Increased pentane and carbon disulfide in the breath of patients with schizophrenia

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Abstract

Aims—To determine the concentrations of pentane (a marker of lipid peroxidation) and other volatile organic compounds in the breath of patients with schizophrenia.

Methods—Volatile organic compounds were assayed by gas chromatography/mass spectroscopy (GC/MS) in 88 subjects—25 with acute schizophrenic psychosis, 26 with psychiatric disorders other than schizophrenia, and 37 normal volunteers.

Results—The mean alveolar gradients of pentane and carbon disulfide (CS2) were significantly higher in the patients with schizophrenia than in the control groups.

Conclusions—Schizophrenia may be accompanied by accelerated lipid peroxidation in cell membranes, as well as increased manufacture of CS2, a known neurotoxin.

Schizophrenia is a common and devastating psychotic illness: it affects nearly 1% of the population of all cultures, and often culminates in severe disability and premature death.1-4 The aetiology of schizophrenia is still unknown, and researchers have used many different investigative techniques to determine a biochemical basis for the disordered cognition and behaviour that characterise the disease. Kovaleva et al assayed the breath of acutely psychotic schizophrenic patients and found raised concentrations of pentane which varied with the clinical severity of the condition, then rapidly returned to normal during a course of treatment.5,6

Alkanes in the breath (principally ethane and pentane) result from cellular injuries which cause an intracellular accumulation of oxygen free radicals and accelerated peroxidation of polyunsaturated fatty acids.7,8 The peroxidation of lipids may result in membrane injury, with dysfunction and death of the affected cells. Raised alkane concentrations in the breath have been reported in patients with a number of conditions, including acute myocardial infarction,9 rheumatoid arthritis,10 and nutritional deficiencies of vitamin E.11 The toxic effects of oxygen free radicals may represent the final common pathway of several different pathogenic agents, including chemical toxicity, inflammation, and ischaemia.

Methods

The method for collection of volatile organic compounds has been described before.12 A mobile apparatus was used which sampled 10 litres of alveolar breath over 5 minutes while the donor was breathing in chemically purified air. The breath was drawn through a stainless steel trap in which the volatile organic compounds were captured by adsorption to activated carbon and molecular sieve.

The volatile organic compounds captured from alveolar breath were thermally eluted from the trap in a microprocessor controlled automatic desorber, concentrated by two stage cryofocusing at −150°C, then assayed by gas chromatography and mass spectroscopy using an ion-trap detector. Each volatile organic compound was identified by its mass spectrum, quantified by area under curve, and its alveolar gradient was determined (concentration in alveolar air minus concentration in inspired air). Standard curves for volatile organic compounds were obtained using adsorptive traps loaded with vapour standards prepared by the method of Morris et al.13

Table 1 Diagnostic categories of non-schizophrenic psychiatric disorders

<table>
<thead>
<tr>
<th>DSM-III-R Category</th>
<th>Diagnosis</th>
<th>No of subjects</th>
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</thead>
<tbody>
<tr>
<td>296.3</td>
<td>Major depression, recurrent unspecified</td>
<td>5</td>
</tr>
<tr>
<td>296.33</td>
<td>Major depression, recurrent, severe, without psychotic features</td>
<td>4</td>
</tr>
<tr>
<td>296.44</td>
<td>Bipolar disorder, manic, with psychotic features</td>
<td>3</td>
</tr>
<tr>
<td>296.53</td>
<td>Bipolar disorder, depressed, severe, without psychotic features</td>
<td>1</td>
</tr>
<tr>
<td>296.54</td>
<td>Bipolar disorder, depressed, with psychotic features</td>
<td>1</td>
</tr>
<tr>
<td>296.62</td>
<td>Bipolar disorder, mixed, moderate</td>
<td>1</td>
</tr>
<tr>
<td>300.40</td>
<td>Dysthymic</td>
<td>1</td>
</tr>
<tr>
<td>305.00</td>
<td>Alcohol abuse</td>
<td>3</td>
</tr>
<tr>
<td>309.00</td>
<td>Adjustment disorder with depressed mood</td>
<td>4</td>
</tr>
<tr>
<td>311.00</td>
<td>Depressive disorder not elsewhere classified</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2 Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal controls</th>
<th>Psychiatric controls</th>
<th>Patients with schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>37</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>37±4 (9-0)</td>
<td>41±0 (10-0)</td>
<td>35±8 (12-3)</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>50</td>
<td>53±1</td>
<td>48</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

No significant differences among the three groups were observed.
Figure 1 Alveolar gradients of pentane. Solid bar = group mean, error bar = standard error of mean.

Figure 2 Alveolar gradients of carbon disulfide. Solid bar = group mean, error bar = standard error of mean.

Eighty eight volunteers were studied at St Vincent’s Medical Center of Richmond and Bayley Seton Hospital in Staten Island, New York. Breath collections were performed between 07 00 h–1100 h. Criteria for acceptance to the study included: (1) willingness to cooperate with the breath collecting procedure; (2) age between 18 and 70 years; and (3) no known acute or chronic medical illness. All subjects gave their signed informed consent to participate in this research, which was approved by the institutional review boards of both institutions.

A group of 37 normal volunteers was drawn from the medical and nursing staff of both institutions. None gave any history of psychiatric illness.

Twenty six subjects were recruited from the psychiatric inpatient units of both hospitals. All had been diagnosed as having a psychiatric illness other than schizophrenia by DSM-III-R criteria (table 1).

Breath samples were collected from 25 subjects who had fulfilled DSM-III-R criteria for the diagnosis of schizophrenia during previous hospital admissions. All had experienced a recent acute psychotic exacerbation that was sufficiently severe to require admission to a psychiatric unit, where they shared the same environment and diet as the psychiatric controls. All had been treated with neuroleptic drugs for at least 24 hours before the breath collection.

Using multiple regression, the alveolar gradient of each volatile organic compound was treated as a dependent variable, and the diagnostic group (schizophrenic, normal, or psychiatric controls) as the independent variable. Where significant differences between groups were observed, the effects of cigarette smoking, race, neuroleptic drugs, sex and age were evaluated as potential contributory factors using multiple correlations. A subgroup of treated patients with schizophrenia (n = 8) was studied a second time before discharge; the two assays were compared using Student’s paired t test.

Table 3 Effects of smoking and neuroleptic drugs on alveolar gradients of pentane and CS₂

<table>
<thead>
<tr>
<th></th>
<th>Normal controls</th>
<th>Psychiatric controls</th>
<th>Acutely psychotic schizophrenic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 11</td>
<td>-10.90 ± 7.16</td>
<td>11.55 ± 4.09</td>
<td>37.27 ± 18.43</td>
</tr>
<tr>
<td>Non-smokers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 26</td>
<td>-2.86 ± 3.58</td>
<td>17.48 ± 10.63</td>
<td>11.33 ± 3.86</td>
</tr>
<tr>
<td>CS₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>-23.57 ± 6.46</td>
<td>-8.61 ± 8.18</td>
<td>10.34 ± 14.12</td>
</tr>
<tr>
<td>Neuroleptic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 0</td>
<td>12</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>CS₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 15</td>
<td>18.80 ± 7.37</td>
<td>23.78 ± 8.31</td>
<td>11.01 ± 8.10</td>
</tr>
<tr>
<td>No neuroleptic drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 37</td>
<td>5.25 ± 2.19</td>
<td>9.15 ± 5.40</td>
<td></td>
</tr>
<tr>
<td>CS₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 37</td>
<td>-23.28 ± 5.34</td>
<td>-5.85 ± 4.72</td>
<td></td>
</tr>
</tbody>
</table>

The mean concentration of pentane and CS₂ in each subgroup is shown, expressed in mmoI/l, with the standard error of the mean in parentheses. There were no significant differences among the three groups between the smokers and the non-smokers, or between the psychiatric controls treated or not treated with neuroleptic drugs.

Results
Breath samples were obtained from all subjects without any adverse effects. Characteristics of the three groups are shown in table 2; no significant differences were observed in the distribution of age or sex.

The alveolar gradients of five volatile organic compounds in the breath were significantly higher in the patients with schizophrenia than in the controls: pentane (p < 0.003) (fig 1); carbon disulfide (CS₂) (p < 0.001) (fig 2); benzene, 2-methylbutane, and tetrachloroethene (p < 0.05). Only CS₂ was significantly increased when the patients with schizophrenia were compared with the psychiatric controls (p < 0.05). No significant difference in the alveolar gradient of either pentane or CS₂ was associated with tobacco smoking, treatment with neuroleptic drugs (table 3), or racial group. No significant changes in any volatile organic compounds were observed in the subjects with schizophrenia who were studied a second time.

Discussion
Microanalysis of the breath opens a non-invasive window on to the chemical composition of the blood. Volatile organic compounds diffuse passively across the pulmonary
alveolar membrane; like water running down a hill, they flow rapidly from the compartment with the higher vapour pressure to the compartment where it is lower. A positive alveolar gradient is evidence that the vapour pressure of a volatile organic compound is higher in the venous blood than in the inspired air, while a negative alveolar gradient shows the opposite.

The mean alveolar gradient of pentane was highest in the acutely psychotic schizophrenic patients, a finding consistent with the study by Kovaleva et al. The lack of a significant difference between the schizophrenic patients and the psychiatric controls may have been a consequence of experimental design. We studied schizophrenic patients who had been treated with neuroleptic drugs for 24 hours or longer, while Kovaleva et al. studied untreated patients who may have been more acutely psychotic with higher concentrations of pentane in their breath.

In addition, the mean alveolar gradient of CS₂ was positive in the patients with schizophrenia and negative in the two control groups. These observations indicate that the vapour pressure of CS₂ in venous blood was significantly increased in the schizophrenic patients. This was an unexpected finding which, to our knowledge, has not been reported before. It is unlikely that the differences in breath CS₂ could have been due to environment or diet, because the patients with schizophrenia and the psychiatric controls all resided in the same ward areas and ate similar meals. Tobacco smoke and some neuroleptic drugs (thioridazine and mesoridazine) contain sulfur, but neither significantly affected the alveolar gradient of CS₂. Nor was breath CS₂ significantly affected by age, sex, or racial group.

It has been known for more than 100 years that industrial workers exposed to high levels of CS₂ can develop acute toxicity with psychiatric manifestations, including acute psychotic episodes with mania, rapid mood changes, extreme irritability, uncontrollable anger, and suicidal tendencies. Magos has suggested that these toxic effects may be due to the reaction of CS₂ with amines or thiols; the resulting chelation of metals (mainly zinc and copper) may inhibit the activity of dopamine β-hydroxylase and cause disturbances of catecholamine metabolism.

The negative alveolar gradient of CS₂ observed in the two control groups was consistent with normal catabolism of CS₂, which can occur via several different pathways, including conjugation with glutathione, formation of dithiocarbamates, and by mono-oxygenase catalysed generation of reactive sulfur. Conversely, the positive alveolar gradient observed in the patients with schizophrenia indicated that more CS₂ had been expired than inspired through the lungs. In the absence of any apparent exposure to CS₂ from other sources, this finding was consistent with endogenous manufacture of CS₂.

The origin of the endogenous CS₂ is still unknown. One possible source is the large bowel, where volatile organic compounds containing sulfur may be produced by the metabolism of micro-organisms. The breath of patients with advanced hepatic cirrhosis and fetor hepaticus contains increased concentrations of various volatile organic compounds, principally methyl mercaptan and dimethyl sulfide. Porto-systemic shunts in patients with cirrhosis have also been associated with increased breath concentrations of short chain fatty acids originating in the large bowel, principally acetic and propionic acids. The manufacture of pentane and CS₂ in the large bowel merits further study as a possible explanation for our observations.

The air inspired from the breath collecting apparatus was partially but not completely purified. Even with a large filtration bed of activated carbon, it was not possible to extract completely all traces of airborne volatile organic compounds present in room air in picomolar concentrations. In practice, this did not present a serious problem, because the level of volatile organic compound background in the inspired air was reduced to a fairly consistent low concentration which neither exhibited any significant fluctuation during the course of the study, nor between the three groups of human subjects. The presence of these low yet consistent background concentrations of pentane and CS₂ was ultimately beneficial, because it showed the presence of negative alveolar gradients which would not have been observed had the air been completely purified.

Neuroleptic drugs introduced an extrinsic chemical source into this study which could have affected the findings. It was not ethically possible to withhold these drugs from the patients with schizophrenia, who were admitted as acute psychiatric emergencies. It probably also would not have been possible to secure their cooperation for breath testing without treatment. About half of the psychiatric control patients, however, were also treated with neuroleptic drugs, and there were no significant differences in either pentane or CS₂ concentrations between the treated and the untreated patients (table 3). Consequentially, drug treatment did not seem to influence the findings.

In conclusion, patients with schizophrenia exhibited increased production of CS₂, a known neurotoxin, and pentane, a marker of lipid peroxidation. Further studies are required to determine if these observations were a consequence of the schizophrenic illness or some factor other than the disease itself.

We thank Sheldon Blackman, PhD, for advice on the statistical analysis of data.

using gas chromatography. *Zh Nerpnapd Pakhiaor*
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This sumptuously produced atlas is subdivided into two main sections with three chapters devoted to "clinical aspects" and seven chapters allocated to "pathology." This strategy may be convenient in a multi-author text, but it has resulted in a clinical section which is pathologically naive and a pathological section impoverished by the paucity of clinicopathological correlation.

The wide-ranging introductory chapter, which covers epidemiology, genetics, molecular biology, early diagnosis, and screening for ovarian cancer, provides a useful overview of the subject, although the emphasis placed on ultrasonography is excessive. The two ensuing chapters, both rather lengthy and repetitive, are devoted to management of ovarian carcinoma and non-epithelial tumours, respectively.

The pathology section comprises four chapters devoted to primary epithelial neoplasms including a whole chapter on the interesting but controversial subject of ovarian intraepithelial neoplasia. Other chapters deal with sex cord-stromal tumours, germ cell tumours, and metastatic tumours. This section is well illustrated with adequate photographs and gross photographs of excellent quality. However, many entities are skimpily and unctually described with no attempt to evaluate the taxonomic over-enthusiasm displayed by recent authors in this field. On the other hand, many rare but well-established entities are not included. An even more serious drawback for a book aimed at the practising histopathologist is the lack of consideration given to possible differential diagnoses.

In conclusion, this new atlas is unlikely to fulfill the need for a comprehensive, authoritative, and up to date reference text on ovarian neoplasms. It cannot be recommended as a bench book for the reporting room.

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Massachusetts General Hospital, Harvard Medical School

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