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Guaifenesin has no effect on sputum volume or sputum properties in adolescents and adults with an acute respiratory tract infection

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ABSTRACT (214 words)

Background: Guaifenesin (GGE) has been studied as a cough suppressant and as an expectorant; however published studies to date have failed to find a consistent benefit.

Methods: An 8-day, multi-center, clinical trial conducted to study the effect of two 600 mg extended-release GGE tablets twice daily for 1 week, on cold symptoms,sputum volume and properties in adolescents and adults with productive cough from an acute respiratory tract infection (RTI). The study enrolled 378 subjects (GGE n=188, placebo n=190) who were otherwise healthy and had an RTI for up to 5 days before enrollment. Subjects suffered from at least two of three symptoms of cough, thickened mucus, and chest congestion. A total of 151 GGE and 144 control subjects completed the full protocol.

Evaluations: Single sputum samples were collected from each subject on days 1, 3, 4 and 8 of the study. The rheology and interfacial tension of sputum was measured and 24 hour collected samples from days 1 and 4 were analyzed for total volume and hydration.

Results: Symptoms in both the guaifenesin and placebo groups improved to a similar degree over time. There were no significant differences between the GGE and placebo groups for sputum volume ($p = 0.408$), percent solids ($p = 0.694$), interfacial tension ($p = 0.881$), elasticity ($p = 0.710$), viscosity ($p = 0.447$), or mechanical impedance ($p = 0.749$).*Conclusion:* The recommended dosage of GGE had no measurable effect on sputum volume or properties and is unlikely to be an expectorant or mucolytic when used to treat acute RTI.

Clinical trials registration: clinicaltrials.gov NCT01046136.

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43 **Key message**: Although guaifenesin (GGE) has been used for decades to treat lower respiratory
44 infections (LRTI); there are no clinical data establishing effectiveness or lack of effectiveness.
45 We demonstrate in this large RCT that GGE is neither a mucolytic nor an expectorant and that
46 GGE has no effect on sputum volume or sputum properties in adults with an acute LRTI.

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Introduction

Guaifenesin (3-(2-methoxyphenoxy)-1,2-propanediol), or glyceryl guaiacolate ether (GGE), was originally derived from the guaiac tree. It has been used to treat respiratory disease since the 19th century. GGE is approved by the US Food and Drug Administration (FDA) as an over-the-counter expectorant with the Mucinex extended release brand (Reckitt Benckiser) sales accounting for approximately $135 million US annually. Guaifenesin has been studied both as a cough suppressant and as an expectorant; however published studies have failed to find a consistent benefit (1-5). The only available assessment of guaifenesin's effect in young adults with natural (i.e. community acquired) colds found no objective changes in sputum properties (6).

The purpose of this study was to determine whether two 600 mg extended-release GGE tablets (Mucinex®) or matching placebo given twice daily for 7 days to subjects with an acute respiratory tract infection (RTI), i.e. a common cold, would improve the volume or properties of sputum, and to evaluate the effect of GGE on subjective assessments of symptoms and symptom-associated quality of life. We are reporting only the objective measures of sputum volume and properties in this manuscript.

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Materials and Methods

Subject*s*

This was an 8-day multi-center, double-blind, randomized, placebo-controlled trial comparing the administration of two 600 mg GGE tablets every 12 hours for 7 days with placebo. Subjects were over age 12 and met the following inclusion criteria, as judged by their primary physician/investigator: 1) a respiratory tract infection for 5 days or less; 2) a productive cough beginning within 72 hours of the first dose of study medication; 3) at least two of three target symptoms of cough, thickened mucus, and chest congestion; 4) if female, a negative pregnancy test; 5) likely to comply with the study requirements. Exclusion criteria included respiratory symptoms due to chronic pulmonary disease (e.g. cystic fibrosis, chronic obstructive pulmonary disease, asthma, allergic rhinitis), had been febrile (> 101^{o}F) in the previous 7 days, or clinically significant physical or psychiatric disorders that could interfere with adherence or study outcomes. Subjects were also excluded if there were taking prescription or over the counter cough or cold medications. Concomitant nasal symptoms were not regarded as an exclusion criterion. Randomization of consented eligible subjects was done by sequential number assignment, blocked by study site to achieve an approximately equal distribution for each group. Masking was achieved by packaging GGE and matching placebo product in identical blister packs, and delivering them to each study site with no identifying markings other than the protocol and randomization numbers. Codes were withheld from patients and personnel until the study was completed and data locked.

Approval by the Institutional Review Board at each study site was obtained before study initiation, and written informed consent was obtained from each subject before enrollment. This study is registered with clinicaltrials.gov under the number NCT01046136. Analysis of symptom

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scoring and quality of life data is not within the scope of this paper. A portion of those results have been published (7).

Sputum collection

Spontaneously produced sputum was collected from each subject during morning clinic on days 1, 3, 4 and 8. On days 1, 3, and 4 sputa were collected immediately before the test drug was administered and at 3 and 6 hours after. On day 8 only a baseline sample was collected. Sputum was visually separated from saliva and detritus, and samples were stored at -80°C until analysis. 24-hour collections of sputa were done by subjects at home on days 1-2 and 4-5, stored in a sputum cup in home refrigerators, and returned to the clinic on days 2 and 8 where they were frozen at -80°C. Sputa were thawed on ice for analysis. Analysis of sputum biophysical properties was performed immediately upon thawing.

Sputum samples were analyzed only if they met visual examination criteria for sample integrity. Samples that were clearly watery and degraded or contaminated by saliva were not analyzed. Ideal samples were also free of food or other detritus. Samples that were not identifiable because of missing or illegible labels were discarded without analysis. The median number of sputum samples provided by each subject was 6 (interquartile range 4 – 7). The sample distribution per collection is outlined in Table 1.

Rheology

To assess whether GGE improves the properties of sputum that influence the effectiveness of sputum clearance, each of the serially collected sputa was evaluated for dynamic

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rheology using the AR1500ex rheometer (TA Instruments, New Castle, DE). Parallel plate geometry was used across the dynamic frequency range of stress-strain of a 20 μL sputum sample over driving frequencies 0.01 to 1000 rad/sec using non-destructive creep transformation and triplicate analyses. Rheology measurements were made if the measured strain from 1 to 100 rad/sec was linear and reproducible (8). By *a priori* determination, specimens with non-reproducible results indicated by greater than 20% variability in the linear testing range were considered degraded or contaminated with air and results were not recorded.

Interfacial tension

Interfacial tension was evaluated using a deNuoy ring distraction method. A 90% platinum-10% iridium ring was pulled from the mucus at a distraction velocity of 10 mm/sec until separation was achieved. Interfacial tension was measured at the air-mucus interface by a strain gauge connected to the ring (Fischer Tensiomat Model #21, Fischer Scientific, Pittsburgh, PA). A purpose-made calibrated ring of circumference 1.7145 ± 0.0381 cm was used as previously described (9). Interfacial tension measures the force separating a gel and gas phase; this is called surface tension when measured at the interface between a liquid and air. The work of adhesion of a sessile drop can be calculated by Young's equation as $Wad = \gamma (1+ \cos \theta)$ where γ is the interfacial tension of mucus in air and θ is the contact angle of mucus on the epithelium. Sputum with high Wad is more difficult to clear by cough.

Mucus hydration and volume

To determine if GGE worked as an expectorant and increased the volume of mucus or fluid produced, the total weight and the hydration (% solid material) of the two 24-hr samples

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was calculated by measuring the wet weight of the sputum, then freeze-drying the sputum and determining the ratio of the solid weight to wet weight to obtain the percent solids (10).

Statistical analysis

Pre-study sample size determination was based on previous studies of sputum properties so that 120 subjects per group was needed to detect a 50% change in sputum dynamic viscosity with 80% power. The goal of the study was to enroll at least 375 subjects and collect data on 300 to provide a sufficient sample to have an 80% power to detect a statistically significant ($p < 0.05$) difference between groups for both rheology and sputum volume outcome measures. Although we were unable to collect uncontaminated sputum from all participants, at every time point, *post hoc* power calculation, using the means and variability of the results obtained, confirmed that the study sample size was sufficient to detect any clinically significant changes in the volume or biophysical properties of the sputum (11).

Data were logged in spreadsheets, analyzed by JMP v 9.0 or StatView v 5.0 (both from SAS Institute, Cary NC). Analyses were based on per protocol subjects defined as those who met all inclusion and exclusion criteria, were 100% dosing compliant from Day 1 through Day 4, had 80% of their diaries complete on Day 1 and Day 4, with some diary information on Day 2 and Day 3, and who had their visits on the designated study day. Subject demographics and sputum were compared between groups using t-test for continuous variables, Mann-Whitney U test for categorical variables and Chi-square for proportions. Sputum rheology and interfacial tension were analyzed by Two-way ANOVA. *Post-hoc* correction for multiple comparisons was calculated using the Sidak method. Where applicable, results are reported as means ± standard error of the mean. Significance was defined as $p < 0.05$.

Results

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Patient demographics

The study enrolled 378 subjects (GGE n=188, placebo n=190) at 12 centers who were otherwise healthy and who had symptomatic RTI for up to 5 days before enrollment. There were 295 subjects (151 GGE, 144 Control) who completed the full protocol. There were no significant differences between groups for demographics (Table 2).

Rheology

By two-way ANOVA, there were no significant differences with respect to changes from baseline across the study between the GGE and Placebo subjects for elasticity (G') (Figure 1), $p = 0.710$, viscosity (G") (Figure 2), $p = 0.447$, and mechanical impedance (G*), $p = 0.749$.

Interfacial tension

There were no significant treatment differences between groups with respect to interfacial tension at any time point (Figure 3) $p = 0.881$.

Mucus hydration and volume

There were differences in the wet weight between groups at the first collection (GGE 4.70 ± 0.471 g, Placebo 6.083 ± 0.754 g, $p = 0.117$), second collection (GGE 4.292 ± 0.460 g, Placebo 4.718 ± 0.518 g, $p = 0.538$), nor in the change between the two collections (GGE -0.981 ± 0.593 g, Placebo -2.081 ± 0.821 g, $p = 0.138$). Similarly, there was no significant difference in the percent solids between the groups (Figure 4) at the two collections or in the change between the collections (GGE 0.191 ± 0.223 %, Placebo 0.042 ± 0.236 %, $p = 0.649$).

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Discussion

In this large randomized clinical trial, there were no consistent statistically significant changes in sputum properties comparing placebo to GGE for therapy of acute symptoms of RTI in subjects without underlying chronic pulmonary disease. The absence of changes in sputum rheology and interfacial tension, independent of within-group symptom decrease over time, makes it unlikely that GGE is a mucolytic or that GGE therapy would improve mucociliary or cough clearance. Similarly the lack of difference in sputum wet volume or dry weight makes it unlikely that GGE has significant expectorant properties in healthy subjects with an acute URTI.Although the upper respiratory quality of life improved over the couse of the study in both the GGE and placebo groups, this improvement was not accompanied by changes in sputum properties.

We conclude that extended-release GGE (Mucinex®) administered at the recommended dose is no more effective than placebo in changing sputum properties. Although it is possible that the negative results could be due to an inadequate dose, the drug dosage studied here is consistent with current dosing recommendations for GGE as an over-the-counter medication to ease cold symptoms. It is also possible that the drop off in sample number over the course of the study could confound the results as those with improvement in their productive cough or sputum production may have been omitted from the sampling. Another possible limitation is that the diagnosis of RTI was made clinically and not confirmed by microbiological testing. However, the clinical assessment was performed by experienced clinicians and the clinical conditions matched those for which GGE is most commonly used.

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It has been suggested that GGE is either an expectorant or a mucolytic (requiring very different mechanisms of action) and that changes in sputum properties are responsible for clinical effectiveness. If GGE were an expectorant (4, 12, 13) we would expect to see increased sputum volume and hydration. This was not supported by the results of sputum volume or percent solids measurements in our study. If GGE was a mucolytic (14, 15) we would expect to see decreased viscoelasticity. In our study, we saw no evidence of either expectorant or mucolytic properties either for GGE or for the placebo. Furthermore there were no changes in sputum properties that would be expected to improve mucocilary or cough clearance. In contrast to an earlier *in vitro* study showing that GGE decreased mucociliary transport when applied directly to a ciliated epithelium (14), a more recent study showed a dose-dependent increase in mucus transport when GGE was introduced into airway cell culture (15). These data from a clinical study in subjects with an acute URTI clearly shows no evidence of mucolytic properties and no temporal or drug associated changes in viscoelasticity or sputum volume.

The results of this study, which focused on the properties of mucus during RTIs, are consistent with published studies showing that GGE is not an effective medication for treating acute respiratory tract infection (1-5). The placebo effect has been reported to be as high as 85% in studies of cough therapy (16) and, given the absence of biophysical findings, this may be the best explanation for any perceived benefits for GGE.

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Ms Hoffer-Scheafer performed the analysis of the biophysical properties of secretions, she wrote the initial draft of the manuscript, and was involved in statistical analysis of the data. Dr Rozycki helped to revise several versions of the manuscript. He was involved in data analysis and preparation of data tables and graphs. Ms Yopp was the study coordinator. She collected and catalogued all sputum specimens and subject data, she organized sputum analysis procedures and logging, and she helped to revise the manuscript. Dr. Rubin was principal investigator and he is guarantor of the paper. He designed the study and was involved in all data analysis and prepared the final version of the submitted manuscript.

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278 **Figure 1 legend -** Changes in elasticity from baseline in the placebo and GGE groups over the
279 course of the study. There were no significant differences in the change from baseline between
280 the two groups by ANOVA p = 0.710.
281
282 **Figure 2 legend -** Changes in viscosity from baseline in the placebo and GGE groups over the
283 course of the study. There were no significant differences in the change from baseline between
284 the two groups by ANOVA p = 0.447.
285
286 **Figure 3 legend -** Changes in interfacial tension from baseline in the placebo and GGE groups
287 over the course of the study. There were no significant differences in the change from baseline
288 between the two groups by ANOVA p = 0.881.
289
290 **Figure 4 legend –** Sputum percent solids for 1st Collection (Days 1-2) and 2nd Collection (Days
291 4-5) in Control and GGE groups. By t-test, there were no significant differences between the two
292 groups in either the 1st Collection (p = 0.694) or the 2nd Collection (p = 0.334).
293

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Table 1 Sample Distribution

	GGE (n = 188)	Placebo (n = 190)	Total (n = 378)
Day 1 – Pre-Drug	118	117	235
Day 1 – 3 Hour	51	62	113
Day 1 – 6 Hour	107	109	216
Day 3 – Pre-Drug	99	95	194
Day 3 – 3 Hour	55	62	117
Day 3 – 6 Hour	85	82	167
Day 4 – Pre-Drug	106	110	216
Day 4 – 3 Hour	44	43	87
Day 4 – 6 Hour	84	86	170
Day 8	40	47	87

Table 1 – Number of sputum samples available for analysis for each scheduled collection, by group assignment.

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Table 2: Subject Demographics				
		GGE (N=188)	Placebo (N=190)	p-value
Age		41.15 ± 13.91	40.79 ± 15.04	p=0.41
Sex	Male	49%	56%	p=0.22
	Female	51%	44%	
Ethnicity	Hispanic/Latino	7%	5%	p=0.41
	Not Hispanic/Latino	93%	95%	
Race	White	61%	61%	p=0.34
	Black	37%	33%	
	Asian	2%	4%	
	American Indian/Alaska Native	1%	2%	
	Native Hawaiian/Pacific Islander	1%	0%	

Table 2: Subject demographics for the placebo and GGE groups.. There were no statistically significant differences between groups by Chi-square analysis or t-test for age. Age is in years **±** standard deviation.

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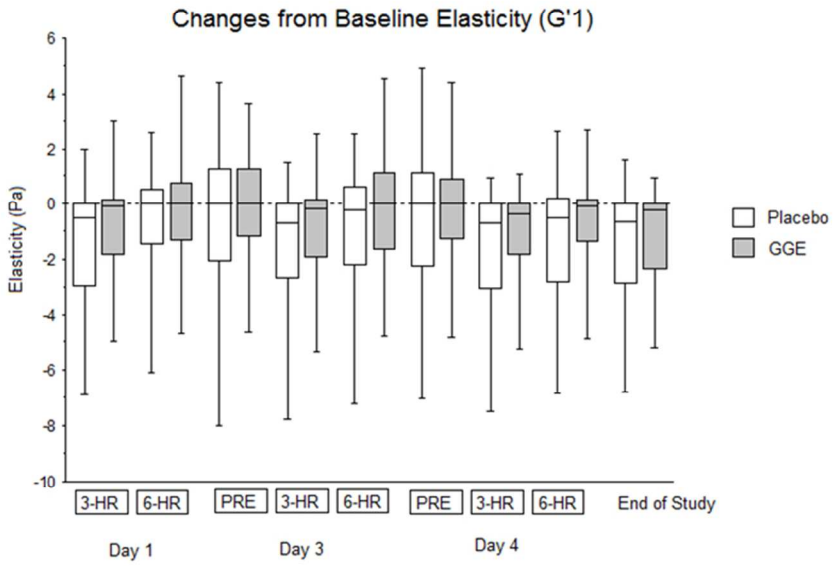

Changes in elasticity from baseline in the placebo and GGE groups over the course of the study. There were no significant differences in the change from baseline between the two groups by ANOVA.
219x145mm (96 x 96 DPI)

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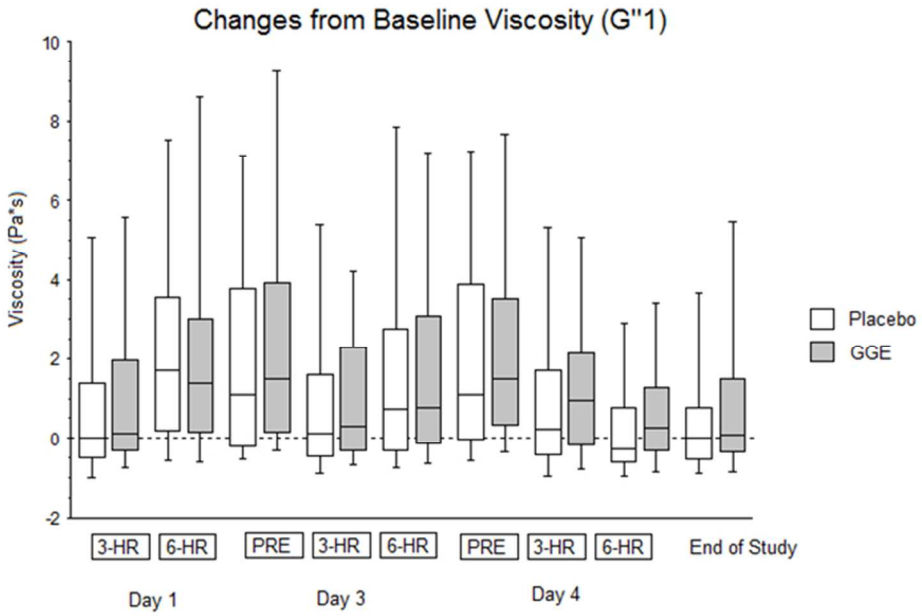

Changes in viscosity from baseline in the placebo and GGE groups over the course of the study. There were no significant differences in the change from baseline between the two groups by ANOVA.
199x132mm (96 x 96 DPI)

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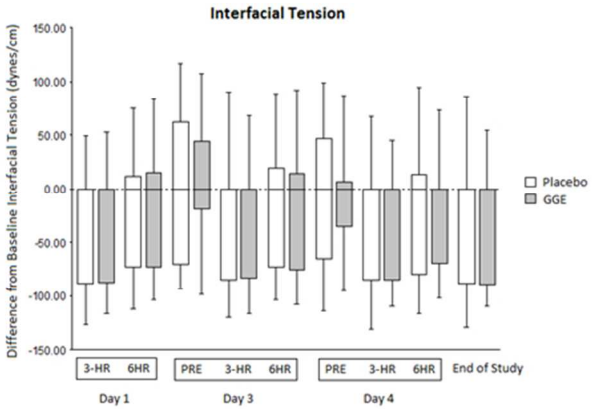

Changes in interfacial tension from baseline in the placebo and GGE groups over the course of the study. There were no significant differences in the change from baseline between the two groups by ANOVA after correction for multiple comparisons.
199x132mm (96 x 96 DPI)

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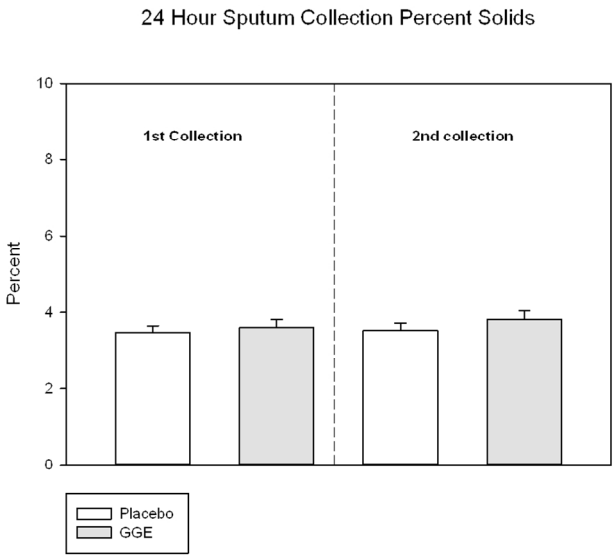

Sputum percent solids for 1st Collection (Days 1-2) and 2nd Collection (Days 4-5) in Control and GGE groups. By t-test, there were no significant differences between the two groups in either the 1st Collection ($p = 0.694$) or the 2nd Collection ($p = 0.334$).
215x279mm (150 x 150 DPI)