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Headspace screening of fluid obtained from the gut during colonoscopy and breath analysis by proton transfer reaction-mass spectrometry: A novel approach in the diagnosis of gastro-intestinal diseases

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Abstract

Background: The diagnosis of many gastro-intestinal diseases is difficult and can often be confirmed only by using invasive diagnostic means. In contrast, the headspace screening of fluid obtained from the gut during colonoscopy and the analysis of exhaled air may be a novel approach for the diagnosis of these diseases.
Materials and methods: The screening was performed by using proton transfer reaction-mass spectrometry (PTR-MS) which allows rapid and sensitive measurement. Fluid samples obtained from the gut during colonoscopy were collected from 76 and breath samples from 70 subjects. Mass spectra of healthy controls were created. Afterwards these spectra were compared with those of patients suffering from inflammatory bowel diseases (IBD; Crohn's disease and ulcerative colitis; $n = 10$) and irritable bowel syndrome (IBS; $n = 7$).
Results: Significant differences in the mass spectra could be observed both in the headspace of the fluid and in the exhaled air comparing patients with healthy controls.
Conclusions: This study is the first describing headspace screening of fluid obtained from the gut during colonoscopy, possibly presenting a novel diagnostic tool in the differential diagnosis of gastro-intestinal diseases.
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Keywords: Mass spectrometry; PTR-MS; Colonoscopy; Breath test; Fluid; Inflammatory bowel disease; Irritable bowel syndrome

1. Introduction

Over the past decade, the analysis of volatile organic compounds (VOC) has witnessed an enormous boost. Several studies dealing with this topic demonstrated that various diseases, e.g. lung cancer [1,2], breast cancer [3], schizophrenia [4] and recent smoking behaviour [5], [6] are associated with a specific VOC-profile either in human exhaled air, or in the headspace of body fluids [7]. These patterns differ explicitly from that of healthy subjects and are specific for the mentioned diseases.

Whereas breath analysis has been performed in numerous studies, no publication describes the mass spectrum in the headspace of fluid obtained from the gut during colonoscopy. This fluid is obtained easily and without additional risks or expenses in the course of the procedure. Normally being discarded at the end, the analysis of these spectra could possibly represent a novel methodical approach in diagnosing gastro-intestinal diseases and/or finally contribute to a better understanding of their pathophysiology.

The aim of our current study is to describe the mass spectrum in the headspace of the "colonoscopy-fluid", and in the exhaled air of healthy controls, and to compare this pattern with that of patients suffering from inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS). It might become possible to attribute a specific mass spectrum to both

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inflammatory bowel diseases and irritable bowel syndrome and to allow a rapid diagnosis by measuring real-time and analyzing the mass spectrum within minutes. In contrast, conventional methods, e.g. the histopathological evaluation of a biopsy, are far more time consuming.

2. Materials and methods

2.1. Patients

All patients who were enrolled in the study underwent elective colonoscopy. The preparation for the procedure included the application of a laxative the day before and all patients had to fast over night. Before the colonoscopy test subjects were asked to exhale into a sample bag (Adtech, Gloucestershire, UK) as previously described [8]. In brief, patients had to discard the first part of expired air and exhaled only the deep portion into the sample bag. Then colonoscopy-fluid was obtained from the gut and filled into glass tubes (Hecht Assistent, Germany). The tubes were stored at 8 °C for a maximum of 24 h before the measurement was performed.

Fluid samples were collected from 76 subjects, and breath samples from 70. Ten patients suffered from inflammatory bowel diseases (IBD; Crohn's disease, ulcerative colitis; $n = 10$, mean age = 40 years, S.D. = 11.4). Diagnosis was confirmed by biopsy and histopathological evaluation. The second group included patients with irritable bowel syndrome (IBS; $n = 7$, mean age = 47 years, S.D. = 17.72). A reliable diagnosis of IBS is extremely difficult [9]. Thus, only the suspicion was considered an including criteria. The healthy controls (fluid sample: $n = 59$, mean age = 59 years, S.D. = 12.4; breath sample: $n = 53$, mean age = 60 years, S.D. = 11.95) underwent a routine colonoscopy. None had intestinal diseases and none had a noted infection with *Helicobacter pylori*.

2.2. Analysis

Fluid samples were diluted 1:2 with distilled water. Both the fluid samples and the air samples were heated to 37 °C. The gas in the headspace of the fluid and the air in the sample bags were siphoned off by a heated Teflon tube and fed into the apparatus. The analysis of samples was performed using proton transfer reaction-mass spectrometry. This technique uses H_3O^+ as a chemical ionization reagent to measure volatile compounds in the parts per billion by volume (ppbv) to parts per trillion by volume (pptv) range. Protonated water, H_3O^+, reacts with neutral molecules (M) according to $H_3O^+ + M \rightarrow MH^+ + H_2O$. This reaction only occurs if these neutral molecules have larger proton affinities than H_2O. Almost all compounds have larger affinities and therefore proton transfer occurs on every collision with rate constants k, having typical values of $1.5 \times 10^{-9}\ cm^3\ s^{-1} < k < 4 \times 10^{-9}\ cm^3\ s^{-1}$. Due to fragmentation (most commonly by the loss of a thermodynamically favoured neutral molecule like H_2O), it is possible that additional ions are generated in the drift tube. The count rate of ions is determined in the ion detection system. There is a linear relationship between the recorded normalized count rate of ions at different m/z values (according to our settings: 21–229) and the concentration of M in the original trace gas, so that the latter can be calculated. The formula includes the normalized count rate of ions and of the primary ion H_3O^+, the temperature in the drift tube, the pressure in the drift tube, the mass dependent transmission efficiency, the rate constant, and the reaction time [10].

After baseline conditions had been established, each sample was measured at least three times and the calculated average of these measurements was used for further statistical analysis.

2.3. Statistics

Statistical analysis was performed using the software package SPSS (v.11.0, Chicago, USA). The calculated concentrations are expressed as mean ± S.D. in parts per billion volume (ppbv) for each subgroup. Statistical significance of difference in the calculated concentrations between the groups was determined with the unpaired t-test after obtaining normal distribution in each group. Statistical significance was assumed at $p < 0.05$, and $p < 0.01$ were considered highly significant.

3. Results

Initially proton transfer reaction-mass spectra in the headspace of the fluid and in the exhaled air of healthy controls were created. These spectra were compared with those of patients suffering from IBD and IBS. The concentration of ions at 209 different m/z values was evaluated. For the most part, the corresponding substances are thought to be hydrocarbons, in particular alkanes, alkenes, alcohols, ketones, and organic acids. The mean concentration is given in parts per billion volume (ppbv).

In the headspace of the fluid which was obtained from the gut during colonoscopy ions at 12 certain m/z values were found consistently in all 59 healthy controls, namely ions at m/z values of 31, 33, 41, 43, 45, 47, 55, 59, 60, 61 and 73. The proton transfer reaction-mass spectrum in the headspace of the sample fluid of the average healthy subject is demonstrated in Fig. 1.

Comparing the previously mentioned profile of healthy controls with that of patients suffering from IBD, the concentrations of ions at the m/z value of 57 and 83 showed an increase that was highly significant ($p < 0.01$; Table 1). The concentration of ions at the m/z value of 46 and 47 were also found to be elevated (NS). In Fig. 2, the mean concentration of the ions at the aforementioned m/z values corresponding to the two different collectives are presented. In contrast to the above mentioned results, no significant differences were

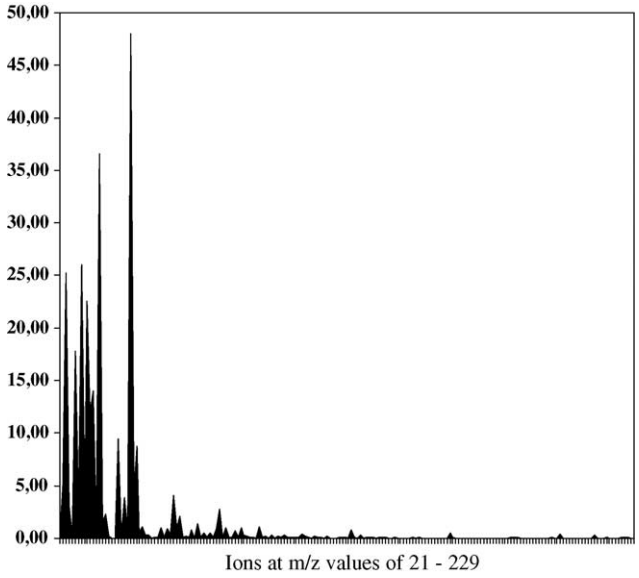

Fig. 1. Proton transfer reaction-mass spectrum of the average healthy subject (control group) in the headspace of fluid obtained from the gut during colonoscopy. *X*-axis: ions at *m*/*z* values of 21–229; *Y*-axis: calculated concentration [ppbv].

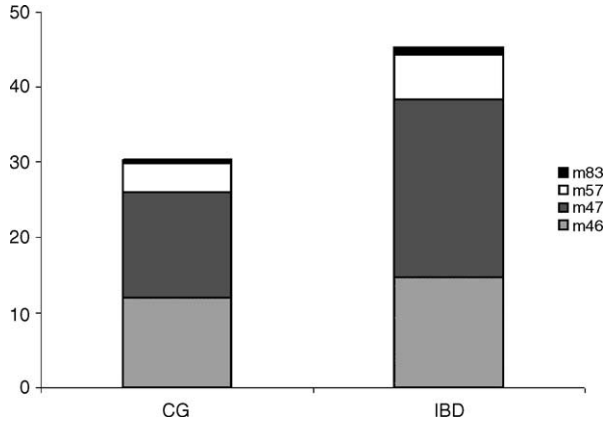

Fig. 2. Calculated mean concentrations [ppbv] of ions at the *m*/*z* value of 46, 47, 57, and 83 in the headspace of fluid obtained from the gut during colonoscopy comparing control group (CG) to patients suffering from inflammatory bowel diseases (IBD).

found after correlating the two spectra (healthy controls versus IBD) in exhaled air.

In addition to IBD, we also included patients with IBS in our current study. The comparison of the fluid patterns obtained from healthy controls with that of patients suffering from IBS showed no significant difference. But using exhaled air, the concentration of ions at the *m*/*z* value of 31 was found to be significantly elevated in the IBS group. An equal trend was observed regarding the concentration of ions at *m*/*z* 77 (Table 2).

4. Discussion

This study is the first describing the headspace proton transfer reaction-mass spectrum of fluid obtained from the gut during colonoscopy. The following could be observed: only ions at 12 (of the 209 evaluated) *m*/*z* values were detected in all subjects. Ions at each of the 209 evaluated *m*/*z* values (with the exception of *m*/*z* 179) could be found in the headspace of at least one sample, even though partly in very low concentrations. The results also show that the concentration of ions at certain *m*/*z* values showed large inter-individual differences in healthy subjects.

It should be kept in mind that "colonoscopy-fluid" represents residual human stool, remaining in the lumen of the gut after routinely performed laxation. These residuals are obtained from the gut during colonoscopy. The variations in the composition between samples mentioned before are mainly influenced by four factors: food intake, bacterial flora in the gut, their metabolites and by the exchange of substances between the intestinal mucosa and the lumen. It is important to take note that nutrition influences the composition of the bacterial metabolites significantly [11].

One similar study should be mentioned, describing the headspace screening of human stool. Probert et al. collected stool samples from 35 patients suffering from infectious diarrhoea and from six healthy controls. Depending on the causative organisms, different specific patterns could be de-

Table 1
IBD group vs. control group (fluid samples)

m/*z*	IBD				Control group				*P*	*F*	Variance of homogenity
	n	Mean	S.D.	S.E.	*N*	Mean	S.D.	S.E.			
57	10	5.95	2.5	0.79	59	3.9	2.16	0.28	0.008	0.718	+
83	10	1.06	0.56	0.18	59	0.49	0.58	0.08	0.006	0.032	+

Table 2
IBS group vs. control group (exhaled air)

m/*z*	IBD				Control group				*P*	*F*	Variance of homogenity
	n	Mean	S.D.	S.E.	*N*	Mean	S.D.	S.E.			
31	7	13.48	3.85	1.46	53	9.97	4.23	0.58	0.042	0.41	+
77	7	9.95	6.18	2.34	53	5.76	6.6	0.91	0.118	0.168	+

scribed and raise hope that in the near future infectious diarrhoea can be diagnosed faster using this novel tool [13].

In our case, the proton transfer reaction-mass spectrum was created from volatile compounds in the headspace of fluid obtained from healthy controls serving as a reference in order to be compared with the samples obtained from patients with gastro-intestinal diseases, such as IBD and IBS. As described, the concentration of ions at the *m*/*z* value of 57 and 83 were highly significantly elevated comparing fluid samples from healthy controls to patients with IBD. Due to the limitation of the PTR-MS method, we can just speculate about the origin of these ions. Butene or acroleine might possibly contribute to the concentration of the ions at the *m*/*z* value of 57. But it seems that there is no relation between the pathogenesis of inflammatory bowel diseases and buthene or acroleine. However, the recent literature supposes that hydrogen sulphide (H_2S) and other sulphur compounds seem to play an important role in the pathogenesis of these diseases [14–16]. Both in this case or in the case of *m*/*z* 57 and 83, a conclusive statement can only be made if ions at the different *m*/*z* values are identified.

In exhaled air, differences in the proton transfer reaction-mass spectrum between healthy controls and patients with IBD were not observed. Moreover, in exhaled air the standard deviation was much higher. Therefore, no conclusive statement can be made.

Contrary to our expectations, no significant differences between healthy subjects and patients with IBS were found using fluid samples. The presumption that significant changes in the mass spectrum could be observed does not seem unfounded, especially because modifications in the composition and colonization of the intestinal flora have previously been described [9,17]. But comparing breath samples, the concentration of ions at *m*/*z* 31 was found to be significantly elevated in the IBS group. These ions most probably represent protonated formaldehyde or ethane and the ions detected at *m*/*z* 77 could be protonated acetone with an attached water molecule.

Finally, some potential limitations of the study should be briefly addressed. The sample sizes are partly rather small but taking the proportion of the sample sizes between healthy controls and patients into account, the statistically confirmed differences appear to be more relevant. One limitation of the method is the fact that the ions at certain *m*/*z* values can represent various substances or rather fragments. The use of a gas chromatography–mass spectroscopy (GC–MS) in combination with PTR-MS would help contribute to a more detailed identification of these substances. However, PTR-MS has been shown an excellent screening tool before [18], featuring a rapid real-time measurement and a highly sensitive analysis.

5. Conclusion and perspectives

In the future, it might become possible to collate a specific mass spectrum to the above-mentioned diseases. Nevertheless, much effort has to be made and the reliability of this approach has to be proven. Different methods like GC–MS (gas chromatography–mass spectroscopy) will help identify the exact substances and contribute to the basic understanding of gastro-intestinal diseases.

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