SOME BIOLOGICAL PROPERTIES OF
THIAMINIC ACID ANALOGUES

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Thiaminic acid (TnA) has been found as a new oxidation product of thiol-type
thiamine and is of much interest because of its possible occurrence in the body as
a normal metabolite of thiamine derivatives.

The authors have already reported that the compound does not convert to
thiamine by treatment with cysteine or thiosulfate, and is negative for thiochrome
reaction.

In this paper, some biological properties of TnA and its O-benzoyl derivative
will be described. Since the derivatives of TnA do not convert to thiamine
nonenzymatically, they were assumed to have no biological activity for thiamine,
but the possibility of antithiamine could not be excluded considering its chemical
structure. Therefore as a preliminary experiment its activity on love-birds was
tested and it was found that they had no antithiamine activity. Further, acute
toxicity experiment was performed and it was found that they were by far less
toxic than known the thiamine derivatives.

EXPERIMENTAL

1. Effect on Love-birds

Male love-birds, Uroloncha striata var. domestica, weighing 12—13 g, were
divided into 4 to 6 groups, each group consisting of 4 to 5 birds. They were fed
a thiamine-deficient diet as shown in Table 1. Daily dose of the diet was 4 g each
and besides during the preliminary period 200 mg of yeast powder was supplemented.

| TABLE 1 |
| Thiamine-Deficient Diet |

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polished rice powder (free from thiamine)</td>
<td>100 g</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>25.0 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>Inositol</td>
<td>100 mg</td>
</tr>
<tr>
<td>Choline-HCl</td>
<td>100 mg</td>
</tr>
<tr>
<td>Calcium lactate</td>
<td>1.25 g</td>
</tr>
<tr>
<td>Liver oil (per week per bird)</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

1 Studies on Thiamine Disulfide. XL.
2 内海 勇，原田 清，河野啓一，塚本悟郎.
TnA and O-benzoyl thiaminic acid (BTnA), 3 and 600 µg thiamine equivalents dissolved in 0.05 ml water, respectively, were given orally once a day. To the thiamine-deficient group was given 0.05 ml water and to the thiamine group 0.05 ml aqueous solution containing 3 µg thiamine. Change of body weight and the prolongation of life were observed. Total thiamine content in the deficient diet was less than 0.2 µg per 4 g of daily dose and was proved satisfactory for this experiment. The cages were placed in an air-conditioned room kept at 20 ± 1° and 50 ± 10% humidity. Water was given *ad libitum*.

2. Acute Toxicity for Mice

Groups of DD strain mice, weighing 20 ± 1 g, each group consisting of 6 to 10 animals, were given various amounts of TnA dissolved in water, pH 4.5 and 7.0 or of BTnA, pH 7.0. After 72 hours, LD₅₀ of the compound was calculated from the death rate according to Weil (1).

RESULTS

1. Effect on Love-birds

*Administration of a Small Dose*—Prolongation of life following administration of 3 µg TnA is shown in Fig. 1. The thiamine group (3 µg) lived over three weeks without remarkable loss of the body weight, while the control group receiving water instead of thiamine solution as well as TnA and BTnA groups showed after 7 days roughness of the feather, slowness of the movement, and ataxia. In about one-third of the birds, a typical convulsion and opisthotonos appeared and all died after 9—10 days. The mortality of the birds was undoubtedly caused only by thiamine deficiency, since the birds survived for several days after oral administration of 100 µg of thiamine prior to death.

*Administration of a Large Dose*—Effects of 600 µg thiamine-equivalents of TnA derivative with or without 3 µg of thiamine were shown in Fig. 2. The
average prolongation of life of the TnA or BTnA group was found to be insignificantly different from that of the thiamine-deficient group.

On the other hand, simultaneous administration of 3 μg thiamine and 600 μg thiamine-equivalents of TnA or its derivative resulted in the prolongation of three weeks or more except for one case of accidental death. There was no difference in the fall of the body weight between the thiamine and the TnA group.

From the above findings TnA and BTnA were confirmed to have no thiamine activity on love-birds when given orally. Administration of about 200 thiamine equivalents of TnA or its derivative to the thiamine deficient or the sufficient group gave no effect on the prolongation of life or the change of body weight; a finding which shows that TnA or its derivative has no antithiamine activity. In such experiment, however, the absorbability from the digestive tract might be taken into consideration. TnA and its derivative are thiamine derivatives having a hydrophilic SO₃H group and have possibility of poor absorbability and might show no antithiamine activity because of their poor absorption. Anyhow it is evident that a large dose of TnA and its derivative given orally has no inhibitory effect against the absorption or biological activity of thiamine simultaneously administered.

2. Acute Toxicity for Mice

As shown in Table 2, the toxicity of TnA and its derivative was extremely

<table>
<thead>
<tr>
<th>Administration</th>
<th>TnA pH 4.5</th>
<th>TnA pH 7.0</th>
<th>BTnA pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>3.86 (3.44 to 4.34) µg</td>
<td>&gt; 4</td>
<td>1.87 (1.63 to 2.16) µg</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Orally</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
</tr>
</tbody>
</table>

*Parenthesized figures represent 95 per cent fiducial limits.
low. The LD_{50} of BTnA (pH 7.0, aqueous solution, intravenously injected) was 1.86 g per kg body weight, and no animal died after oral administration of 4 g per kg body weight of TnA (pH 7.0, aqueous solution). Thus TnA and its derivative are shown to be the compounds extraordinarily low toxic substance among those related to thiamine.

The pharmacological properties of these compounds have partially been reported from Fujimura’s Laboratory, Institute of Chemistry, University of Kyoto (2). According to the report, TnA, BTnA, thiamine, and thiamine disulfide had all an analgetic effect similar to aspirin when intraperitoneally injected to mice. As for the anti-inflammation effect, the inhibitory effect of TnA or its derivative against Carrageenin edema following intraperitoneal injection in mice was greater than that of thiamine disulfide and almost as strong as aspirin. In this effect, thiamine has been reported to be by far less active.

SUMMARY

Thiaminic acid (TnA) and its O-benzoyl derivative (BTnA) had no thiamine activity on love-birds. Oral administration of large dose, daily 600 μg thiamine-equivalents, with or without thiamine (3 μg daily), resulted in no significant difference in body weight and prolongation of life between the control and the given groups. This finding suggests that the compounds have no antithiamine activity in love-birds when given orally.

The toxicity was found to be very low among the compounds related to thiamine. The LD_{50} of BTnA for mice by intravenous injection (pH 7.0, aqueous solution) was 1.87 g/kg and that of TnA (pH 7.0, aqueous solution) over 4 g/kg. No toxic effect was observed after either oral or intraperitoneal administration.

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REFERENCES