Implication of ‘Harmful’ Intestinal Microflora in the Pathogenesis of Diseases with Immune Dysfunction

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The implication of an abnormal spectrum of intestinal microflora in the pathogenesis of diseases with immune disorders was examined in patients with sternocostoclavicular hyperostosis, rheumatoid arthritis and atopic dermatitis. In these patients, frequent episodes of severe constipation or diarrhea were observed before the onset of the diseases. Serum biotin levels in the patients were low and remained almost unchanged even after oral administration of biotin. However, supplementary administration of a probiotic agent with biotin significantly increased serum biotin levels and maintained the vitamin levels high enough to improve clinical manifestations. Biotin is mainly synthesized by members of the microflora in the intestine and absorbed from the intestine into the circulation. Therefore, low serum biotin levels may be attributable to the proliferation of ‘harmful’ intestinal microflora to degrade or ingest biotin. The results presented here suggest that biotin-limited conditions produced by ‘harmful’ intestinal microflora in the patients with immune disorders play a causal role in the pathogenesis of the diseases. Biotin administration with a probiotic agent may provide a therapeutic effect on the diseases.

Key words: intestinal microflora; immune dysfunction; probiotic agent; biotin deficiency

INTRODUCTION

Since an etiopathological implication of intestinal microflora for rheumatoid arthritis (RA) was proposed by Bennett (2), several investigators (4, 8, 14–16, 18, 20, 21) have tried to establish a causal role of intestinal microflora in the pathogenesis of RA. Among them, Shinebaum et al. (21) compared the fecal microflora in patients with RA with that in healthy controls and suggested that bacterial products or compounds triggering the immune system might induce the formation of immune complexes and the deposition of degradable bacterial compounds in the joint tissues, causing inflammation symptoms such as those found with RA. However, to date, no particular microorganism or its degradable compound responsible for the induction of joint inflammation has been identified. In this study on joint inflammation and spondyloarthropathy such as RA and sternocostoclavicular hyperostosis (SCCH), a high incidence of constipation or diarrhea was found in the majority of patients with SCCH and RA before the onset of the diseases, although they did not present pronounced abdominal symptoms. Patients with atopic dermatitis (AD) also had similar abnormal stool profiles. These abnormal stool profiles could be related to a pathogenic spectrum of intestinal microflora and resultant immune disturbances.

On the other hand, our previous studies (9–13) showed low serum biotin levels in patients with SCCH, RA and AD. The studies (9–13) also demonstrated a close inverse correlation between serum biotin levels and the ratios of CD4 cells/CD8 cells in T lymphocytes, a hallmark of immune dysfunction, in the patients. Experimentally induced biotin deficiencies, such as following egg-white treatment of rats, resulted in immune disturbances (9, 19). Since biotin is synthesized by intestinal microflora, a pathogenic spectrum of the microorganisms may cause biotin deficiency, resulting in immune disturbances. In other words, the diseases with immune disturbances could be considered to be a kind of biotin deficiency. Therefore, this study was undertaken to determine whether an abnormal spectrum of intestinal microflora plays a role in triggering the pathogenesis of the diseases.

PATIENTS AND METHODS

Patient population. The study was performed on 183 patients with SCCH, 90 males and 93 females; 35 female patients with RA; and 195 patients with AD, 98 males and 97 females. Before their first visit to our
clinic, all patients had been treated with one or a combination of non-steroidal antiinflammatory or antiallergic drugs and/or topically with corticoid ointments without any favorable effect on their clinical manifestations, immunologic profiles, radiologic features and/or outcomes. The diagnoses of the diseases were established orthopedically or dermatologically, respectively, on the basis of the presence of clinical radiologic findings defined according to the criteria of Sonozaki et al. (22) for SCCH, the American Rheumatism Association (1) for RA and Hanifin and Rajka (7) for AD. The patients were obliged not to take any topical and systemic medication for at least four weeks before blood sampling, unless otherwise indicated. Sixty-four control subjects for this study had no clinical, metabolic or immunologic symptoms or complaints, and did not take any medication including antibiotics. The purpose of this study was explained to all patients and their consent was obtained before starting. Blood sampling was performed in the morning after an overnight fast and avoidance of smoking.

Measurement of serum biotin levels. Serum biotin levels were measured in 118 patients with SCCH, 58 males and 60 females; 35 female patients with RA; and 58 patients with AD, 30 males and 28 females. To determine whether there are significant differences in serum biotin levels between diseases, the vitamin levels of patients with other immune disorders, such as psoriasis vulgaris, IgA nephropathy and systemic lupus erythematoses, were measured.

Biotin administration. In the trial study on the effect of biotin administration on therapeutical assessments, the following treatments were employed:

1. Six patients, 2 with SCCH, 2 with RA and 2 with RA, and 2 control subjects were administered at a dose of 9 mg/day of biotin (three times a day) and then monitored daily by measuring serum biotin levels for one week. Thereafter, the biotin levels were measured biweekly for five weeks.

2. In the second set of the study, another 40 patients, 20 with SCCH and 20 with AD, participated in this study. The patients were randomized into two groups to receive either oral administration of biotin, 9 mg/day, or intramuscular injection of biotin, 1 mg/day, for four weeks followed by a crossover to the other treatment after a four-week washout period.

3. Subsequent to the second set, 6 patients with SCCH were randomly divided into three groups and assigned to receive biotin, 9 mg/day, or a combination of biotin, 9 mg/day, and a probiotic agent; Biofermin® (Biofermin Pharmaceutical Co., Streptococcus faecalis, 10⁸/g), 3 g/day, or Miya-BM® (Miyarisan Pharmaceutical Co., Clostridium butyricum Miyairii, 10⁸/g), 3 g/day, each given to inhibit the proliferation of 'harmful' intestinal microflora to degrade or ingest the administered biotin.

4. Before starting the biotin treatment, long-term clinical assessments on the therapeutical efficacy of oral administration of biotin, 9 mg/day, were done on 2 patients with SCCH. In this study, the following variables were evaluated: (a) serum biotin level; (b) severity of skin lesions; (c) objective complaints, especially anterior chest pain; and (d) radiographic figures related to the presence of sternocostoclavicular joint inflammation.

Assay procedure. Serum biotin level was measured microbiologically (6). The test microorganism was Lactobacillus plantarum (ATCC 8014).

Statistical analysis. All results are expressed as mean ± SEM. Statistical analysis was performed by the two-tailed Student's t-test for paired and unpaired data, with p < 0.05 accepted at the level of significance.

RESULTS

Serum Biotin Levels

As shown in Table 1, serum biotin levels in patients with SCCH, RA and AD were significantly lower than those in control subjects and showed no differences between sexes. Similarly, the biotin levels were low in patients with immune dysfunction such as psoriasis vulgaris, IgA nephropathy and systemic lupus erythematoses and Sjögren's syndrome. However, there were no significant differences in the biotin levels between the patients with non-IgA nephropathy and the control subjects.

Oral Administration of Biotin

In control subjects, the oral administration of biotin, 9 mg/day, significantly increased serum biotin levels up to 4-fold of their basal values one day after administration (Fig. 1). Two days after administration, the biotin levels increased 6- to 8-fold, and 3 days after, 8- to 10-fold. On the contrary, in patients with SCCH and RA, the biotin levels remained almost unchanged despite continued administration. In patients with AD, the biotin levels increased slightly but greater than those in patients with SCCH and RA in response to biotin administration.

Intramuscular Injection of Biotin

The patients were divided into two groups and administered biotin orally, 9 mg/day, or parenterally with
Table 1. Serum biotin levels in normal subjects and patients with various diseases

<table>
<thead>
<tr>
<th></th>
<th>Serum biotin (nmol/liter)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>(N=64) 96.5 ± 3.1</td>
</tr>
<tr>
<td>Sternocostoclavicular hyperostosis (118)</td>
<td>34.3 ± 1.7*</td>
</tr>
<tr>
<td>Rheumatoid arthritis (35)</td>
<td>58.9 ± 4.5*</td>
</tr>
<tr>
<td>Atopic dermatitis (58)</td>
<td>44.6 ± 3.6*</td>
</tr>
<tr>
<td>Psoriasis vulgaris (28)</td>
<td>51.7 ± 6.1*</td>
</tr>
<tr>
<td>IgA nephropathy (34)</td>
<td>64.6 ± 3.0*</td>
</tr>
<tr>
<td>Non-IgA nephropathy (29)</td>
<td>88.0 ± 2.6</td>
</tr>
<tr>
<td>Systemic lupus erythematoses (10)</td>
<td>47.1 ± 2.9*</td>
</tr>
<tr>
<td>Behçet’s disease (15)</td>
<td>50.5 ± 8.6*</td>
</tr>
<tr>
<td>Sjögren’s syndrome (5)</td>
<td>41.2 ± 4.2*</td>
</tr>
<tr>
<td>Crohn’s disease (5)</td>
<td>56.3 ± 3.5*</td>
</tr>
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Data are mean ± SEM.

* Significant difference from normal group at p < 0.001.

Fig. 1. Rates of increase in serum biotin levels in normal subjects and patients with various diseases after oral administration of biotin (9 mg/day).

In patients with SCCH, serum biotin levels remained almost unchanged after the oral administration of biotin alone, 9 mg/day. However, the patients responded to a combination of biotin, 9 mg/day, and a probiotic agent, 3 g/day, with a significant increase in the biotin levels. As with Biofermin®, the biotin levels reached the maximum, 4-fold basal values, three months after administration (Fig. 3). Miya-BM® supplementation to the biotin treatment resulted in a remarkable increase in the biotin levels, 6- to 8-fold basal value, one month after supplementation, and maintained values high.

Probiotic Agent Supplementation

In patients with SCCH, serum biotin levels remained almost unchanged after the oral administration of biotin, 1 mg/day, for four weeks. In the group of patients who were administered biotin orally, serum biotin levels remained almost unchanged or increased only slightly, while in the other group, of which patients were injected intramuscularly, the biotin levels increased dramatically up to 4-fold their basal values. After a 4-week washout period, crossover administration was conducted on these groups and the same results were achieved (Fig. 2).
Fig. 4. Changes in serum biotin levels in patients with sternocostoclavicular hyperostosis after oral administration of biotin and a probiotic agent.

Table 2. Incidences of diarrhea or constipation in patients with various diseases prior to the onset of the diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Case</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sternocostoclavicular hyperostosis</td>
<td>183</td>
<td>72.1</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>35</td>
<td>68.6</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>195</td>
<td>92.0</td>
</tr>
</tbody>
</table>

enough to improve clinical manifestations. The supplementation-induced increase in serum biotin levels was significantly higher in Miya-BM® group as compared with that in the Biofermin® group (p < 0.001).

Long-Term Administration of Biotin and Probiotic Agent

After the oral administration of biotin alone, 9 mg/day, to patients with SCCH, changes in serum biotin levels were small and transient with a slight improvement of their clinical manifestations. The biotin levels began to decrease, and the clinical manifestations worsened again to the basal conditions despite continued administration (Fig. 4). Then, Miya-BM®, a probiotic agent, was administered supplementarily at a daily dose of 3 g with the biotin treatment. As shown in the figure, serum biotin levels increased rapidly in response to the supplement administration and maintained high values, causing complete improvement of their clinical, metabolic, immunologic and radiologic manifestations. No adverse events occurred during the study.

Abnormal Stool Profiles

Although the onset of the diseases was insidious and progression was subclinical in our patients, abnormal profiles of stool antedated or coincided with bone and/or skin manifestations. Of 183 patients with SCCH, 134 (72.1%), and of 35 patients with RA, 24 (68.4%) had been suffered from severe constipation or diarrhea prior to the outbreak of clinical manifestations (Table 2). Similarly, in 174 (92.0%) of 195 patients with AD, frequent episodes of green-colored and greasy foul-smelling diarrhea preceded the onset of disease in their suckling period. As to serum biotin levels, there were no significant differences between the patients with and without episodes of abnormal stool profiles.

DISCUSSION

This study demonstrated that patients with SCCH, RA and AD had low serum biotin levels. The study also showed low biotin levels in patients with immune disorders, such as psoriasis vulgaris, IgA nephropathy, systemic lupus erythematoses and Sjögren’s syndrome, as compared with biotin levels in the control subjects. As reported previously (9–13), there was an inverse correlation between serum biotin levels and the ratios of CD4 cells/CD8 cells in peripheral T lymphocytes in the patients with immune disorders and experimentally induced biotin-deficient rats, in which the ratios were significantly higher than those in the control men and animals. Immunologic dysfunction has been reported for several children with biotinidase deficiency, of which skin symptoms and some of the metabolic and neurological manifestations were similar to those in biotin-deficient state (3, 5, 19). Biotin treatment corrected these abnormalities including immunologic dysfunction. This evidence supports the proposal that low serum biotin levels contribute to immune dysfunction. This study also gives additional clinical support to this proposal.

Biotin is mainly synthesized by intestinal microflora, absorbed from the intestine into the circulation and excreted into urine; therefore, it may be suggested that low serum biotin levels in patients can be attributed to a disturbance of this process at some stage, as follows: (a) reduced biosynthesis of biotin by poor development of ‘useful’ intestinal microflora, (b) increased degradation or ingestion of the vitamin due to an excess of ‘harmful’ intestinal microflora, (c) impaired absorption of the vitamin from the intestine into the blood, (d) excessive presence of biotin-binding substances such as avidin in the intestine, (e) large loss of biotin into the urine, (f) absence of biotin-binding protein in the blood or (g) a combination of or all of these. As described previously, biotinidase deficiency may be another factor to cause low serum biotin levels (3, 5, 19). Biotinidase is necessary for recycling biotin by cleaving it from biocytin and biotinyl-peptides, the final products of protein-bound biotin (17, 23). Therefore, its defi-
iciency requires exogenous biotin for the prevention of clinical, metabolic and immunologic manifestations of biotin deficiency from the suckling period. However, the clinical presentation in our patients and the time of its onset may exclude the possibility that low serum biotin levels were due to biotinidase deficiency.

Serum biotin levels increased rapidly in the control subjects in response to the oral administration of biotin, while the response to administration was poor in the patients as evidenced by a low rate of increase in the biotin levels. This poor response in the patients may be explained by the degradation and/or ingestion of the administered biotin by proliferated ‘harmful’ microflora in the intestine, impaired absorption of biotin from the intestine or both. A significant increase in serum biotin levels in the patients could be observed after the parenteral administration of biotin because the intestinal affection of the vitamin levels could not be seen when biotin was given parenterally.

In order to ascertain this evidence, antibiotic agent supplementation was tried with the biotin treatment to inhibit the proliferation of ‘harmful’ microflora in the intestine. This combination treatment significantly increased serum biotin levels with a faster onset of clinical improvement (data not shown). However, the beneficial response to the treatment was not long-lasting. Serum biotin levels again decreased to their basal values despite continued treatment, and the therapeutic effectiveness wore off; presumably by not only inhibiting the development of ‘useful’ microflora to synthesize biotin but also promoting the proliferation of antibiotic-resistant ‘harmful’ microflora, resulting in poor therapeutical benefit and the frequent occurrence of undesirable side effects such as colonization by methicillin-resistant microorganisms and drug-induced intoxication. Instead of antibiotics, the supplementary administration of a probiotic agent with biotin significantly increased serum biotin levels in the patients and maintained vitamin levels high enough to improve clinical, metabolic, immunologic and radiologic manifestations with no evidence of hematological, renal or hepatic toxicity.

Another study on biotin requirement for the growth of ‘medical’ microorganisms, the main constituents of probiotic agents used in this study, showed that the inoculation of Streptococcus faecalis into a medium containing biotin resulted in a 30–40% reduction in the vitamin content, while Clostridium butyricum Miyairii reduced vitamin content only slightly. Similarly, lactobacillus employed for microbioassay to determine serum biotin levels consumed a large amount of the vitamin (unpublished data). This evidence, together with the results of our study, indicates that Clostridium butyricum Miyairii is expected to be a useful probiotic agent for supplementation, because this agent serves not only to inhibit the proliferation of ‘harmful’ microflora but also does not ingest the administered biotin, inducing sufficient clinical improvement.

Abnormal profiles of stool observed for the patients provided an important approach to investigate the implication of intestinal microflora in the pathogenesis of diseases with immune dysfunction. The patients had frequently suffered from severe constipation or chronic diarrhea prior to the onset of the diseases. It was also found that the stool of most patients with AD consisted of green-colored and greasy foul-smelling diarrhea, exhibiting symptoms characteristic of watery gut actions such as susceptibility to the presence of ‘harmful’ intestinal microflora.

A definitive conclusion as to the implication of ‘harmful’ intestinal microflora to degrade or ingest biotin in the pathogenesis of diseases with immune dysfunction must await the isolation and identification of such unknown microorganisms in the patients. However, it appears likely that low serum biotin levels in patients with immune disorders may be attributable to an abnormal spectrum of indigenous intestinal microflora different from that of normal subjects and that ‘harmful’ microorganisms may be dominant in the intestine of the patients, thereby inducing clinical, metabolic, immunologic and radiologic disturbances. At present, analyses of the microflora in the patients are in progress.

REFERENCES


