Note

Effect of Deaerated Water on Serum Biochemical Values and on the Cecum Concentration of Short-chain Fatty Acids in the Rat

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The effects of drinking deaerated water on serum biochemical values, and on the concentrations of short-chain fatty acids (SCFAs) derived from bacterial fermentation in the colon were examined in rats. Drinking deaerated water decreased the levels of serum alkaline phosphatase (SAP) and serum urea nitrogen (SUN), and increased the serum potassium (SK) and serum phosphorus (SP) levels. Although the concentration of propionic acid in the cecum was decreased by drinking deaerated water, the concentrations of isobutyric, valeric, and isovaleric acids in the cecum were increased.

Key words: deaerated water; drinking water; functional water; physiological effects; short-chain fatty acid

Various kinds of water such as electrolyzed, magnetized, ceramic-filtered and deaerated water are used for drinking or cooking. However, the physicochemical properties of these types of water have not been determined. Moreover, there is little information available regarding their in vivo effects on the human body. Deaerated water is widely used for food production; for example, in producing boiled rice and beans, tofu and soup stock. In this study, we determined the values of the serum biochemical parameters to evaluate the physiologic effects of deaerated water on rats, and examined the composition of SCFAs fermented by anaerobic bacteria in the intestinal tract.

Deaerated water

Deaerated water was produced from tap water by using a gas separation system consisting of several tens of thousand hollow fiber membranes encapsulated in a decompression case (Miura Co., Ltd., Matsuyama, Japan). Each hollow fiber membrane had an inside diameter of 0.2 mm and a length of 500 mm. Feed water enters each hollow fiber and passes through in only a few seconds. Air dissolved in the water is removed through the membrane wall of the hollow fiber by the action of a vacuum pump. Oxygen dissolved in the water was reduced to less than 0.5 ppm by this procedure, while dissolved nitrogen was similarly separated by the deaeration process. The concentrations of nitrogen and oxygen in tap water 16 ppm and 8 ppm, respectively, while those in the deaerated water reduced to 1.5 and 0.5 ppm, respectively. Since the hydrogen ion concentration (pH) of the water was increased by 0.1–0.3 to pH 7.3 by deaeration, it is likely that free carbon dioxide dissolved in the water would be separated similarly to oxygen and nitrogen. However, the concentration of dissolved carbon dioxide could not be assayed because of the presence of bicarbonate in the water. The concentration of residual chlorine was not different between tap and deaerated water, which may have been due to the large molecular size of chlorine. No difference was observed in levels of other parameters between tap and deaerated water; i.e., temperature, conductivity, sulfate, silica, calcium, magnesium, sodium, potassium, dry residuals, nitrates, nitrites, permanganate value, iron, manganese, copper, zinc, phosphorus, chloride, and fluoride. These physicochemical properties may not have been changed by decompression for only a few seconds.

Animal experiments

Twenty male Sprague-Dawley rats (8 weeks old; Japan Clea Inc., Tokyo, Japan) were used after being acclimatized to the laboratory conditions for 4 days. The rats were divided into two groups, each of which received deaerated water or tap water, and housed in individual cages. The animals were fed on CE-2 commercial pellets obtained from Japan Clea Inc., and on deaerated or tap water for 6 weeks. Water was supplied by polyester packages equipped with a feed valve (Musashi Co., Ltd., Saitama, Japan) which did not allow the entry of any air from drinking. Dissolved oxygen was present in the tap and deaerated water at 7.0–8.0 ppm and at less than 0.5 ppm, respectively, throughout the experiments. The water in the supply packages was renewed twice a week, and food and water were provided ad libitum. Body weight, and the food and water intake were recorded twice a week for all the rats. At the end of the feeding period, the rats were decapitated, blood then being collected from the neck and centrifuged immediately. Biochemical tests were performed on the serum at Otsuka Assay Institute, Otsuka Pharmacy Co., Ltd., Tokyo, Japan. The liver, kidneys, femurs, and cecum were dissected out, weighed and placed in a laboratory freezer until required for further analysis. Lactic, formic, acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids in the cecum contents were extracted with 20% (v/v) perchloric acid and assayed with a carbonic acid analyzer (S-14, Tokyo Rika Co., Ltd., Tokyo, Japan). The data were statistically analyzed by using Student’s t-test.

The initial body weight, body weight gain, food and water intakes, and liver, kidney, femur, cecum and cecum content weights were no different between the tap and deaerated water groups: initial body weight (g), 280 ± 2 in both groups; body weight gain (g/day), 4.9 ± 0.2, 4.8 ± 0.2; food intake (g/day), 26 ± 0.7, 24.5 ± 0.5; water intake (g/day), 39 ± 0.4, 44 ± 1; liver weight (g), 17.9 ± 0.8, 17.0 ± 0.7; kidney weight (g), 3.3 ± 0.1, 3.1 ± 0.1; femur weight (g), 1.3 ± 0.06, 1.3 ± 0.03; cecum weight (g), 1.2 ±

Abbreviations: SCFAs, short-chain fatty acids; SAP, serum alkaline phosphatase; SUN, serum urea nitrogen; SK, serum potassium; SP, serum phosphorus; pH, hydrogen ion concentration.
Table I. Effects of Deoxygenated Water on the Serum Biochemical Parameters in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tap water</th>
<th>Deoxygenated water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase (SAP)</td>
<td>(IU) 498 ± 38</td>
<td>386 ± 31*</td>
</tr>
<tr>
<td>Urea nitrogen (SUN)</td>
<td>(mg/dl) 21 ± 10</td>
<td>18 ± 0.4*</td>
</tr>
<tr>
<td>Potassium (SK)</td>
<td>(mEq/l) 6.7 ± 0.1</td>
<td>7.1 ± 0.1*</td>
</tr>
<tr>
<td>Phosphorus (SP)</td>
<td>(mg/dl) 8.1 ± 0.2</td>
<td>9.0 ± 0.2**</td>
</tr>
</tbody>
</table>

Ten rats were included in each group.

*Significantly different from the tap water group by 5% (p < 0.05), and
**by 1% (p < 0.01).

Values of the other serum biological parameters assayed were no different between the two groups. The other biological parameters assayed were as follows: total bilirubin, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, choline esterase, leucine aminopeptidase, creatine phosphokinase, total cholesterol, β-lipoprotein, triglyceride, creatinine, uric acid, sodium, chloride, calcium, iron, and amylase.

Table II. Concentrations (μmol/g) of Lactic Acid and Short-chain Fatty Acids in the Cecum Contents of Rats

<table>
<thead>
<tr>
<th>Acid</th>
<th>Tap water</th>
<th>Deoxygenated water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>0.6 ± 0.05</td>
<td>0.6 ± 0.08</td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.1 ± 0.01</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>15.6 ± 0.8</td>
<td>16.3 ± 0.6</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>2.1 ± 0.14</td>
<td>0.8 ± 0.07**</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>8.5 ± 0.4</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>0.1 ± 0.02</td>
<td>0.2 ± 0.03**</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>0.2 ± 0.01</td>
<td>0.4 ± 0.03**</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>0.1 ± 0.02</td>
<td>0.3 ± 0.04**</td>
</tr>
<tr>
<td>Total acids</td>
<td>27.4 ± 1.4</td>
<td>28.3 ± 1.2</td>
</tr>
</tbody>
</table>

Ten rats were included in each group.

**Values are significantly different from the tap water group by 1% (p < 0.01).

* Total acids = lactic + formic + acetic + propionic + butyric + isobutyric + valeric + isovaleric acids

0.03 in both groups; cecum content weight (g), 6.3 ± 0.3, 5.6 ± 0.3. Table I shows results of common biochemical assays of the serum. The levels of SAP and SUN in the rats given deoxygenated water were lower than those in the animals given tap water. However, the SK and SP levels were higher in the deoxygenated water group than in the tap water group. No differences were observed in the other biochemical parameters between these two groups. Table II summarizes the effects of deoxygenated water and tap water on the concentrations of individual SCFAs fermented by colonic bacteria in the cecum. The deoxygenated water group showed a lowered concentration of propionic acid, but increased concentrations of isobutyric, valeric and isovaleric acids relative to the equivalent values in the tap water group. The concentrations of other acids and total acids (lactic + formic + acetic + propionic + butyric + isobutyric + valeric + isovaleric acids) were not different between these two groups.

SCFAs produced by colonic bacterial fermentation regulate the intestinal digestive motion. In addition, SCFAs absorbed through the wall of the large intestine not only serve as an energy source, but are also related to the metabolism of lipids and saccharides. Drinking deoxygenated water altered the SCFA profile in the cecum. Thomsen et al. and Koseki et al. have reported that high-fat diets increased the concentrations of butyric and valeric acids in the cecum, and decreased that of propionic acid in the cecum. The SCFA profile in the cecum for the rats which drank deoxygenated water was similar to that of rats fed on a high-fat diet. Therefore, the effects of drinking deoxygenated water on the intestinal tract environment may have something in common with those of a high-fat diet.

Although increases in alkaline phosphatase activity are related to some diseases and physiological changes, the consequences of the decreased activity have not been clearly demonstrated. Therefore, we are unable to evaluate the significance of the reduced activity of alkaline phosphatase in the serum of the rats given deoxygenated water in this study. Since alterations in the levels of SUN, SK, and SP are related to the kidney function, deoxygenated water might also affect the kidney function, although details of this possible correlation could not be clarified here. Alterations in these biochemical parameters should be fairly good indicators of the physiological effects of deoxygenated water.

Food and water were given ad libitum to investigate their respective intakes. No differences were observed between the two test groups, although pair-feeding experiments will be necessary to precisely determine the effects of deoxygenated water.

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References