Salivary Level of Volatile Fatty Acids as an Index of the Status of the Human Oropharynx - Normal Microflora System

V. N. Babin, N. V. Chen, O. A. Kondrakova, Yu. I. Lyakhovetskii, I. V. Dobryakova, N. F. Dmitrieva, and A. V. Dubinin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 494-496, May, 1995
Original article submitted May 23, 1994

Gas-liquid chromatographic determination of the levels and profiles of volatile fatty acids (acetic, propionic, and butyric acids) in saliva samples from healthy volunteers and patients with acute or chronic tonsillitis revealed lowered concentrations of these acids in the saliva of patients and the presence of disease-specific fatty acid profiles.

Key Words: *volatile fatty acids; microbiocenosis; anaerobic microflora; epithelium; metabolism; sugar fermentation*

Recent years have witnessed the emergence of a new approach to the correction of dysbiotic shifts in various biotopes of the human body. This approach relies on the knowledge of molecular mechanisms underlying the transition from symbiotic relationships, which maintain the epithelium-autochthonous microflora system in a state of metabolic equilibrium, to dysbiotic relationships that disrupt this equilibrium. In this connection, increasing interest is being shown in relatively simple bacterial metabolites, in particular volatile fatty acids, including acetic, propionic, and butyric acids, produced in large quantities by autochthonous saccharolytic anaerobic microorganisms such as *Bacteroides, Clostridium, Propionebacterium, Fusobacterium,* and *Bifidobacterium.* A balanced exchange of metabolites is presumed to occur between the epithelium and autoflora, with oligosaccharide fragments of mucosal glycoproteins serving as food substrates for saccharolytics and the volatile fatty acids (VFA) they produce serving as respiratory metabolites for epithelial cells. Moreover, it is believed by some - and this belief is supported by *in vitro* experiments - that the energetics of the large intestine epithelium is largely based on metabolic products of symbiotic saccharolytic microflora [1,2].

To what extent the mechanism outlined above may be a general one, i.e., whether it also operates in other biotopes of the human body, in particular the oropharynx, remains unknown. VFA are present in the oropharyngeal secretion [3,4], and it is therefore interesting to find out whether and how their production changes in disease states.

In the present study, gas-liquid chromatography (GLC) was used to measure the levels and proportions of acetic, propionic, and butyric acids in the total pool of monocarboxylic acids contained in the saliva of healthy volunteers and patients with acute or chronic tonsillitis.

MATERIALS AND METHODS

Fasting saliva samples were collected in the morning from healthy volunteers, patients with acute (follicular or lacunar) tonsillitis, and individuals

Ultrasan Research Company, Moscow. (Presented by I. V. Domaradskii, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0005-0477$12.50

Table 1. Levels of Major VFA in Saliva Samples from Healthy Subjects and Patients with Tonsillitis

Group	No. of samples	VFA, μmol/ml				Percent of normal value		
		AA	PA	BA	Σ	AA	PA	BA
Healthy	29	11.2±2.3	2.4±0.5	0.4±0.08	14.0±2.88	100	100	100
Acute tonsillitis	19	6.0±1.6	1.7±0.5	0.3±0.07	8.0±2.17	54	71	75
Chronic tonsillitis	13	6.9±1.5	1.1±0.2	0.1±0.03	8.1±1.73	62	46	28

Note. Here and in Table 2: *AA* = acetic acid, *PA* = propionic acid, *BA* = butyric acid.

with chronic tonsillitis. All samples were stored frozen until analyzed by GLC.

The analyses were performed on a chromatograph with a flame ionization detector using a 3 mm×1.2 m stainless steel column packed with 15% Carbowax 20 M modified by 1.5% terephthalic acid and with N-AW Chromaton (0.16-0.20 mm). The temperature of the column thermostat was 85°C and that of the evaporator 200°C. The carrier gas was nitrogen having a pressure of O.57 atmosphere at the column entry; its flow rate was 35 ml/min.

Saliva samples of 1-1.5 ml were each diluted with distilled water to a volume of approximately 20 ml and then alkalized with 0.01 N NaOH solution to pH 9-10 using a general-purpose indicator. The water was removed with a rotor evaporator and 3 ml of ether that had been passed through an aluminum oxide layer of second-degree activity (after Brockman) were added to the dry residue. The ether was then acidified with 90% sulfuric acid to pH 3, care being taken to prevent the formation of a second layer. The mixture components were thoroughly mixed. Next, 1-2 μl of the material thus prepared was introduced into the evaporator of the chromatograph. Finally, 1 ml of an ether solution containing a known amount of butyric acid was added to each sample and the samples were placed in the chromatograph. Details of the procedure used to process the results will be given in a separate communication.

RESULTS

Saliva samples were found to contain acetic, propionic, isobutyric, butyric, isovaleric, valeric, and caproic acids. The first five of these metabolites were detected in all samples. The largest quantitative contributions to the total pool of monocarboxylic acids were made by acetic, propionic, and butyric acids (Table 1).

Total concentrations of these three acids in saliva samples from normal subjects ranged from 11 to 17 μmol/ml (values comparable to those reported in the literature [4]), but were significantly lower in samples from patients with acute or chronic tonsillitis. This may be taken as evidence that anaerobic fermentation of sugars is inhibited in tonsillitis and also, probably, as an indication of altered infrastructure of the microbiocenosis in this state. As shown in Table 1, the greatest differences from values obtained for normal subjects occurred in the concentration of acetic acid in acute tonsillitis and in the concentrations of propionic and butyric acids in chronic tonsillitis.

Within each group, differences between samples in the concentration ratios of individual acids to their total concentration, unlike differences in the concentrations themselves, were slight, which means that the statistical significance of the differences between these ratios is higher. Each of the three states (i.e., health, acute tonsillitis, and chronic tonsillitis) was found to have a characteristic profile of the three major VFA (Table 2). As can be seen in this table, the concentration ratio of propionic plus butyric acid to acetic acid is increased in acute tonsillitis and decreased in chronic tonsillitis relative to that in health.

Propionic acid is produced in the process of propionic fermentation by anaerobic saccharolytic microorganisms from the genera *Bacteroides* and

Table 2. Profiles of Major VFA in Saliva Samples from Healthy Subjects and Patients with Tonsillitis

Group	No. of samples	Proportions of VFA			(PA+BA):AA ratio
		AA	PA	BA	
Healthy	29	0.793±0.002	0.172±0.10	0.035±0.002	0.26
Acute tonsillitis	19	0.738±0.004	0.232±0.003	0.030±0.008	0.35
Chronic tonsillitis	13	0.829±0.003	0.147±0.003	0.024±0.004	0.21

Propionebacterium (the latter organisms can be cultured from the oropharynx only very rarely). Bacteroides possess cell-associated neuraminidases and are capable of splitting off terminal sugars from the glycoproteins of saliva and of the glycocalyx. The main products of sugar fermentation by various bacteroides are acetic, propionic, and succinic acids. The last acid is a propionate precursor in the propionic pathway of fermentation, which is a typical pathway for bacteroides. This suggests that the major producers of propionic acid are members of the *Bacteroides* genus. In the oropharynx this genus is represented by *B. oralis*, *B. melaninogenicus*, *B. gingivalis*, *B. ruminicola*, and some other species [5]. Sources of butyric acid in the oropharynx may be fuso- and eubacteria which can be cultured from this biotope. Possibly, butyric acid is also formed by clostridia inhabiting gingival pockets. In butyric fermentation, acetic acid is usually produced in addition to butyric acid. Acetic acid can be generated in any type of saccharolytic fermentation.

Thus, individual VFA are most likely to be produced by different bacterial species, so that the activities of particular fermentation pathways may change in an independent manner when dysbiotic shifts occur. It is therefore possible to gain information on the relative contributions of individual anaerobic sugar fermentations to the total VFA pool.

Acetic acid can be regarded as a more oxidized "aerobized" metabolite than propionic or butyric acid (the atomic ratio of carbon to oxygen in acetic acid is 1 as compared to 1.5 and 2 in propionic and butyric acids, respectively), and the concentration ratio of propionic and butyric acids to acetic acid (Table 2) may therefore be used as an index of the extent to which this system of acids is anaerobic. It is seen in Table 2 that the index is shifted to the anaerobic side in acute tonsillitis and to the aerobic side in chronic tonsillitis. These findings suggest a greater inhibition of the propionic and butyric fermentation pathways in chronic tonsillitis, which may be a consequence of alterations in the infrastructure of the oropharyngeal microbiocenosis as a result of the inhibition of propionic and butyric acid producers.

The higher relative concentrations of propionic and butyric acids in acute tonsillitis against a background of inhibited aerobic fermentation do not probably signify activation of propionic and butyric fermentations, but may be attributed to a selectively reduced consumption of these two acids by epithelial cells to meet the needs of epithelial energy metabolism or to the suppression of the latter in cases of mucosal inflammation or atrophic changes.

This view is consistent with the reported altered strategy of cell metabolism in the large intestine epithelium affected by inflammation [6]. It has been shown that intestinal inflammation is associated with "autonomization" of epithelial metabolism in the large intestine, whereby the epithelium ceases to consume the respiratory substrates such as butyrate supplied by anaerobic microflora. Conceivably, the oropharyngeal epithelial tissues, which are mainly represented by stratified epithelium, have a greater need for these metabolites than does the unstratified columnar epithelium of the large intestine, since nutrients from the regional bloodstream find it more difficult to diffuse to the former epithelium than to the latter.

REFERENCES

1. A. V. Dubinin, P. M. Raevskii, B. V. Kirkin, *et al., Klin. Med.*, № 7, 140-143 (1987).
2. J. H. Cummings, *Gut*, **22**, 763-779 (1981).
3. J. H. Cummings, N. J. Neil, and W. P. T. James, *Gut*, **19**, 819-822 (1978).
4. A. Heimdahl and C. E. Nord, *Scand. J. Infect. Dis.*, **11**, 233-242 (1979).
5. M. G. Newman and R. Nisengard, in: *Oral Microbiology and Immunology*, Philadelphia (1988), pp. 111-128.
6. W. E. W. Roediger, Gut, **21**, 793-798 (1980).