500 rpm. A 500 μl aliquot of the supernatant was transferred to an autosampler vial and 4 μL were injected into the HPLC system.

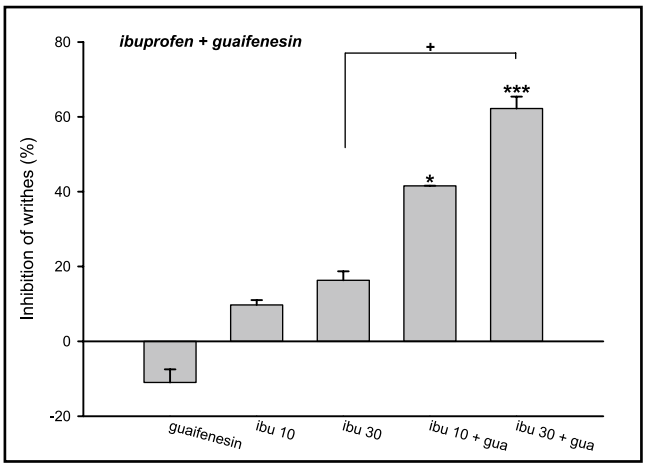

Fig. 1. Analgesic effect of ibuprofen (ibu) 10 and 30 mg/kg p. o., guaifenesin (gua) 200 mg/kg and combinations of both on acetic acid-induced writhing in mice. The test was performed 30 min after p.o. administration of drugs. A proportional inhibition from the control number of writhes (40–60 writhes during 20 min) is shown. Each column represents the mean of nine animals. Asterisks and crosses denote the significance level as compared with the control group and among groups, respectively (ANOVA followed by Bonferonni´s test);
$^{*}p < 0.05$, $^{***}p < 0.001$ between the experimental group and controls; $^{+}p < 0.05$ between experimental groups.

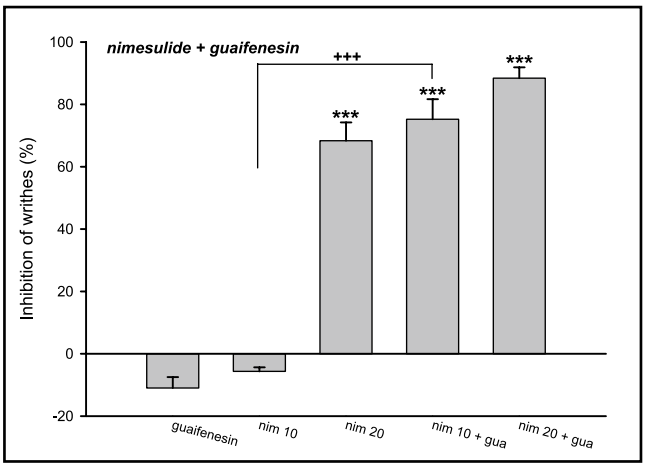

Fig. 2. Analgesic effect of nimesulide (nim) 10 and 20 mg/kg p. o., guaifenesin (gua) 200 mg/kg and combinations of both on acetic acid-induced writhing in mice. The test was performed 30 min after p.o. administration of drugs. A proportional inhibition from the control number of writhes (40–60 writhes during 20 min) is shown. Each column represents the mean of nine animals. Asterisks and crosses denote the significance level as compared with the control group and among groups, respectively (ANOVA followed by Bonferonni´s test);
$^{***}p < 0.001$ between the experimental group and controls; $^{+++}p < 0.001$ between experimental groups.

2.4.4. Calibration curves

Quantitative calculations were based on external calibration. The calibration curve was constructed in the 1–80 μg/mL range to cover the concentrations of real samples. Peak areas were used for quantification. Five standards, with different concentrations, were used for the construction of the calibration curve. The curve was linear with a correlation coefficient $r^2 = 0.999$.

Within-day and day-to-day precision expressed by relative standard deviations was better than 4%. Inaccuracy did not exceed 7%. The limit of quantitation (LOQ), based on the signal to noise ratio S/N ≥ 10, was 0.5 μg/mL.

2.5. Data analysis

Statistical analysis was carried out using a one-way ANOVA (analysis of variance) with a post-hoc Bonferroni t-test and the Student-Newman-Keuls method. All results are expressed as mean values ± SEM (standard error of mean). $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Acetic acid writhing

3.1.1. Ibuprofen (Fig. 1)

A one-way ANOVA showed a significant effect of treatment (F 10.030, $p < 0.001$) on the total number of writhes. The subsequent Bonferroni t-test showed a statistically significant decrease in the total number of writhes in mice treated with ibuprofen and guaifenesin when compared to controls (t = 3.021, $p = 0.041$ and t = 5.616, $p < 0.001$ for mice treated by guaifenesin in combination with ibuprofen 10, and 30 mg/kg, p.o., respectively). Additionally, a significant difference between ibuprofen 30 mg/kg alone and ibuprofen 30 mg/kg with guaifenesin was observed (t = 3.188, $p = 0.026$). No significant difference was found between ibuprofen 10 mg/kg alone and ibuprofen 10 mg/kg with guaifenesin (t = 2.520, $p = 0.154$). Neither guaifenesin (200 mg/kg), nor ibuprofen 10 or 30 mg/kg administered alone produced a significant analgesic effect when compared to saline.

3.1.2. Nimesulide (Fig. 2)

A one-way ANOVA showed a significant effect of treatment (F 60.477; $p < 0.001$) on the total number of writhes. Guaifenesin (200 mg/kg) alone and nimesulide (10 mg/kg) alone produced no inhibition of writhing, but a combination of the same doses of guaifenesin and nimesulide produced significant inhibition of writhing both in comparison to the control mice (t = 8.981; $p < 0.001$) and nimesulide alone (t = 9.688; $p < 0.001$). Nimesulide (20 mg/kg) alone inhibited the number of writhes in comparison with controls both when given alone (t = 8.150; $p < 0.001$) and in combination with guaifenesin (t = 10.561; $p < 0.001$).

3.1.3. Celecoxib (Fig. 3)

A one-way ANOVA showed a significant effect of treatment (F 10.689, $p < 0.001$) on the total number of writhes. Guaifenesin (200 mg/kg) alone and celecoxib (1 mg/kg) alone did not produce any significant inhibition of writhing in comparison to the control mice.

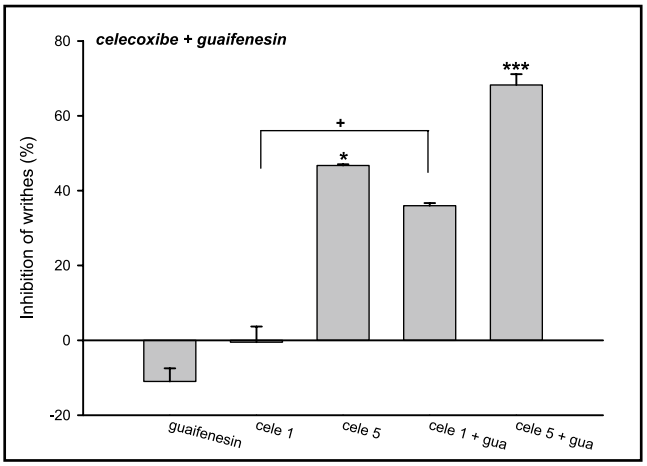

Fig. 3. Analgesic effect of celecoxib (cel) 1 and 5 mg/kg p. o., guaifenesin (gua) 200 mg/kg and combinations of both on acetic acid-induced writhing in mice. The test was performed 30 min after p.o. administration of drugs. A proportional inhibition from the control number of writhes (40–60 writhes during 20 min) is shown. Each column represents the mean of nine animals. Asterisks and crosses denote the significance level as compared with the control group and among groups, respectively (ANOVA followed by Bonferonni´s test);
*$p < 0.05$, ***$p < 0.001$ between the experimental group and controls; $^{+}p < 0.05$ between experimental groups.

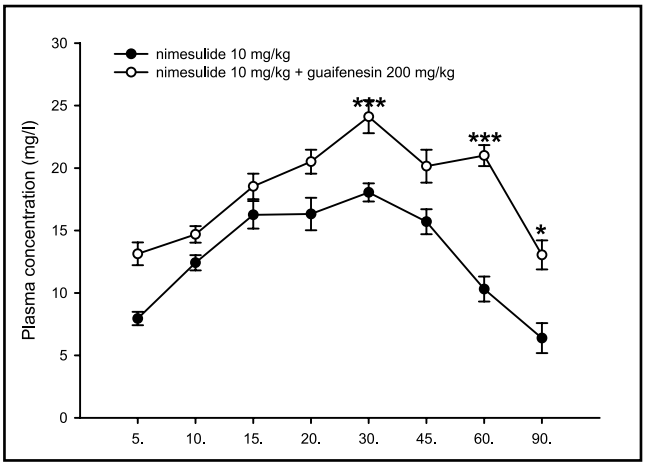

Fig. 4. Plasma levels of nimesulide 10 mg/kg and nimesulide 10 mg/kg + guaifenesin 200 mg/kg in mice. Each point represents the mean of ten animals with S.E.M. The asterisks denote the significance levels as compared among groups (ANOVA followed by Bonnferoni´s test);
*$p < 0.05$, *** $p < 0.001$.

However, celecoxib 5 mg/kg administered together with guaifenesin produced a significant difference when compared to controls (t = 4.850; $p < 0.001$). Moreover, a significant difference between celecoxib 1 mg/kg and celecoxib 1 mg/kg with guaifenesin 200 mg/kg was observed (t = 3.084, $p = 0.039$) as was a significant difference between celecoxib 5 mg/kg and controls (t = 3.317, $p < 0.021$). No other significant differences were found in this experiment.

However, when the results were subsequently evaluated by Student-Newman-Keuls method, the following significant differences were observed relative to controls: q = 3.607, $p = 0.015$; q = 4.691, $p = 0.006$ and q = 6.859, $p < 0.001$ for celecoxib 1 mg/kg + guaifenesin, celecoxib 5 mg/kg, and celecoxib 5 mg/kg + guaifenesin, respectively. There were also significant differences between celecoxib 1 mg/kg given alone and its combination with guaifenesin (q = 4.361, $p = 0.011$) and between both combinations of celecoxib and guaifenesin (q = 3.636, $p = 0.038$). No other investigated differences were revealed by this method.

3.2. Plasma levels of nimesulide (Fig. 4)

In general, plasma levels of nimesulide combined with guaifenesin were above those of nimesulide given alone: 5 minutes, 13.13 ± 0.91 mg/l vs. 7.95 ± 1.24 mg/l; 10 minutes, 14.70 ± 0.66 mg/l vs. 12.42 ± 0.61 mg/l; 15 minutes, 16.49 ± 0.87 mg/l vs. 16.43 ± 0.95 mg/l; 20 minutes, 19.75 ± 1.15 mg/l vs. 15.48 ± 1.20 mg/l; 30 minutes, 23.50 ± 1.11 mg/l vs. 15.61 ± 1.00 mg/l; 45 minutes, 19.60 ± 1.32 mg/l vs. 15.70 ± 1.01 mg/l; 60 minutes, 19.87 ± 0.89 mg/l vs. 9.79 ± 0.66 mg/l; and 90 minutes, 13.03 ± 1.16 mg/l vs. 6.38 ± 1.19 mg/l.

Several significant differences using an ANOVA with subsequent Bonferroni's t-test (F 18.819, $p < 0.001$) at 30, 60, and 90 minutes were found as followed: t = 6.646, $p < 0.001$; t = 8.480, $p < 0.001$ and t = 3.757, $p = 0.027$, respectively (Fig. 4).

When using the Student-Newman-Keuls method (F 18.819, $p < 0.001$), the difference at minutes 5 also revealed a statistical significance: t = 3.923, $p = 0.044$.

4. DISCUSSION

The present study showed that the simultaneous administration of guaifenesin with subanalgesic doses of ibuprofen, nimesulide or celecoxib resulted in a significant antinociceptive effect in the model of visceral pain (writhing test) in mice. Guaifenesin alone did not produce any antinociceptive effect. The plasma levels of nimesulide were significantly higher in combination with guaifenesin 30 to 90 min after administration of the drug combination.

These data corroborate and extend previous findings indicating that guaifenesin could increase analgesic activity of paracetamol (Dolezal & Krsiak, 2002; Kršiak *et al.,* 1980b) and acetylsalicylic acid (Kršiak *et al.,* 1980b).

The mechanism of enhancement of antinociceptive potency of analgesics by guaifenesin is unknown. Pharmacokinetic and pharmacodynamic mechanisms may be involved.

Pharmacokinetic factors appear to contribute to these effects, considering that guaifenesin increased antinociceptive potency in analgesics with various chemical structures and pharmacological effects as well

as the plasma levels of nimesulide, which were shown, in the present study, to be higher in combination with guaifenesin. This observation is supported by a clinical pharmacokinetic study, where guaifenesin almost doubled the rate of paracetamol absorption in healthy volunteers (Perlik *et al.*, 1988).

Pharmacodynamic factors of this interaction cannot be excluded either, especially in light of the central myorelaxant and sedative properties of guaifenesin (Haga *et al.*, 2000; Maier, 1980a; Matthews *et al.*, 1997). However, despite these properties, guaifenesin did not influence motor performance of mice, in an activity cage or in the rota-rod test, at doses ranging from 100 to 400 mg/kg, p.o. (Dolezal & Krsiak, 2002).

Thus, the present results suggest that guaifenesin may increase analgesic potency of various non-steroidal anti-inflammatory drugs. It would be interesting to find out whether guaifenesin also increases other effects of these drugs (e.g. their antipyretic or anti-inflammatory effects) as well as their analgesic effects as measured with other models of pain.

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REFERENCES

1 Carter CH (1966). Muscle relaxant properties of glyceryl guaiacolate. West Med Med J West **7**: 206–211.
2 Collier HO, Dinneen LC, Johnson CA, Schneider C (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. **32**: 295–310.
3 Dicpinigaitis PV, Gayle YE (2003). Effect of guaifenesin on cough reflex sensitivity. Chest **124**: 2178–2181.
4 Dolezal T, Krsiak M (2002). Guaifenesin enhances the analgesic potency of paracetamol in mice. Naunyn Schmiedebergs Arch Pharmacol. **366**: 551–554.
5 Gorski R, Kuchler G (1971). [Action of guaiacol glycerol ether on isolated skeletal muscle]. Acta Biol Med Ger **26**: 141–150.
6 Haga HA, Moerch H, Soli NE (2000). Effects of intravenous infusion of guaifenesin on electroencephalographic variables in pigs. Am J Vet Res. **61**: 1599–1601.
7 Hendershot LC, FORSAITH J (1959). Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. J Pharmacol Exp Ther. **125**: 237–240.
8 Hiramatsu M, Inoue K, Ambo A, Sasaki Y, Kameyama T (2001). Long-lasting antinociceptive effects of a novel dynorphin analogue, Tyr-D-Ala-Phe-Leu-Arg psi (CH(2)NH) Arg-NH(2), in mice. Br J Pharmacol. **132**: 1948–1956.
9 Kršiak M, Tomašíková Z, Líkarová E, Fousková H, Sajvera J, Elis J (1980a). Analgetická účinnost a toxicita směsi paracetamolu s guaifenesinem. [Analgesic activity and toxicity of a combination of paracetamol with guaifenesin]. Čs fysiol. **29**: 56.
10 Krsiak M, Tomasikova Z (1979). Analgetická účinnost a toxicita směsi kyseliny acetylosalicylové s guaifenesinem. [Analgesic activity and toxicity of a combination of aspirin with guaifenesin.]. Cs Fysiol **28**: 266.
11 Kršiak M, Tomašíková Z, Líkařová E, Fousková H, Sajvera J, Elis J (1980b). Analgetická účinnost a toxicita směsi paracetamolu s guaifenesinem [Analgesic activity and toxicity of a combination of paracetamol with guaifenesin]. Cs Fysiol. **29**: 56.
12 Maier RD (1980b). [The detection of guaiacol glyceryl ether, a content of many sedatives and hypnotics in the Federal Republic of Germany]. Arch Toxicol. **45**: 123–131.
13 Maier RD (1980a). [The detection of guaiacol glyceryl ether, a content of many sedatives and hypnotics in the Federal Republic of Germany]. Arch Toxicol. **45**: 123–131.
14 Matthews NS, Peck KE, Mealey KL, Taylor TS, Ray AC (1997). Pharmacokinetics and cardiopulmonary effects of guaifenesin in donkeys. Journal of Veterinary Pharmacology and Therapeutics **20**: 442–446.
15 Millan MJ (1994). Serotonin and pain: evidence that activation of 5-HT1A receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. Pain **58**: 45–61.
16 Perlik F, Janku I, Kordac V (1988). The effect of guaiphenesin on absorption and bioavailability of paracetamol from composite analgesic preparations. Int J Clin Pharmacol Ther Toxicol. **26**: 413–416.
17 Ptacek P, Macek J, Klima J (2001). Rapid and simple high-performance liquid chromatographic determination of nimesulide in human plasma. J Chromatogr B Biomed Sci Appl. **758**: 183–188.
18 Rubin BK, 2007). Mucolytics, expectorants, and mucokinetic medications. Respir Care **52**: 859–865.
19 Smith SM, Schroeder K, Fahey T (2008). Over-the-counter medications for acute cough in children and adults in ambulatory settings. Cochrane Database Syst Rev. CD001831.
20 Taylor EV, Baetge CL, Matthews NS, Taylor TS, Barling KS (2008). Guaifenesin-ketamine-xylazine infusions to provide anesthesia in donkeys. Journal of Equine Veterinary Science **28**: 295–300.