Effect of pantothenic acid and pantethine on intestinal motility in aging rats

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Atonic constipation, which is induced by intestinal decompression and stagnation of intestinal motility, is a serious problem in the elderly. In clinical medicine, pantethine (PaSS), a disulfide type of pantetheine (PaSH), is widely used in this condition to increase intestinal motility1). The present clinical test indicates that pantothenic acid (PaA) is involved in the intestinal motility because PaA and PaSH are the precursors of CoA. Because synthesis from PaA to CoA is decreased, the rate of the biosynthetic reaction in which PaSH is obtained from PaA influences aging2).

In this study, in order to identify the involvement of PaA deficiency in aging, we determined the effects of PaA and PaSS in aging rats.

Methods

Animals: Twenty-three-month-old male rats of the Wistar strain were obtained from the Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan). The animals were housed under the conditions of constant temperature (24°C ± 2°C) and humidity (55% ± 20%) and a 12-h light-dark cycle (0800–2000). After adaptation to a PaA-deficient diet for 3 weeks, the rats were divided into three groups and allowed free access to experimental diets and water for 4 weeks. The composition of the test diets was the same as that of the AIN-93M diet except for the PaA source3). The amounts of PaA (in mg 100 g vitamin mixture) were as follows: 160 in the control (PaA-intake diet) and 320 in the PaSS-intake diet; PaA was not added in the PaA-deficient diet. PaA and PaSS were in equimolecular amounts. PaSS was obtained from Daichii Pure Chemical (Tokyo, Japan) and the other components were purchased from Oriental Yeast (Tokyo, Japan). All the experiments were performed according to the Guide for Care and Use of Laboratory Animals, Showa Women’s University.

Determination of PaA deficiency: PaA deficiency was determined by comparing the CoA concentration in the rats.

Measurement of CoA: The CoA concentrations in the rat hearts were measured by the following method. The pretreatment method for measuring the CoA concentration was a modified protocol4). The 24-month-old rats were anesthetized with diethyl ether. Subsequently, the hearts were mixed with ice-cold saline for 1 min to remove the blood, frozen at liquid nitrogen temperatures, and stored at –70°C. They were weighed, mixed in 1 ml of buffer A (20 mM NaH2PO4, 5 mM tetra-n-butyl ammonium bromide, adjusted to pH 5.5) containing 1% perchloric acid, 2 mM dithiothreitol, and 1 mM propionyl-CoA. The mixture was added to 1 ml buffer A containing 2 mM mercapto ethanol, homogenized with 10 strokes of a motor-driven Teflon pestle, and centrifuged at 5000 rpm for 5 min. For the total CoA assay, the supernatant was adjusted to pH 5.5 using sodium chloride, centrifuged at 12,000 rpm for 2 min, filtered through a 0.45-μm membrane filter, and analyzed by HPLC. All procedures were performed below 4°C.

The CoA compounds were separated with an Eicom products HPLC system (Eicom, Kyoto, Japan) equipped with

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a dual pump, UV detector, and a 5-μm Inertsil ODS-3 column (40°C, 150 × 4.6 mm; GL Science Inc, Tokyo, Japan). The mobile phase composition was 63% buffer A and 37% buffer B (methanol; (v/v) at a flow rate of 1 ml/min. The absorbance was detected at 260 nm. The standard solutions of CoA contained 2.85 or 5.70 mM/L free CoA and 2.70 or 5.40 mM/L Propionyl-CoA, which functioned as an intestinal standard (Sigma, Japan). The recovery of individual CoA from the hearts was >90%. A maximum difference of 5% was found in the detected CoA between the duplicated samples.

**Measurement of food transit time:** The 24-month-old rats were fed 10 g of the marker diet (Co-EDTA 600 mg/kg diet) 1 h before the dark cycle. Feces of all the rats were collected every 1 h after dosing. Food transit time (TT) was determined as the time of the first appearance of the marker after dosing. The concentrations of Co in the feces were determined by a previously described method.⁵

**Statistical analysis:** Data were expressed as means ± SE and analyzed with one-way analysis of variance (ANOVA) between groups. If a significant difference was established, Fisher's PLSD test was used to compare data from each group. Difference were considered significant at $p<0.05$.

**Results and Discussion**

The PaA deficiency was determined by comparing the CoA concentrations in the rat hearts. As shown in Fig. 1, the CoA concentration in the PaA-intake group slightly increased as compared to that of the PaA-deficient group. On the other hand, the CoA concentration in the PaSS-intake group increased significantly and aided recovery from PaA deficiency. These results suggest that the conversion of PaA to PaSH is delayed in aging rats.

We analyzed the TT to identify the degree of intestinal motility (Table 1). TT indicates the time from intake to the excretion of Co. The TT delays in normal rats have an average of 7–9 h. PaA-deficient aging rats had a TT of over 10 h. The TT in the PaA-intake group did not decrease; in contrast, it decreased in the PaSS intake group. Intestinal motility recovery takes place with PaSS and the biosynthesis of CoA is high, but not with PaA; CoA concentration correlates with intestinal motility. This result supports the results obtained in clinical medicine studies in which PaSS affected atonic constipation.

In conclusion, PaSS is more effective than PaA in aiding the recovery of PaA-deficient aging rats. These results showed that the rate of biosynthesis of pantetheine from PaA was delayed.

![Fig. 1](image-url) Concentration of CoA in hearts of aging rats.
PaA: pantothenic acid. PaSS: pantetheine. §1 indicates significant difference between PaA deficient group and PaSS intake group ($p<0.01$). §2 indicates significant difference between PaA intake group and PaSS intake group ($p<0.05$).
Table 1  Effects of pantothenic acid deficiency on transit time

<table>
<thead>
<tr>
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<th>PaA deficiency</th>
<th>PaA-intake</th>
<th>PaSS-intake</th>
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<tbody>
<tr>
<td>Transit time (hr)</td>
<td>11.2 ± 2.4</td>
<td>100 ± 29</td>
<td>7.3 ± 0.7 **</td>
</tr>
</tbody>
</table>

PaA: pantothenic acid, PaSS: pantethine. Values are means ± SD.

a) Significantly different from the control values at p<0.05.
b) Significantly different from the PaA deficiency values at p<0.01.

Although these results have been obtained using rats, we can speculate that the CoA concentration decreases in a similar manner in the elderly. Our results indicate that atonic constipation in the elderly is closely related to PaA deficiency.

Reference


