Published in final edited form as:

Am J Gastroenterol. 2010 June ; 105(6): . doi:10.1038/ajg.2009.655.

Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS

Ashok Attaluri, Michelle Jackson, Jessica Paulson, and Satish SC Rao, M.D., Ph.D., FRCP (LON)

Department of Internal Medicine Division of Gastroenterology/Hepatology 200 Hawkins Drive, 4612 JCP

Abstract

Background: About 35% of humans have methane producing gut flora. Methane-producing IBS subjects were generally constipated. In animal models, methane infusion slows intestinal transit. Whether methanogenic flora alters colonic transit or stool characteristics and its relationship to constipation is unclear.

Aim: To examine the prevalence and association of methanogenic flora in patients with slow transit constipation (ST) and normal transit constipation (NT) and non-constipated controls.

Methods: Ninety six consecutive subjects with chronic constipation (Rome III) were evaluated with radio-opaque marker (ROM) transit studies and were classified as slow transit (>20% ROM retention) or normal transit. All constipated subjects and 106 non constipated controls underwent breath tests to assess methane production. Baseline CH₄ of 3 ppm was used to define presence of methanogenic flora. Stool frequency and consistency were assessed using a prospective stool diary. Correlation analyses were performed.

Results: Forty eight subjects had ST and 48 had NT. Prevalence of methanogenic flora was higher ($p < 0.05$) in ST (75%) compared to NT (44%) or controls (28%). ST patients had higher methane production compared to NT and controls ($p < 0.05$). NT patients also produced more methane compared to controls ($p < 0.05$). There was moderate ($p < 0.05$) correlation between baseline, peak and AUC of methane response with colonic transit but not with stool characteristics.

Conclusions: Presence of methanogenic flora is associated with chronic constipation. Methane production following carbohydrate challenge and its prevalence were higher in ST than NT, although stool characteristics were similar in both groups. Methane production correlated with colonic transit, suggesting an association with stool transport but not with stool characteristics.

Keywords

methane; constipation; colon transit

INTRODUCTION

What is known: Adults with constipation predominant irritable bowel syndrome and children with constipation and soiling have higher prevalence of methanogenic flora, but whether subjects with non-IBS chronic constipation have higher prevalence of methanogenic flora, is not known.

What is new:

- Adults with chronic constipation without irritable bowel syndrome have higher prevalence of methanogenic flora compared to non-constipated controls
- Constipated subjects with slow transit have higher prevalence of methanogenic flora compared to those with normal transit constipation.
- There is no correlation between methane production and stool frequency or stool consistency

Fermentation of unabsorbed carbohydrates by colonic anerobic microflora produces hydrogen (H₂), carbondioxide (CO₂), methane (CH₄), as well as short chain fatty acids and sulfites (1). A variable proportion of H₂ and CH₄ are absorbed, transported to the lungs and exhaled through breath, and the rest expelled in flatus (1). Methane gas is produced by enteric bacteria in 30–62% of healthy humans (1-6).

For many years, methane was thought to be physiologically inert. However, there is increasing evidence from clinical and epidemiological studies that methanogenesis may be associated with changes in gut physiology. Methane production in healthy humans has been shown to inversely correlate with stool frequency and colonic transit (4). Children with constipation and soiling have higher prevalence of methanogenic flora and relatively slower colonic transit (7). Likewise, patients with irritable bowel syndrome (IBS) and methane production (after a lactulose load) were reported to invariably have IBS-C (8, 9). Furthermore, the degree of methane production during a lactulose breath test was reported to correlate with the degree of constipation in IBS subjects (10).

There is some evidence from animal experiments that methane may have effects on small bowel and colonic motility. Recently, methane has been shown to increase non-propagating small bowel contractile activity and decrease small bowel transit in animal models (11). The aforementioned studies suggest an association between the degree of methane production and alterations in stool frequency in both healthy humans and subjects with IBS. However, the prevalence of methanogenic flora in non-IBS chronic constipation (CC) and its effect on colonic transit and stool characteristics has not been assessed.

We hypothesized that subjects with chronic constipation have higher prevalence of methanogenic flora when compared to non-constipated controls and methane production in response to a carbohydrate substrate correlated with colonic transit.

Therefore, our aims were: 1. To evaluate the prevalence of methanogenic flora in subjects with chronic constipation and its pathophysiologic subtypes and compare this with non-constipated controls. 2. To examine the correlation between methane production, colonic transit and stool characteristics in subjects with and without constipation.

METHODS

Subjects

We examined consecutive subjects with chronic constipation (Rome III) who were referred to a tertiary care center (12). We excluded patients with severe cardiovascular disease, chronic renal failure, neurologic diseases, recent use of antibiotics (< 6 weeks) or those with previous gastrointestinal surgery. Patients with alternating constipation and diarrhea and those who fulfilled the Rome-III criteria for irritable bowel syndrome were also excluded. Patients using laxatives, tegaserod or lubiprostone were asked to discontinue these medications at least 2 weeks prior to study.

We also recruited a control group of subjects referred to our tertiary care center for evaluation of unexplained abdominal pain, bloating, excessive flatulence or nausea, but without any history of constipation or diarrhea. This study was approved by the Institutional Review Board of the University of Iowa.

Protocol

All subjects maintained a prospective stool diary for 7 days. Information on stool frequency and stool consistency (Bristol Stool scale) was recorded. On day 2 of the 7 day period, subjects ingested a Sitmarker capsule (see below) and the colon transit assessment was done concurrently with the stool diary. Subsequently, about 1-4 weeks later, subjects underwent a glucose breath test to evaluate the presence of methanogenic flora.

Colonic transit study: A single capsule containing 24 radio-opaque markers (Sitzmarks®, Konsyl Pharmaceuticals, Fort Worth, Texas) was administered on day 1 and a plain abdominal x-ray was obtained on day 6 (120 hours later)(14)(15) (16). Retention of > 20% markers (>6 markers) on day 6 (120 hrs) was considered abnormal (16) and indicative of slow transit constipation.

Glucose breath test: All subjects underwent breath testing with glucose. One day prior to the test, subjects were asked to consume a carbohydrate restricted diet to avoid high baseline values of breath H₂ or CH₄ from ingestion of previously unabsorbed carbohydrates. No food or drink was allowed for at least 12 hours before the study. A baseline breath sample was obtained and a solution of glucose (75 gm in 250cc water) was administered to the patient. End expiration breath samples were collected using a modified (Haldane-Priestley) bag (Quin Tron, Milwaukee, WI) and analyzed for the concentration of H₂ and CH₄ using a gas chromatography analyzer (Quintron Microlyzer Self Correcting Model SC, Quin Tron, Milwaukee, WI). Breath samples were collected at 15 minute intervals for 2 hrs (17, 18).

DATA ANALYSIS

Presence of methanogenic flora was defined as a baseline CH₄ value of at least 3 ppm on two separate breath samples. Previous studies (4) have used 1 ppm to define the presence of methanogenic flora; we chose a higher level to avoid any spurious levels from technical or mechanical errors.

Patients were categorized into two groups: (1) Slow transit constipation(ST) (≥ 20% retention of ROM at 120 hrs), (2) Normal transit constipation (NT) (<20% retention of ROM at 120 hrs)

Breath H₂ and CH₄ responses for each subject were analyzed for the following parameters: (1) Baseline value (in parts per million), (2) Peak values (in parts per million), (3) Area under the curve (AUC) (in parts per million.hours). For each subject, we examined the breath H₂ and CH₄ data obtained from the glucose breath test and used this as evidence for the presence of methanogenic flora.

Statistical analysis

Students' t test was used to compare the baseline, peak and AUC of the breath H₂ and CH₄ responses between the controls, ST and NT groups. Spearman test was used to correlate colonic transit (% retention of markers at 120 hours), stool frequency (BM/week) and stool consistency (Bristol scale 1-7) with breath CH₄ responses in each group. Correlation analysis was performed within the methane producers in each group. A p value < 0.05 was considered significant. Next, multiple regression analyses were done to evaluate for

interaction between stool consistency, stool frequency, colonic transit and methane values, in a combined sample of all methane producing constipated subjects (ST and NT). Adjusted r^2 values were calculated for the correlation between methane values and stool characteristics & transit. We used a commercially available software package (Prism 3.0 and InStat; GraphPad Software, Inc., San Diego, California, USA) to facilitate statistical analysis.

RESULTS

Subject demographics and characteristics

We examined 96 patients (M/F=7/89, mean age 47.4 years, range 21-75 years) with chronic constipation and 106 non-constipated subjects (M/F=36/70, mean age 55.4 years, range 21-86 years). There was no statistically significant difference ($p > 0.05$) in the mean age but there were significantly ($p < 0.05$) more females in the constipated group compared to the non-constipated group.

In the constipated group, forty eight subjects had normal colonic transit and 48 patients had slow colonic transit. There was no significant difference ($p > 0.05$) in either the age or gender distribution between the NT and ST groups (Mean age $\pm$ SD: 45.2 ± 24.5 years vs 48.8 ± 26.6 years; M/F = 3/45 and 4/44 respectively)

Prevalence of methanogenic flora

We found that 30/106 (28%) controls had methanogenic flora, which is similar to the prevalence in the general population reported previously (4, 5). In the patient groups, 21/48 (44%) normal transit patients and 36/48 (75%) slow transit patients had methanogenic flora (Figure 1). The prevalence of methanogenic flora was significantly higher ($p = 0.02$) in the ST group, when compared to either the NT group or controls. The prevalence of methanogenic flora was similar ($p > 0.05$) in the NT group and controls.

Furthermore, the baseline levels of methane (prior to administration of carbohydrate) were significantly higher in the ST group when compared to the NT group and controls respectively (mean $\pm$ SD: 23.1 ± 14.6 ppm vs 10.7 ± 6.1 ppm vs 13.5 ± 6.9 ppm, $p = 0.01$) (Table 1)

Methane production after administration of carbohydrate substrate

Methane production after administration of glucose may serve as a surrogate measure of methanogenic flora. Following administration of glucose, subjects in the ST group had significantly higher methane response (peak levels and area under the curve) when compared to the NT group and controls ($p < 0.05$). (Table 1). H_2 production was higher ($p < 0.05$) in the controls compared to the NT group and ST group, and there was no statistically significant difference between the NT and ST groups.

Within methane producers in the ST and NT groups, in order to assess the correlation between level of methane production and constipation severity, we performed correlation analyses. Multiple linear regression analysis was performed, with methane levels as the dependent variable and stool consistency, stool frequency and colon transit as predictors. There was a significant overall relationship ($R^2=53.4\%$ for baseline methane, $p < 0.0001$) and colon transit was the only significant contributor ($R^2= 52.5\%$, $p < 0.0001$), while stool consistency and stool frequency did not contribute significantly to the model ($p > 0.3$) (Table 2).

The proportion of subjects with a positive glucose breath test (≥ 20 ppm rise of methane and or hydrogen above baseline, in association with symptoms) was similar in controls (13/106; 12%), and the NT group (6/48, 12%), while the ST group had higher prevalence (17/48; 35%).

Stool characteristics

The prospective 7 day stool diary revealed no differences in the stool frequency/week in the ST and NT groups (4/week and 4.8/week respectively, $p > 0.05$). Similarly, there was no difference in mean stool consistency on the Bristol stool form scale (2.8 in ST group, and 2.9 in NTC group respectively, $p > 0.05$). Therefore, although the groups had different colonic transit times, there was no objective difference in the severity of constipation, as assessed by stool characteristics, between the two groups.

DISCUSSION

In this study, we found that subjects with chronic constipation have higher prevalence of methanogenic flora when compared to non constipated subjects. Furthermore, the prevalence of methanogenic flora was higher in constipated patients with slower colonic transit than those with normal colonic transit. In response to a carbohydrate substrate, CH_4 production was higher in patients with slower transit than those with normal transit or controls. Also, among methane producers in the slow transit group, CH_4 production correlated with colonic transit, but not with stool consistency or stool frequency.

Therefore, these results show a *qualitative* correlation between chronic constipation and the presence of methanogenic flora, as well as a *quantitative* correlation between the degree of methane production and colonic transit. Interestingly, there was no relationship between methane production and some objective measures that are commonly used to grade the severity of constipation such as stool frequency and stool consistency. Previous studies have reported a relationship between colonic transit, stool consistency and stool frequency (4, 7, 10). In a recent study, colonic transit in constipated patients correlated well with stool consistency but not with stool frequency (19). It is unclear how many of these subjects had methanogenic flora. Methane increases non-propagating small bowel contractile activity and decrease small bowel transit in animal models (11). Based on the above data, the pathogenesis of methane associated constipation is unclear; methane may have physiologic effects on both the small bowel and colon that retards chyme transport.

We wish to emphasize that although this study suggests an association between methanogenesis and constipation, causality cannot be established on the basis of our data. However, prevalence of methanogenic flora and methane production were both higher in the slow transit group than in the normal transit group, despite no apparent differences in their stool frequency or stool consistency. Based on this data, one could speculate that methanogenic flora may predispose to slower colonic transit in patients with constipation. However, it is equally possible that higher levels of methanogenic flora may be a consequence, rather than a cause of slow transit constipation, and this merits further study.

Children with constipation and soiling have been shown to have a higher prevalence of methanogenic flora and relatively slower colonic transit (7). Patients with irritable bowel syndrome (IBS) and methane production (after lactulose load) were also reported to invariably have IBS-C (8). Our findings are consistent with these studies in that we can confirm a relationship between the presence of methanogenic flora and constipation. However, it is worth pointing out that 27% of subjects with ST and 56% of subjects with NT had non-methanogenic flora. Thus, the presence of methanogenic flora may be one of the predisposing factors for the development of constipation and many constipated patients have

non- methanogenic flora. Also, there appears to be a trend towards higher hydrogen production in controls and NT subjects, probably because of lower conversion to methane by methanogenic flora (1).

Our finding of an association between methanogenesis and slower colonic transit and the absence of an association with stool characteristics is intriguing and the reasons are unclear. We speculate that the underlying pathogenesis of slow transit constipation is different from that of normal transit and that methane may play a more important role in slow transit constipation. The exact mechanisms require further study.

One of the limitations of our study was that glucose was used as the carbohydrate substrate. Although this substrate does not affect prevalence data, methane production following glucose primarily reflects fermentation by small bowel flora and therefore does not accurately reflect colonic methanogenic flora loads. A higher proportion of the ST patients had a positive glucose breath test compared to either the NT patients or controls, and this could partly explain increased methane production in this group. Also, while the stool diary was maintained concurrently with the colon transit test, the breath test was performed at a later date.

It has been reported that treatment with neomycin improves constipation in IBS-C and that the improvement depends on the presence and elimination of methane as examined by a lactulose breath test (9). While these findings are intriguing and may be true, it is debatable whether the improvement in symptoms was due to the elimination of methanogenic flora. Also, is it unclear whether colonic flora can be altered by antibiotics and if such a change can be sustained in the long term. Nonetheless, our study provides further evidence for an association between methanogenesis and constipation, and in particular, the relationship between slower colonic transit and constipation. Whether these findings are due to altered colonic microbiota merits further appraisal.

A. Who is the guarantor of the paper?

Satish Rao, MD, PhD is the guarantor of the paper

B. What is EACH AUTHOR'S contribution to the paper?

Ashok Attaluri and Satish Rao were involved in study design, subject enrollment, data analysis and manuscript preparation. Michelle Jackson was involved in performing breath tests. Jessica Paulson was involved in identifying and enrolling patients, as well as data analysis.

C. What financial support was received?

None

D. What may be the potential competing interests?

None

Acknowledgments

This research was supported in part by NIH grant RO1 DK 57100-05

References

1. Levitt MD, BJ H Jr. Volume, composition, and source of intestinal gas. *Gastroenterology*. Dec; 1970 59(6):921–9. [PubMed: 5486278]

2. Cloarec D, Bornet F, Gouilloud S, Barry JL, Salim B, Galmiche JP. Breath hydrogen response to lactulose in healthy subjects: Relationship to methane producing status. *Gut*. Mar; 1990 31(3):300–4. [PubMed: 2323593]
3. Levitt MD. Production and excretion of hydrogen gas in man. *N Engl J Med*. 1969; 281:122–7. [PubMed: 5790483]
4. Levitt MD, Furne JK, Kuskowski M, Ruddy J. Stability of human methanogenic flora over 35 years and a review of insights obtained from breath methane measurements. *Clin Gastroenterol Hepatol*. Feb; 2006 4(2):123–9.
5. Levitt MD. Intestinal gas production. *J Am Diet Assoc*. 1972; 60:487–96. [PubMed: 4554579]
6. McKay LF, Eastwood MA, Brydon WG. Methane excretion in man--a study of breath, flatus, and faeces. *Gut*. Jan; 1985 26(1):69–74. [PubMed: 3965369]
7. Soares AC, Lederman HM, Fagundes-Neto U. Breath methane associated with slow colonic transit time in children with chronic constipation. *J Clin Gastroenterol*. 2005; 39:512–5. [PubMed: 15942438]
8. Pimentel M, Mayer AG, Park S, Chow EJ, Hasan A, Kong Y. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig Dis Sci*. Jan; 2003 48(1):86–92. [PubMed: 12645795]
9. Pimentel M, Chatterjee S, Chow EJ, Park S, Kong Y. Neomycin improves constipation-predominant irritable bowel syndrome in a fashion that is dependent on the presence of methane gas: Subanalysis of a double-blind randomized controlled study. *Dig Dis Sci*. Aug; 2006 51(8):1297–301. [PubMed: 16832617]
10. Chatterjee S, Park S, Low K, Kong Y, Pimentel M. The degree of breath methane production in IBS correlates with the severity of constipation. *American Journal of Gastroenterology*. 2007; 102(4):837–41. [PubMed: 17397408]
11. Pimentel M, Lin HC, Enayati P, van den Burg B, Lee HR, Chen JH, et al. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am J Physiol Gastrointest Liver Physiol*. Jun; 2006 290(6):G1089–95. [PubMed: 16293652]
12. Drossman D. The rome foundation and rome III. *Neurogastroenterol Motil*. 2007; 19:783–786. [PubMed: 17883428]
13. Rao SS, Tuteja AK, Vellema T, Kempf J, Stessman M. Dyssynergic defecation: Demographics, symptoms, stool patterns, and quality of life. *J Clin Gastroenterol*. Sep; 2004 38(8):680–5. [PubMed: 15319652]
14. Diamant ND, Kamm MA, Wald A, Whitehead WE. AGA technical review on anorectal testing techniques. *Gastroenterology*. 1999; 116:735–60. [PubMed: 10029632]
15. Nam YS, Pikarsky AJ, Wexner SD, Singh JJ, Weiss EG, Nogueras JJ, et al. Reproducibility of colonic transit study in patients with chronic constipation. *Dis Colon Rectum*. Jan; 2001 44(1):86–92. [PubMed: 11805568]
16. Hinton JM, Lennard-Jones JE, Young AC. A new method for studying gut transit times using radio-opaque markers. *Gut*. 1969; 24:123–6.
17. Read NW, Al-Janabi MN, Bates TE, Holgate AM, Cann PA, Kinsman RI, et al. Interpretation of the breath hydrogen profile obtained after ingesting a solid meal containing unabsorbable carbohydrate. *Gut*. Aug; 1985 26(8):834–42. [PubMed: 4018650]
18. Solomons NW. Evaluation of carbohydrate absorption: The hydrogen breath test in clinical practice. *Clinical Nutrition*. 1984; 3:71–8.
19. Saad R, Chey WD, Rao SSC. How well does stool form and stool frequency correlate with colonic and whole gut transit in healthy controls and constipated patients? *Gastroenterology*. 2008; 134(4, Supp 1):M1794.

Figure 1. Prevalence of methanogenic flora

Prevalence of methanogenic flora in controls, constipated subjects with normal transit and constipated subjects with slow transit

* significantly different from controls and NT ($p < 0.05$)

Figure 2. Correlation between colon transit and AUC of methane production
Area under the curve of methane (in ppm.hours) are shown on the y axis. Colon transit (percentage retention of markers at 120 hrs) is shown on the x axis.

Table 1
BREATH CH₄ & H₂ PRODUCTION AFTER A CARBOHYDRATE CHALLENGE

Baseline hydrogen and methane values, and the peak & area under the curve of gas production after carbohydrate challenge in methane producing subjects are shown. Values are represented as mean (in ppm) ± SD.

	CH ₄			H ₂		
	Baseline (ppm)	Peak (ppm)	AUC (ppm X hr)	Baseline (ppm)	Peak (ppm)	AUC (ppm X hr)
Controls n=30	13±7	24±10	102±45	4± 3	36±17	117±49
Normal Transit n=21	11±6	19±13	110±44	4±3	24±14 *	85±46 *
Slow Transit n=36	23±14 ^{*/+}	43±24 ^{*/+}	218±126 ^{*/+}	9±6 *	19±13 *	79±49 *

Mean ± SD,

* significantly different from controls (p < 0.05),

⁺ significantly different from NT (p < 0.05)

Table 2
CORRELATION BETWEEN CH₄ PRODUCTION, STOOL CHARACTERISTICS & COLONIC TRANSIT

The correlation between stool consistency, stool frequency, colonic transit and methane production is shown for methane producing constipated subjects. Adjusted correlation coefficients (R^2) are shown.

	Baseline CH ₄	Peak CH ₄	AUC CH ₄
Stool consistency	$R^2 = 0.01$	$R^2 = 0.07$	$R^2 = 0.01$
Stool frequency	$R^2 = 0.03$	$R^2 = 0.02$	$R^2 = 0.02$
Colonic transit	$R^2 = 0.52^*$	$R^2 = 0.54^*$	$R^2 = 0.56^*$

*
 $p < 0.0001$