Accumulation of Quinolinic Acid in Uremic Serum and Its Removal by Hemodialysis

Toshimitsu Niwa, Hideo Yoshizumi,1 Yutaka Emoto, Takashi Miyazaki, Naosumi Hashimoto, Naohito Takeda,1 Akira Tatematsu,1 and Kenji Maeda

Quinolinic acid was first identified in uremic serum by use of gas chromatography/mass spectrometry. Quantification by selected ion monitoring revealed that the serum concentration of quinolinic acid was markedly increased in the chronic hemodialysis patients, and that the acid could be removed by conventional hemodialysis. The serum concentration of quinolinic acid was weakly but significantly correlated with the serum uric acid concentration. Accumulation of quinolinic acid in uremic blood may be involved in the pathogenesis of anemia, suppressed immune system, and uremic encephalopathy.

Additional Keyphrases: gas chromatography/mass spectrometry, selected ion monitoring, uremic encephalopathy

Several endogenous metabolites that are ordinarily excreted in urine accumulate in blood in uremia; among these are albumin-bound furancarboxylic acids (1) and indoxyl sulfate (2). Accumulation of these metabolites in uremic blood is considered to account for certain uremic symptoms. Quinolinic acid, a metabolite of tryptophan, is excreted in urine in healthy subjects and is thought to accumulate in the blood of uremic patients. Quinolinic acid shows various pharmacological effects in vitro and in vivo, such as convulsions in mice (3), inhibition of gluconeogenesis in an isolated perfused liver (4), and inhibition of drug binding, erythropoiesis, and lymphocyte blast formation (in vitro) (5). Therefore, accumulation of quinolinic acid in blood may be related to anemia, a suppressed immune system, and convulsions in uremic patients. Quinolinic acid has been quantified in urine by reversed-phase high-performance liquid chromatography (6) and in plasma, cerebrospinal fluid, and brain by gas chromatography/mass spectrometry (GC/MS) (7-9).1 To our knowledge, however, there has been no report on quantifying quinolinic acid in serum of uremic patients.

In this study we identified and quantified quinolinic acid in uremic serum by GC/MS to determine the possible pathogenetic role of quinolinic acid in hemodialysis (HD) patients.

Materials and Methods

Chemicals

Quinolinic acid and 3,5-pyridinedicarboxylic acid were purchased from Tokyo Kasei Co., Tokyo, Japan. Bond Elut SAX, an anion-exchange resin in 1-mL columns, was obtained from Analyticchem International, Harbor City, CA. The other chemicals used were of analytical grade.

Subjects

The study included 54 patients receiving HD, 4 h three times a week. They had been undergoing periodic dialysis for 5.4 (SD 4.4) years (range 0.3-16.3 years). Their mean age was 53.9 (SD 11.7) years (range 26-78 years); 31 were men and 23 were women. Blood samples were taken before and after HD and allowed to clot. The controls were a group of 10 healthy subjects, five of each sex. All serum samples were stored at -20 °C until they were analyzed.

Sample Preparation for GC/MS

After addition of 30 nmol of 3,5-pyridinedicarboxylic acid as an internal standard, 1 mL of serum was deproteinized with 2 mL of ethanol. After centrifugation at 1500 × g for 15 min, the supernate was collected, then evaporated in a rotary evaporator. The residue was dissolved with 1 mL of distilled water. The Bond Elut SAX column was pre-activated with 1 mL of 0.5 mol/L HCl reagent, washed with 1 mL of methanol, and further washed with 2 mL of distilled water. The dissolved residue was applied to the pretreated column, and the acids were eluted with 1 mL of 0.5 mol/L HCl reagent. The eluate was evaporated in a rotary evaporator. The extract residue was methylated with diazomethane in ether at room temperature for 30 min. After evaporating the derivative under nitrogen stream, we dissolved the sample in 20 μL of an equimol solution of methanol/chloroform, then injected 2 μL of this into the GC/MS.

To extract quinolinic acid not bound to serum protein, we deproteinized serum with a cone membrane filter (CF-25; Amicon, Lexington, MA) after addition of 30 nmol of 3,5'-pyridinedicarboxylic acid as an internal standard. The ultrafiltrate was applied to the pretreated Bond Elut SAX column and treated in the same way as the supernate of the ethanol-deproteinized serum.

GC/MS

The methylated sample was analyzed with a moving-needle injection system of a Model GC-9A gas chromatograph combined with a double-focusing mass spectrometer (Model 9020-DF; both from Shimadzu, Tokyo, Japan). The data were stored and processed in a Shimadzu PAC-1100 data processing system. The GC column was a 25-m fused-silica column, coated with OV-101 (Gasukuro Kogyo Co., Tokyo, Japan), and was programmed from 100 to 220 °C at 6 °C/min. The injection

Received July 3, 1990; accepted November 16, 1990.

1Department of Internal Medicine, Nagoya University Branch Hospital, 1-1-20, Daiko-minami, Hisagahi-ku, Nagoya 461, Japan.
2Faculty of Pharmacy, Meijo University, Yagoto, Nagoya 468, Japan.
3Nonstandard abbreviations: GC/MS, gas chromatography/mass spectrometry; HD, hemodialysis; El, electron-impact ionization; and SIM, selected ion monitoring.

CLINICAL CHEMISTRY, Vol. 37, No. 2, 1991 159
temperature of GC was 300 °C, the separation temperature 280 °C. Electron-impact ionization (EI) mass spectra were recorded at an ionizing energy of 70 eV, a trap current of 60 μA, an acceleration voltage of 3 kV, and an ion-source temperature of 250 °C.

**Selected Ion Monitoring (SIM)**

To quantify quinolinic acid by SIM, we monitored both m/z 165, (M – CH$_3$O)$^+$, and m/z 164, (M – CH$_3$O)$^+$, to confirm the identification of quinolinic acid based on the peak-height ratio at m/z 165 to m/z 164. The molecular ion at m/z 195 was not used as a monitoring ion because of its low relative abundance. The calibration line was obtained with SIM by analyzing standard solutions, as follows: Quinolinic acid (0.3–30 nmol) was added to 1-mL aliquots of distilled water. After addition of 30 nmol of 3,5-pyridinedicarboxylic acid as an internal standard, we treated these solutions as described for sample preparation for GC/MS. The molecular structure and EI mass spectrum of 3,5-pyridinedicarboxylic acid dimethyl ester are shown in Figure 1. The calibration line relating the concentration (y) of quinolinic acid to the peak-height ratio (x) of the acid at m/z 164 to the internal standard at m/z 165 was obtained from the SIM chromatogram. The correlation coefficient of the calibration line for quinolinic acid, y = 8.0x + 2.1, was 0.9999. There were no interfering peaks in the SIM chromatograms of the serum samples for assay of quinolinic acid.

Quantification of quinolinic acid in biological materials by GC/MS has been reported in which 2,4-pyridinedicarboxylic acid (9) or [$^{18}$O]quinolinic acid (7, 8) was used as the internal standard. However, [$^{18}$O]quinolinic acid is not commercially available. We selected 3,5-pyridinedicarboxylic acid as an internal standard, because its retention time (9.3 min) is closer to that of quinolinic acid (8.7 min) than that of 2,4-pyridinedicarboxylic acid (10.1 min), and because the mean recovery of quinolinic acid relative to that of 3,5-pyridinedicarboxylic acid (93%) was much higher than that relative to 2,4-pyridinedicarboxylic acid (84%). The minimum sensitivity of our assay was 10 pmol, which is enough to quantify the concentration of quinolinic acid even in serum from nonuremic subjects. Quinolinic acid was derivatized to dimethyl ester instead of dihexafluoro-

roisopropyl ester (7, 8), because methylation with diazomethane is easy to perform, even at room temperature. All data are expressed as mean ± SD. Statistical analysis was by Student's t-test.

**Results**

**Identification of quinolinic acid in uremic serum by GC/MS.** Figure 2 shows the gas chromatogram of methylated acids extracted from ethanol-deproteinized uremic serum. The EI mass spectrum of a peak in the gas chromatogram is shown in Figure 3b. We identified the peak as quinolinic acid dimethyl ester, because its retention time (8.7 min) and EI mass spectrum (Figure 3b) were identical with those of authentic quinolinic acid dimethyl ester (Figure 3a).

**Quantification of quinolinic acid in uremic serum.** Figure 4 shows the SIM chromatograms of methylated standards, methylated extract from ethanol-deproteinized uremic serum, methylated extract from uremic serum ultrafiltrate, and methylated extract from ethanol-deproteinized normal serum. The concentration of quinolinic acid was markedly increased in uremic serum. Quinolinic acid accumulated in the uremic serum was not bound to serum protein, because the acid was detected in the uremic serum ultrafiltrate (9.0 ± 3.0 μmol/L, n = 10) at the same concentration as in the ethanol-deproteinized uremic serum (9.0 ± 2.9 μmol/L, n = 10). Therefore, the protein binding of quinolinic acid in uremic serum was 0%.

Table 1 shows the concentrations of quinolinic acid in serum samples of chronic HD patients. The mean concentration of quinolinic acid in the pre-HD serum of uremic patients was about 15-fold greater than that in healthy subjects. To determine the rate of removal of quinolinic acid by HD, we quantified the concentration of quinolinic acid in serum samples taken before and after HD. The decrease was as great as 88%, indicating the good dialyzability of the acid. The serum concentration of quinolinic acid in the uremic patients showed a weak but significant correlation with the serum concentration of uric acid (r = 0.298, P < 0.05).

**Discussion**

Quinolinic acid reportedly suppresses erythroid colony formation and lymphocyte blast formation even at 6.0 μmol/L (5), which is comparable with its concentra-

---

180 CLINICAL CHEMISTRY, Vol. 37, No. 2, 1991
---

![Fig. 1. EI mass spectrum of 3,5-pyridinedicarboxylic acid, dimethyl ester (internal standard)](image)

![Fig. 2. Gas chromatogram of methylated acids extracted from ethanol-deproteinized uremic serum](image)
of quinolinic acid in anemia of uremic patients. However, anemia in uremic patients is at present successfully treated with recombinant erythropoietin. Quinolinic acid may be involved in a dysfunction of immune system in uremic patients through its inhibitory effect on lymphocyte blast formation. Quinolinic acid is also reported to inhibit the binding of phenytoin to bovine serum albumin (5). However, our study indicated that quinolinic acid in serum was not bound to serum protein, accumulating in uremic serum as a free form.

As demonstrated in mice (3), quinolinic acid shows the strongest convulsive effects and greatest penetration into brain of the six kynurenines examined: DL-kynurenine and quinolinic acid, anthranilic, xanthurenic, picolinic, and nicotinic acids. Injection of as little as 6.0 nmol of quinolinic acid into brain ventricles caused convulsions in mice. Moreover, quinolinic acid was the only compound that produced motor excitement and convulsions after intraperitoneal injection in mice. Quinolinic acid has been detected in brains of rats by use of GC/MS (7). Quinolinic acid, a neurotoxin, is thought to be involved in the etiology of human neurodegenerative disease, particularly convulsant disorders. Patients with advanced end-stage renal failure, if not treated by dialysis, show symptoms of uremic encephalopathy such as muscle cramp, convulsion, seizure, and coma. Although the cause of uremic encephalopathy is not clearly elucidated, accumulation of quinolinic acid in uremic blood and its penetration into brain is a potential cause of muscle cramp, convulsion, and seizure. Further analysis of quinolinic acid in the cerebrospinal fluids of uremic patients with encephalopathy should be carried out to determine the role of quinolinic acid in uremic encephalopathy.

References

<table>
<thead>
<tr>
<th>Table 1. Concentrations of Quinolinic Acid In Uremic Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Uremic patients</td>
</tr>
<tr>
<td>Pre-HD</td>
</tr>
<tr>
<td>Post-HD</td>
</tr>
</tbody>
</table>

* P < 0.001 as compared with normal.