Cyanide Concentrations in Blood after Cigarette Smoking, as Determined by a Sensitive Fluorimetric Method

Per Lundquist, Hans Roelse, Bo Sörbo, and Lita Tibbling

Using the sensitive fluorimetric method described here, we evaluated the determination of blood cyanide as a method for monitoring exposure to tobacco smoke. The mean concentration of cyanide in blood from eight nonsmokers was 0.098 (SD 0.036) μmol/L. The concentration of cyanide in blood of smokers who had refrained from smoking for at least 2 h before sampling peaked immediately after the subjects smoked a cigarette, then rapidly declined, with a half-life of about 4 min. Its rapid disappearance from blood makes cyanide an unsuitable marker of exposure to tobacco smoke. Because the ability of patients with hepatic dysfunction to detoxify cyanide has been presumed to be impaired, we monitored the concentrations of cyanide in the blood of four patients with severe hepatic insufficiency who smoked a cigarette. The rate of elimination of cyanide from blood after smoking was only slightly less in these patients than in the controls, and the difference was not statistically significant.

Additional Keyphrases: elimination half-life • hepatic dysfunction

Inhaled from tobacco smoke, hydrogen cyanide readily passes through the lungs into the bloodstream and is then converted to thiocyanate in the liver and kidneys. Determination of the latter compound in plasma or serum has been used to indicate exposure to tobacco smoke (1–4). However, measurements of cyanide in blood, erythrocytes, or plasma from smokers and nonsmokers have given conflicting results (5–8). Furthermore, the concentrations of cyanide in blood from nonsmokers appear to be below the true detection limits of the analytical methods currently in use (8).

We have now improved the sensitivity of our previous method (8) for determining cyanide in blood by substituting a fluorimetric step for the colorimetric determination of the König reaction product. Using this method, we have monitored the concentration of cyanide in the blood of subjects who smoke cigarettes and have assessed the ability of smokers with impaired liver function to eliminate cyanide from blood after exposure to tobacco smoke.

Materials and Methods

Materials

Reagents were prepared as earlier described (8). The simple aeration apparatus used for isolation of cyanide from blood (8) had to be modified for satisfactory recovery of the small amounts of cyanide involved. We used silicon tubing instead of rubber latex tubing for connecting sample tubes to NaOH traps, and we soaked the tubing as well as all glassware in 1 mol/L nitric acid for several hours, washing them extensively with de-ionized water before use.

Procedure

The procedure was carried out essentially as described previously (8), up to the detecting step: Mix 1 mL of blood with 10 mL of acid silver sulfate reagent. Then, in the aeration apparatus, add H₂SO₄ to liberate HCN. Transfer the evolved HCN with a stream of nitrogen (flow rate 0.3 L/min, aeration time 2 h) to a trap containing 2 mL of 0.1 mol/L NaOH and initiate the König reaction by adding 0.2 mL of 2 mol/L acetic acid, followed by 50 μL of 5 mmol/L NaClO and 0.25 mL of barbituric acid–pyridine reagent. After 5 to 15 min determine the reaction product by fluorimetry, using an excitation wavelength of 582 nm and an emission wavelength of 600 nm. Ascertain the cyanide concentration in the sample from a standard curve prepared by using cyanide standards of known concentrations.

Subjects. Our first studies on the elimination of cyanide from blood after the smoking of a cigarette involved five otherwise healthy smokers (three men, two women, ages 36 to 55 years) who had abstained from smoking for at least 2 h before the experiment.

Blood was sampled via an indwelling needle in an arm vein and collected into a 10-mL Vacutainer Tube containing heparin (Becton Dickinson, Rutherford, NJ). After baseline samples were collected, the subjects then smoked a "Prince" brand cigarette during 5 min, having been asked to inhale smoke every 30 s, and blood was sampled during the next hour. A single brand of cigarette was used in these experiments, because different cigarette brands show large variations in cyanide production (9). We used a simplified protocol for our studies of four patients with severely reduced hepatic function. Besides the baseline samples after a 2-h morning abstinence from smoking, we collected blood samples immediately after the subjects finished smoking and 15 min later. The diagnosis of each patient (see Table 1) was verified by liver biopsy and by conventional laboratory investigations.

Table 1. Concentrations of Cyanide in Blood after Cigarette Smoking: Normal Subjects and Patients with Hepatic Insufficiency

<table>
<thead>
<tr>
<th>Sex</th>
<th>Basal</th>
<th>Immediately after smoking</th>
<th>15 min after smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.215</td>
<td>0.635</td>
<td>0.280</td>
</tr>
<tr>
<td>F</td>
<td>0.085</td>
<td>0.370</td>
<td>0.110</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td>0.125 ± 0.035</td>
<td>0.484 ± 0.100</td>
<td>0.180 ± 0.042</td>
</tr>
<tr>
<td><strong>Alcoholic cirrhosis patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.185</td>
<td>0.660</td>
<td>0.315</td>
</tr>
<tr>
<td>F</td>
<td>0.250</td>
<td>0.850</td>
<td>0.345</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td>0.160 ± 0.037</td>
<td>0.568 ± 0.117</td>
<td>0.238 ± 0.054</td>
</tr>
<tr>
<td><strong>Biliary cirrhosis patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.125</td>
<td>0.430</td>
<td>0.165</td>
</tr>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td>0.160 ± 0.037</td>
<td>0.568 ± 0.117</td>
<td>0.238 ± 0.054</td>
</tr>
</tbody>
</table>
Results

Analytical procedure. The excitation and emission spectra of the König reaction product obtained from cyanide in the present assay show peaks at 582 and 600 nm, respectively—rather similar to the values, 583 and 607 nm, reported for the König reaction product obtained under somewhat different conditions (10). The standard curve was linear up to concentrations of cyanide in blood corresponding to 0.5 μmol/L. The detection limit of the method, defined as the concentration corresponding to twice the SD of blank determinations, was 5 pmol per assay, corresponding to a blood concentration of 5 nmol/L, a 30-fold increase of sensitivity over that of the earlier colorimetric method (8). The intra-assay precision, evaluated by analysis of five replicate samples from a nonsmoking subject, gave a mean of 0.062 (SD 0.00027) μmol/L (CV = 4.4%). The analytical recovery of cyanide (0.500 μmol/L) added to a blood sample containing 185 nmol of cyanide per liter was 97.0% (range 96.2–97.9%, n = 3). Concentrations of cyanide measured in blood from eight normal nonsmoking subjects (ages 23–41 years; four men, four women) were 0.038 (SD 0.036) μmol/L.

Concentrations of cyanide in blood after smoking in normal smokers. Figure 1 illustrates the blood cyanide profiles obtained after the subjects had smoked a cigarette. Blood cyanide increased rapidly during smoking, to peak at the end of the smoking period at about fourfold the basal concentrations. It then decreased rapidly, with a biological half-life of about 4 min, reaching basal values about 30 min after the end of the smoking period. The rapid clearance of blood cyanide was not caused by a metabolic conversion of cyanide in the bloodstream, because in vitro incubation of blood at 37 °C resulted in a slight but definite increase in cyanide concentration (Figure 2).

Cyanide concentrations after smoking in blood of patients with hepatic insufficiency. Cyanide concentrations after smoking did not decline as rapidly in patients with severely impaired liver function as in controls (Table 1), but the difference was statistically insignificant. We conclude that the ability to eliminate cyanide from blood is not markedly impaired in patients with hepatic insufficiency.

Discussion

The results of the present investigation demonstrate that although there are significant increases in blood cyanide after smoking a cigarette, the rapid elimination of cyanide from blood makes assay of blood cyanide unsuitable as a biological marker of exposure to tobacco smoke. Cyanide in blood is present mostly as cyanomethemoglobin (8), which is converted in vivo to thiocyanate, as demonstrated earlier in animal experiments (11). The somewhat unexpected finding that in vitro incubation of blood increases the concentration of cyanide is explainable by the oxidation of thiocyanate to cyanide by granulocytes (12).

The practically normal rate of disappearance of cyanide from the bloodstream of patients with hepatic dysfunction does not agree with the results reported by Jästico et al. (13) for patients with tobacco amylia and hepatic dysfunction. The very high concentration of blood cyanide they observed in these patients ranged from 35 to 65 μmol/L, about 500-fold higher than the basal cyanide concentrations we found in our patients, who had an apparently similar degree of hepatic dysfunction as the patients studied by Jästico et al. Perhaps the different method of analysis for blood cyanide they used accounts for this discrepancy. It should be noted that our cirrhotic patients had a severely decreased liver parenchyma but nevertheless had only a slightly impaired ability to eliminate cyanide. This may be explained by the very high enzymatic capacity of the mammalian liver to detoxify cyanide by conversion to thiocyanate (14). The fact that partial hepatectomy or chemically induced liver damage did not increase the sensitivity of mice to cyanide (15) is consistent with this hypothesis.

This work was supported by grant no. 13X-5644 from the Swedish Medical Research Council and by grant no. 8608 from Svenska Tobaks AB. We are indebted to Drs. Ricci Gotthard and Bengt-Olof...
Rydén, who kindly allowed us to study their patients with hepatic insufficiency. Thanks are also due to Inga Hellman for excellent secretarial work.

References

CLIN. CHEM. 33/7, 1230–1232 (1987)

Laboratory and Field Evaluation of Three Desktop Instruments for Assay of Cholesterol and Triglyceride
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We evaluated three desktop instruments suitable for decentralizing the assays of blood cholesterol and triglycerides to satellite and physician’s office testing. The instruments, Ektachem DT 60 (E), Reflotron (R), and Seralyzer (S), were used according to their manufacturers’ instructions to assay serum or capillary blood from outpatients at two physicians offices. Accuracy was assessed in the centralized laboratory by using an automated centrifugal analyzer (A). The bias of A was monitored with an international quality-control material. We found all instruments easy to handle. Regression equations for cholesterol determinations were: $E = 0.92A + 0.7$ (n = 331, r = 0.94), $R$ (capillary blood) = $0.96A + 0.3$ (n = 256, r = 0.95), and $S = 0.93A + 0.6$ (n = 260, r = 0.92). For triglycerides we obtained $E = 1.02A$ (n = 331, r = 0.97), $R$ (cap. blood) = $0.88A$ (n = 213, r = 0.97), $R$ = $0.94A + 0.1$ (n = 90, r = 0.99), and $S = 0.96A$ (n = 266, r = 0.98). Duplicate and within-day precision was <8%. Between-day precision (during a month) was <10%. We stress the need of both laboratory and field evaluation and emphasize the benefit of quality control.

Additional Keyphrases: physician’s office testing · centrifugal analysis compared

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Received February 25, 1987; accepted April 13, 1987.

Recent developments in analytical technology have made possible satellite and physician’s office testing (SPOT) (1–3). Such measurements are not in opposition to those produced in the centralized hospital laboratory if the former are found to be reliable, render immediate access to results for the responsible physician, and are subjected to quality control. The importance of identifying and characterizing lipid disorders has been established in numerous epidemiologic investigations that have clearly established a strong association between total blood cholesterol and ischemic cardiovascular disease (4). Furthermore, triglyceride concentrations in serum after fasting have recently been identified as an independent risk factor for myocardial infarction (5).

Because screening for hyperlipidemia should be as widespread as screening for high blood pressure (6), we examined methods for decentralizing assays of blood cholesterol and triglyceride from the hospital laboratory to some primary health-care centers. Here, we report our experience with the laboratory and field evaluation of three instruments intended for SPOT assays.

Materials and Methods

Methods: Blood was obtained from outpatients attending two primary health-care centers or the Department of Internal Medicine. Blood was taken either as capillary blood by pricking the finger tip with a mini-lancet (Ames/Bayer, Gothenburg, Sweden) or as venous blood by venipuncture into evacuated tubes, with or without anticoagulants (EDTA), from Becton-Dickinson, Stockholm, Sweden.

Instruments: We used the automated centrifugal analyzer