

## Use of the Hydrogen Breath Test to Determine the Influence of Antibiotic Prophylaxis on Intestinal Flora

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### ABSTRACT

**Purpose:** This experimental study was designed to use the hydrogen (H<sub>2</sub>) breath test to investigate changes in the intestinal flora of patients that were administered prophylactic antibiotics for 48 hours after surgery.

**Methods:** Altogether, 22 patients were divided into two groups and the antimicrobial prophylactics, cefazolin (3.0 g/day) or sulbactam/ampicillin (4.5 g/day), were administered on induction of anaesthesia for 48 hours after surgery. End expiratory breath samples were collected on the morning of the day of surgery and every morning for 1-6 days after surgery.

**Results:** H<sub>2</sub> breath concentration significantly decreased in each group on day 1 (cefazolin: 1.20 ± 0.39 ppm vs. sulbactam/ampicillin: 1.17 ± 0.34 ppm). On day 2, the H<sub>2</sub> concentration in the sulbactam/ampicillin group was significantly lower than the cefazolin group (cefazolin: 6.4 ± 2.2 ppm vs. sulbactam/ampicillin: 1.0 ± 0.4 ppm, *p* < 0.05). H<sub>2</sub> concentration was still lower in the sulbactam/ampicillin group (1.3 ± 0.3 ppm vs. 3.3 ± 1.0 ppm, *p* = 0.10) on day 3. On days 4-6, H<sub>2</sub> concentration was essentially the same for both groups.

**Discussion:** Colonic anaerobes are thought to be a reservoir of resistant organisms and prolonged antimicrobial treatment is a major cause for the development of resistance. Surgical prophylaxis is basically recommended for use within 24 hours after surgery. The breath H<sub>2</sub> concentration in both groups significantly decreased 24 hours after administration. These results suggest that both antibiotics influence the activity of colonic anaerobes and the duration of surgical antibiotic prophylaxis should be as short as possible.

**Key words:** Intestinal flora, antibiotics, prophylaxis, breath hydrogen

Anaerobic bacteria comprise a large proportion of the normal human commensal gut flora and evidence continues to mount that normal intestinal flora provides protection against a broad range of enteric pathogens, including bacteria such as *Escherichia coli* and *Pseudomonas* spp., anaerobic *Clostridium* spp., and yeasts like *Candida albicans*.<sup>21,22)</sup> The ability to prevent or limit colonization of the intestinal tract by pathogenic microorganisms has been termed as colonization resistance.<sup>53)</sup> Antimicrobial therapy induces rapid and profound changes in the intestinal flora, thereby disrupting colonization resistance.<sup>14,37,52)</sup> Imbalances in the microbial ecosystem caused by the administration of antimicrobial agents may allow colonization or overgrowth of resistant organisms that might spread within the body and cause infection.<sup>12)</sup>

Microorganisms in the lower gut also ferment dietary fibre and produce hydrogen (H<sub>2</sub>), methane, and carbon dioxide gases. A portion of these gases enters the blood stream and is excreted via the lungs.<sup>30,31,39)</sup> The hydrogen breath test, which is based on the premise that H<sub>2</sub> gas in humans is produced exclusively by colonic fermentation, uses levels of expired H<sub>2</sub> as an indirect measure of disturbances in the intestinal flora.<sup>20,29,30)</sup> The test is widely used to detect a battery of non-structural gastrointestinal disorders, particularly carbohydrate malabsorption, small intestinal bacterial overgrowth, and irritable bowel syndrome. The breath test is also used in studies of food metabolism and as various indicators of intestinal flora.<sup>5-8,17,25,32,34,39,42-45,47)</sup>

The prolonged use of antibiotics is associated with an increased risk of drug toxicity, a change

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in the antimicrobial susceptibility pattern of pathogens, and an alteration in intestinal flora.<sup>15)</sup> Therefore, despite the accepted use of antimicrobial prophylaxis in surgery, its use remains controversial, especially regarding the duration of postoperative administration. In general, antimicrobial prophylaxis should last less than 24 hours, and under some circumstances, may consist of a single dose.<sup>13)</sup>

Since the intestinal tract may be a major site for the emergence of drug-resistant organisms due to its frequent exposure to antimicrobial agents, the ecological effects of antibacterial agents on human microflora are of clinical importance.<sup>23,48,50)</sup> This study enrolled surgical patients who were administered antibiotics at the induction of anaesthesia up to 48 hours following surgery. The hydrogen breath test was used to assess changes in their intestinal flora to determine whether a 48-hour administration was an appropriate duration for postsurgical antibiotic prophylaxis.

## PATIENTS AND METHODS

### Basal analysis: fasting breath hydrogen data in healthy Japanese subjects

Thirty-five healthy volunteers (21 men and 14 women, aged 21-65 years) fasted after their usual dinner until the following morning (~08:00) when hydrogen breath tests were conducted at the Hiroshima University School of Medicine. End-alveolar breath samples were obtained by having the subjects exhale end-expiratory samples into 500 ml plastic bags fitted with stopcocks. Samples were analysed for H<sub>2</sub> concentration with a HCMA-T1™ Gas Chromatograph (Abilit Corporation, Osaka, Japan). The data was presented as normalized breath-H<sub>2</sub> concentrations in parts per million (ppm).

### Effect of antimicrobial agents on breath hydrogen

#### Subjects

Twenty-two patients (12 males and 10 females; median age 53 years; range 38-71 years) were recruited to this study. All the patients were treated surgically at the Hiroshima University Hospital. The patients had undergone laparoscopic cholecystectomy (n = 12) and inguinal hernia repair (n = 10). The following exclusion criteria were applied: previous history of bowel resection, antibiotic treatment the month before the study, allergy to beta-lactams, and evidence of diabetes mellitus.

#### Experimental protocol

The patients were divided into 2 groups, each of which was given an antimicrobial prophylaxis treatment (Fig. 1). One group (n = 11) was given cefazolin at a daily dose of 3.0 g (1.0 g × 3). Members of the

second group (n = 11) took sulbactam/ampicillin at a daily dose of 4.5 g (1.5 g × 3). The antibiotics were administered upon induction of anaesthesia and continued up to 48 hours after surgery.

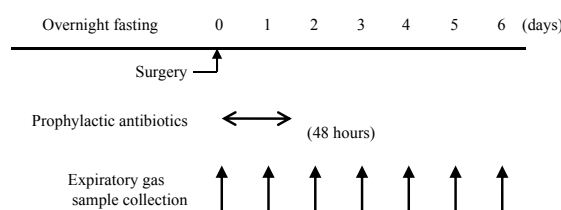


Fig. 1. Experimental protocol

### Hydrogen breath test

End expiratory breath samples were collected the morning of the day of surgery, and then every morning for 1-6 days following surgery. Patients fasted until the following morning (more than 12 hours) after their evening meal. Samples were obtained using the GaSampler System (QuinTron Instruments, Milwaukee, WI) as described previously.<sup>46)</sup> The patients were instructed to exhale as deeply as possible to obtain alveolar air directly into the apparatus via a mouthpiece. A 5 ml aliquot of each breath sample was transferred to a silicone-greased plastic syringe fitted with a 3-way plastic stopcock. Hydrogen concentrations were measured using a gas chromatograph (HCMA-T1®, Abilit Corporation, Osaka, Japan). The data was presented as normalized breath hydrogen concentrations in ppm.

### Statistical analysis

All measured results were expressed as mean concentrations. The data was analysed using the Student's *t*-test and *p* < 0.05 indicated a significant difference.

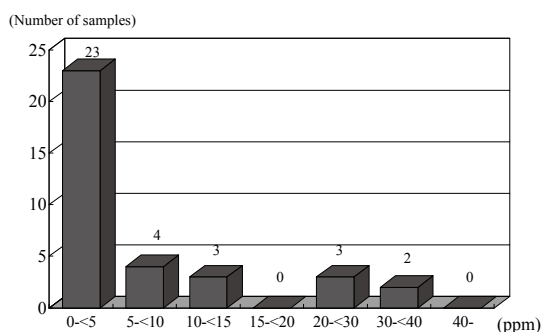
### Ethical considerations

This study was approved by the Medical Ethics Committee of the Hiroshima University School of Medicine and signed informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki.

## RESULTS

### Basal analysis: fasting breath hydrogen data in healthy Japanese subjects

The breath H<sub>2</sub> concentrations of 35 healthy subjects were determined after overnight fasting (Fig. 2). The 5 subjects with an increase in H<sub>2</sub> concentration of more than 25 ppm were classified as having diabetes. The 23 subjects with an increase of less than 10 ppm were classified as normal metabolizers.



**Fig. 2.** Fasting breath hydrogen data in healthy Japanese subjects. The number of healthy subjects (y-axis) grouped against the breath hydrogen concentration in ppm (x-axis).

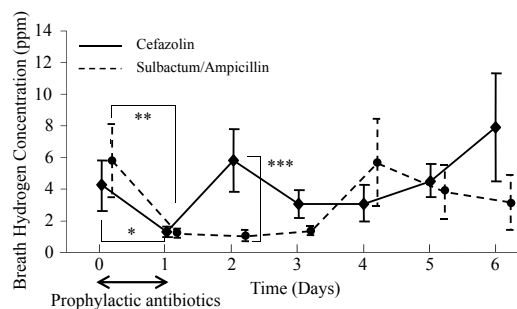
### The effect of antimicrobial agents on breath hydrogen

The subjects were matched according to weight (cefazolin,  $56 \pm 5$  kg; sulbactam/ampicillin,  $51 \pm 7$  kg) and age (cefazolin,  $54 \pm 3$  years; sulbactam/ampicillin,  $49 \pm 6$  years) (Table 1). None of the patients classified as normal metabolizers were excluded. As no adverse effects due to antibiotic use were observed, all patients completed the study.

Changes in the daily breath  $H_2$  concentrations that were observed after receiving the 2 antibiotics are shown in Fig. 3.  $H_2$  concentration before antibiotic administration was not significantly different from that on the day of surgery (day 0; cefazolin:  $4.6 \pm 1.8$  ppm vs. sulbactam/ampicillin:  $6.0 \pm 2.4$  ppm). Breath  $H_2$  concentration significantly decreased in each group on day 1 (cefazolin:  $1.20 \pm 0.39$  ppm vs. sulbactam/ampicillin:  $1.17 \pm 0.34$  ppm). On day 2, the sulbactam/ampicillin group had a significantly lower  $H_2$  concentration (cefazolin:  $6.4 \pm 2.2$  ppm vs. sulbactam/ampicillin:  $1.0 \pm 0.4$  ppm,  $p < 0.05$ ). There was no significant difference in the breath  $H_2$  concentration of the cefazolin group between day 0 and day 2.  $H_2$  concentration was still lower in the sulbactam/ampicillin group ( $1.3 \pm 0.3$  ppm) when compared with the cefazolin group ( $3.3 \pm 1.0$  ppm,  $p = 0.10$ ) on day 3. On days 4-6, there was no significant difference in the breath  $H_2$  concentrations of the two groups.

**Table 1.** Comparison of the cefazolin and sulbactam/ampicillin groups

	Administrated antimicrobial agents	
	Cefazolin	Sulbactam/Ampicillin
No. of patients	11	11
Median age, range (years)	54(38-71)	49(22-72)
Sex ratio, M/F	6/5	6/5
No. of patients consisted of surgery		
Inguinal hernia repair	3	0
Laparoscopy assisted cholecystectomy	8	3
Hemorrhoids	0	1
Resection of small intestine (Crohn's disease)	0	7



**Fig. 3.** Mean  $\pm$  SEM breath hydrogen concentrations (ppm) from 22 patients that received cefazolin ( $\blacklozenge$ ) or sulbactam/ampicillin ( $\bullet$ ) for prophylaxis during and following surgical procedures. \*: day 0 vs. day 1 in the cefazolin group ( $p = 0.05$ ), \*\*: day 0 vs. day 1 in the sulbactam/ampicillin group ( $p < 0.05$ ), \*\*\*: cefazolin vs. sulbactam/ampicillin on day 2 ( $p < 0.05$ ).

## DISCUSSION

Hydrogen is not a by-product of the mammalian cell metabolism. It is only formed in the body due to bacterial fermentation of carbohydrates in the colon. The anaerobic fermentation of carbohydrates results in the production of carbon dioxide, methane, and hydrogen. These gases are consumed by bacteria or are quickly absorbed into the blood stream.<sup>20,29-31,39</sup> Therefore,  $H_2$  production in individuals can be studied by means of a breath test using lactulose (4-*O*-b-D-galactopyranosyl-D-fructose) as a substrate.<sup>16-18,38</sup> This synthetic carbohydrate is not absorbed in the small intestine and is thereby, fermented in the colon. The fermentation and subsequent metabolic processes result in the production of gasses that are absorbed by the colonic mucosa and eventually exhaled. Therefore, breath  $H_2$  measurements provide a semi-quantitative assessment of the quantity of soluble carbohydrate reaching the colon.<sup>4,40</sup>

Levine et al.<sup>34</sup> measured breath  $H_2$  concentration to determine the association between individual faecal microflora and the fermentation of dietary fibres. They were able to associate anaerobic species with  $H_2$  production, suggesting that breath  $H_2$  concentration reflected the activity of anaerobes in the large intestine. Previous measurements regarding the activity of colonic anaerobes were based on the bacterial counts in faeces or mucosal tissues.<sup>48,50</sup> However, a faecal sample from a patient is not easy to collect and the costs of the counts are high. Moreover, bacterial counts do not always reflect the activity of the flora.

The healthy volunteers in this study had breath  $H_2$  concentrations of 0-40 ppm. As most cases were within 10 ppm, the baseline concentration was stable in many patients. However, high  $H_2$  concentrations (more than 25 ppm) were observed in

some cases. These patients were classified as diabetic, since glucose metabolic abnormalities exert a great influence on the concentration of fasting breath  $H_2$ .<sup>40)</sup>

The  $H_2$  breath test was then used to evaluate the influence of antibiotics on colonic flora during surgical prophylaxis. Many antibiotics can affect the colonic flora,<sup>9,33,36,41,48,50)</sup> sometimes leading to adverse outcomes such as *Clostridium difficile* colitis.<sup>1,9,11,19,26,28,35,37)</sup> The elimination of anaerobes should be avoided to prevent such side effects. Another issue is resistant organisms. It has been suggested that colonic anaerobes are a reservoir of resistant organisms<sup>12)</sup> and that prolonged antimicrobial treatment is a major cause for the development of resistance.<sup>2,14,15,23,27,37,49,52,53)</sup> Supporting this view is the finding by Harbarth et al. that associated an increased risk of antibiotic resistance with more than 48 hours of antibiotic prophylaxis.<sup>24)</sup>

Guidelines for the use of surgical prophylaxis recommend that antibiotics should be used within 24 hours of surgery. However, a recent analysis in the U.S. demonstrated that only 40.7% of the surgeons stopped prophylactic antimicrobials within 24 hours after surgery and that 26.7% used prophylaxis beyond 48 hours after surgery.<sup>10)</sup> Many surgeons tend to use antibiotics longer than the period recommended by several published guidelines.

In this study, we chose cefazolin and sulbactam/ampicillin for surgical prophylaxis. Cefazolin is a first generation cephalosporin that acts against aerobes,<sup>37,51)</sup> while sulbactam/ampicillin covers both aerobes and anaerobes.<sup>26)</sup> After only 24 hours of administration, both antibiotics had significantly decreased breath  $H_2$  concentrations, suggesting that both antibiotics could influence the activity of colonic anaerobes.

The reason behind cefazolin's ability to affect anaerobes is unknown. While Akagi et al.<sup>3)</sup> reported that a 3-day administration of cefazolin did not influence total counts of colonic anaerobes, some anaerobic species such as *Bacteroides*, *Eubacterium*, *Lactobacillus*, *Veillonella*, and *Bifidobacterium* were significantly decreased. These results suggest that the first generation cephalosporin might affect colonic anaerobes.

The difference in the effects of cefazolin and sulbactam/ampicillin was observed for 48 hours after surgery. Patients in the cefazolin group recovered from the decrease in  $H_2$  concentration 48 hours after surgery. In contrast, suppression of  $H_2$  concentration in the sulbactam/ampicillin group was not relieved until 4 days after surgery, finally recovering after an additional 72 hours. The anaerobic coverage by this antibiotic is thought to be the reason for the delayed recovery. It is possible that the prolonged suppression of colonic anaerobes by sulbactam/ampicillin might lead to the development

of drug-induced colitis or resistant organisms.

The use of  $H_2$  measurements to assess absorption is painless and the closed system is well tolerated by patients. The measurement of  $H_2$  concentration using gas chromatography is simple and takes only about 3 min. The drawbacks to the widespread use of this technique include the need for relatively expensive equipment and the time required for each study, which consists of sampling every morning (after overnight fasting) to measure the  $H_2$  response to antimicrobial load. In addition, the results of the test can be difficult to interpret. Understanding the factors that influence  $H_2$  production and excretion could have important clinical implications and provide basic information on the regulation of the colonic ecosystem. A better understanding of  $H_2$  physiology would also facilitate a more accurate interpretation of the  $H_2$  breath tests that are widely used for the study of carbohydrate malabsorption, small-bowel transit time, and bacterial overgrowth.<sup>5-8,25,32,42-45,47)</sup>

In this study, breath hydrogen was used as a relative evidence of the bacteriological condition of the colon. As the test is simple, this study could indicate new therapeutic approaches to surgical antibiotic prophylaxis. However, more experiments that assess intestinal microflora using the  $H_2$  breath test are needed because the data obtained in this study was based on a small sample size. Ultimately, further studies will be required to determine the selection and duration of prophylactic antimicrobial agents to prevent postoperative infection and the emergence of resistant organisms.

In summary, we evaluated the influence of cefazolin and sulbactam/ampicillin on the intestinal microflora, and found that the concentration of breath hydrogen was only slightly suppressed under cefazolin treatment when compared to the sulbactam/ampicillin treatment. As these results suggest that both antibiotics influence the activity of colonic anaerobes, the duration of surgical antibiotic prophylaxis should be kept as short as possible.

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