Influence of Zinc Deficiency to the Rats Infected with *Babesia rodhaini*

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ABSTRACT. Zinc deficiency induces a wide range of disorders including immunodeficiency. It is known that microbial infections occur with a high frequency in the zinc-deficient hosts, but the study on the correlation between parasitic infection and zinc status in hosts is scarcely performed. We observed that the influence of zinc deficiency to the rats infected with *Babesia rodhaini*. Experiments of *B. rodhaini* infection were conducted using zinc-deficient (ZD; eat *ad libitum* or 10 g/day on the ZD diet), zinc-adequate (ZA; *ad libitum* on the ZA diet), and diet-restricted (DR; eat 7 g/day on the ZA diet) rats. The findings in this study suggested that the zinc deficiency had deleterious effects on the hemodynamics and mortality of the rats infected with *B. rodhaini*.

KEY WORDS: *Babesia rodhaini*, rat, zinc, zinc-deficient.

Although the importance of zinc in humans had not been noticed before the study of Prasad *et al.* in 1963 [18], it is well known that zinc is found in every cell of living organisms and essential for DNA synthesis and for cell growth and differentiation [6, 19]. Chronic zinc deficiency induces a wide range of disorders including the skin and hair lesions, growth retardation, immunodeficiency, dysmature, wound healing defects, and neuropathy [1–4, 10, 15, 22, 23]. Mild zinc deficiency in humans occurs not only in developing countries with a poor food situation, but also in advanced countries where there are cases due to their inadequate eating habits [15].

Babesiosis, caused by the infection with intraerythrocytic parasitic protozoa of the genus *Babesia*, is one of the most common infectious diseases of worldwide animals, and it is also increasing interest as an emerging zoonosis in humans. Although babesial parasites have infectious capability to a wide range of vertebrates, they require competent invertebrate vector, ixodid ticks, to maintain the transmission cycles. The spectrum of the disease is broad, ranging from an apparently subclinical to a fulminating infectious disease resulting occasionally in death. Determinants in the severity of disease manifestation involve age, nutritional status, and immunocompetence [14]. It was noted that a high frequency of bacterial, viral, and fungal infections occurred in zinc-deficient hosts [16]. However, the study on the correlation between parasitic infection and zinc status in hosts is scarcely conducted, and there is no research paper for the babesiosis in zinc-deficient animals so far. Since it has been reported that antibody-mediated responses are impaired in the zinc-deficient animals [8, 9], it might be expected that *Babesia* parasites would readily proliferate in such animals. However, pathogens are also known to have their own unique metabolic and nutritional requirements [7], so it is also conceivable that the parasite may not proliferate well in a zinc deficient host.

This study was undertaken to distinguish these two possibilities and to obtain the fundamental information of rodent Babesia, *B. rodhaini*, infection dynamics in the zinc deficient rats.

For both experiments I and II, respective ten male 3-week-old Wistar rats, weighing initially 55–60 g, were purchased from Saitama Experimental Animal Supply Co., Ltd. (Saitama, Japan), and fed mash food added 20% casein to acclimatize to mash food for 5 days [13]. They were housed individually in stainless-steel cages with mesh bottoms to inhibit recycling of zinc, and respective five rats were allowed to eat *ad libitum* on the zinc-adequate (ZA) or the zinc-deficient (ZD) diets, which were based on egg albumin (20 %) and D-glucose (64 %), for 3 weeks, respectively [13]. Both ZA and ZD diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). In the experiment II, 5 rats were fed each 7 g of ZA diet (diet-restricted (DR)) and the other five were fed each 10 g of ZD diet per day throughout the experiment period, because the rats in both diet groups had to become about the same body weights at the time of parasite challenge infection. Residual of the diets were discarded daily, and replaced with fresh one. The fresh ultra-pure water purified with Milli-Q system (Millipore Corp., MA, U.S.A.) was offered *ad libitum* daily throughout the experiments.

*B. rodhaini* Australia strain was used for the challenge experiments. Each rat was challenged with 8.5 × 10⁷ parasitized erythrocytes in the experiment I and 5.0 × 10⁷ in the experiment II. In both experiments, all rats were observed their clinical symptoms during 3 weeks, and then necropsied. Parasitemia in 300 erythrocytes was monitored every day by microscopic examination of Diff-Quik (Sysmex Co., Ltd., Hyogo, Japan) stained thin smears of tail blood.
Packed cell volumes (PCVs) were measured by Celltac α (MEK-6358; Nihon Kohden Co., Ltd., Tokyo, Japan) in the experiments I and II.

For statistical analysis, Fisher’s exact test and Student’s t test were used to determine the significant difference in the parasitemia, the PCVs, and thymus weights of the rats.
between in the ZA and ZD groups.

Parasitemia of the ZD rats infected with *B. rodhaini* became bastardized compared with that of the ZA rats in the experiment I (Fig. 1A). Piroplasms of *B. rodhaini* first appeared at 3rd day post infection (PI) in the peripheral blood of the rats in both groups. Parasitemia showed the peak of 12.2 (20.2)% on 8th day PI in the ZA group, while 37.5 (45.2)% on 9th day PI in the ZD group. Parasites in the ZD rats were detected in peripheral blood smears until 14th day PI, but those in the ZA rats were hardly detected at 10th day PI.

PCVs of both groups in the experiment I declined, but it of the ZD group was lower than that of the ZA group through the observation period (Fig. 1B). Although the PCV of the ZA rats began to restore on and after 10th day PI, that of the ZD rats continued to decline and the recovery was late (Fig. 1B and 1C).

All the five rats of the ZA group survived until 21st day PI when they were autopsied, while two rats of the ZD group died at 15th and 16th day PI, respectively (Fig. 1D).

Weights of the rats of the ZA group were always more twice than those of the ones of the ZD group in the experiment I (Table 1).

Parasitemias of the DR and ZD rats in the experiment II were recognized at 2nd day PI and the highest values indicated at the 8th day PI in both DR and ZD groups (Fig. 2A). No parasites became to be detected from the all five rats of ZA group after 12th day PI. The PCVs of both groups rats were allowed to recover from 10th day PI, but the PCV of the ZD group was significantly lower than that of DR group at 7th, 14th, and 21st day PI in the experiment II (Fig. 2B). However, there was a significant difference between in the PCVs of the ZD and DR groups at the parasitic challenge infection in the experiment II as same as the experiment I (p<0.05) (Fig. 1B and 2B). From the point of view at decreasing rate of the PCVs of the both groups, no difference was found between the rates in ZD and DR group rats during the period of protozoan proliferations (until 10th days PI), but the recovery of the rate in the ZD group delayed remarkably after the protozoa disappeared from their blood smears (Fig. 2C). All the five rats of the DR group survived over 14th day PI, but the three rats of ZD group died at the 8th, 11th, and 14th day PI, respectively (Fig. 2D).

Both in the experiments I and II, all the ZA, DR, and ZD rats had swollen spleens at the time of autopsy (data not shown). In addition, thymic atrophy was marked in the ZD group rats in both experiments (Table 1). Thymus/body weight ratio of 1.73 (1.27–2.19) × 10–3 in the ZD group was significantly lower than that of 2.25 (2.11–3.39) × 10–3 in the ZA group in the experiment I (p<0.05). The ratio of 1.43 (1.22–1.64) × 10–3 in the ZD group was also significantly lower than that of 2.49 (2.09–2.89) × 10–3 in the DR group in the experiment II (p<0.05).

Zinc is an essential micronutrient for growth and development in humans and animals [5]. It is reported that the deficiency of the zinc induces various kinds of disorders such as delay of the growth and neuropathy, and reduce the resistance to some infectious diseases [5]. Babesiosis of animals and humans has been known to cause anemia, jaundice, and excretion of the hemoglobinuria [14]. It is reported that the zinc deficient person becomes susceptible to the infection of *Plasmodium falciparum*, resembling Babesia parasite [11, 12], while zinc supplementation to children in Papua New Guinea reduces morbidity to this protozoal infection [21]. However, there are no reports that are studied on the effect of zinc to babesiosis so far. In this study, we experimentally created zinc-deficient rats and examined the influences of the infection with *B. rodhaini* to the rats.

Zinc deficiency made worse parasitemia, PCV, and mortality of the rats infected with *B. rodhaini* in both experiments I and II. The average weight of the ZA rats showed more than a double of that of the ZD rats during *B. rodhaini* infection. It has been already reported that chronic zinc deficiency causes neuropathy, dysesthesia, anorexia, dysosmia, dysgeusia, and then decrease of amount of food ingested decreases resulting body weight loss [13]. In the experiment II, according to our previous knowledge (unpublished data), five rats were restricted to feed 7 g of ZA diet per day and others fed 10 g of ZD diet to make the rat weight even between in both groups. The ZA rats completely ate 7 g of diet every day, but the ZD rats left about 3 g of diet, i.e. they ate about 7 g of diet every day throughout the experimental period. As a result, the difference was hardly found in the average body weight of both groups of the rats at the time of *B. rodhaini* challenge infection. The restore of the PCV accompanied with the amelioration of parasitemia in 12th–14th day PI was slower in the ZD rats than in the ZA and DR rats in both experiments I and II. We thought that delay of recovery from the anemia in the ZD rats induced multiple organ failure, resulting higher mortality of the rats than the ZA and DR rats.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Body weight before infection (g)</th>
<th>Body weight after infection (g)</th>
<th>Thymus weight (g)</th>
<th>Thymus/BODY weight (× 10–3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ZA</td>
<td>210.38 ± 18.13</td>
<td>248.38 ± 16.70</td>
<td>0.56 ± 0.05</td>
<td>2.25 ± 0.14</td>
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<tr>
<td></td>
<td>ZD</td>
<td>87.20 ± 7.30</td>
<td>106.50 ± 11.47</td>
<td>0.19 ± 0.06</td>
<td>1.73 ± 0.46</td>
</tr>
<tr>
<td>II</td>
<td>DR</td>
<td>119.84 ± 2.82</td>
<td>124.98 ± 6.84</td>
<td>0.31 ± 0.05</td>
<td>2.49 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>ZD</td>
<td>103.73 ± 8.57</td>
<td>102.16 ± 10.32</td>
<td>0.15 ± 0.01</td>
<td>1.43 ± 0.21</td>
</tr>
</tbody>
</table>

a) Zinc-adequate group, b) Zinc-deficient group, c) Diet-restricted group.

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Influence of Zinc (−) to *B. Rodhaini* Infection
The atrophy of thymuses in the ZD rats was conspicuous compared with the ZA rats in both experiments I and II. Rat thymic atrophy by zinc deficiency has been also found in the previous studies [13, 17]. Thymic atrophy inhibits maturation of the T cell and/or B cell in acquired immunity and macrophages in innate immunity are considered to be reduced in zinc-deficient animals [20]. It is thought that resistance against infectious diseases is inhibited by zinc deficiency, and thymic atrophy by zinc deficiency impairs overall immune function and resistance to the infection with B. rodhaini.

In spite of the situation that the DR rats which were fed restricted amount of diet would be received nutritional stress, the rats survived without death during the infection with B. rodhaini. On the other hand, it was obviously thought that the death of 3 of 5 ZD rats was caused by their reduced immune functions. The hematopoietic function of 3rd- and 8th-week rats under zinc-deficient reduced in PCV from 50% to 40% approximately in previous [13] and present studies. We speculated that the ZD rats slowly recovered from anemia than the ZA and DR rats in the experiments I and II, because both immune and hematopoietic functions of the rats reduced concurrently. Although the ZD rats took less amount of zinc than the ZA and DR rats, the parasitemia of the ZD rats became bastardized than that of ZA and DR rats. In other words, the proliferation in the ZD rats was superior to that in the ZA and DR rats. It was supposed that the zinc-deficiency of the hosts did not directly affect to the protozoan proliferation.

All the rats which survived until 21st day PI in the experiments I and II showed splenic swelling because of babesial infection. However, no difference was observed in the splenic weight between in the ZA or DR and ZD rats. It was thought to be consequent that the spleen processed B. rodhaini infected erythrocytes.

In this examination, the following facts in the ZD rats confirmed (1) reduction of basal metabolism induced by live weight gain, (2) reduction of immunity induced by thymus atrophy, and (3) reduction of hematopoietic system meant by reduction of PCV. The zinc deficiency in the B. rodhaini infected rat acted on the hemodynamics and mortality in the rats exacerbated by the infection with B. rodhaini. The findings of this study would also supply the important fundamental information for the other hemocytotoxonoses, such as malaria and loucocytozoosis, under the zinc deficient human and animals.

REFERENCES