Effects of Se deficiency on serum histamine concentration and the expression of histamine H₂ receptor in the jejunum of chickens

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Abstract

The present study was designed to investigate the influence of Se deficiency on serum histamine concentration and the expression of histamine receptor in the jejunum of chickens. Forty neonatal chickens were randomly divided into two groups. Experimental chickens were fed a low-Se diet (0.034 mg/kg), whereas chickens in the control group were fed a diet with a Se level of 0.229 mg/kg. Ten chickens were sacrificed on days 30, 45, 60 and 75. Blood and jejunum samples were collected. Histamine concentration in the jejunum was measured by ELISA, the jejunal mast cell (MC) ultrastructure was studied by transmission electron microscopy, and the expression level of histamine H₂ receptor (H₂R) mRNA in the jejunum was examined using real-time PCR. Results: The jejunal histamine concentration in chickens fed the low-Se diet was significantly higher than that in the control group (P < 0.01). Se deficiency induced degranulation of MC in the jejunum of chickens in the low-Se diet group; their cytoplasm was filled with fused granules and vacuoles. The expression level of jejunal H₂R mRNA in chickens fed the low-Se diet was also significantly higher than that in the control group (P<0.01). The results obtained suggest that Se deficiency stimulates MC degranulation and release of histamine, binding H₂R promotes both regulation of digestion and cell proliferation while protects the jejunum from injury induced by Se deficiency.

Key words: selenium deficiency, histamine, histamine H₂ receptor, mast cell, chicken

Introduction

Selenium (Se) is one of the most important antioxidants protecting animal cells against oxidative injury. The biological activity of Se is attained by the maintenance of the activity of glutathione peroxidases (GSH-Px). GSH-Px scavenge reactive oxygen species and play a significant role in defending cells against oxidative stress. Lipid peroxidation and protein denaturation intra-cellular which caused by Se deficiency resulted in injury and apoptosis (Zhu et al. 1996, Bozkaya et al. 2001, Saito et al. 2003). Mast cells
(MC) are widely distributed throughout many different tissues and organs, and play a critical role in inflammatory and immediate allergic reactions (Church and Levi-Schaffer 1997, Metcalfe et al. 1997). MC are important effective cells in the mucous membrane immune system. It has been reported that the activity of GSH-Px descended in rats, however, histamine secreted by MC increased (Gushchin et al. 1990). The hyperoxide in gastric mucosa and 5-hydroxytryptamine (5-HT) in the blood serum of rats which was under the action of compound 48/80 (activator of MC) increased (Ohta et al. 1997), and the activity of GSH-Px degraded. The study above showed that the ischemia caused by MC degranulation resulted in the injury of gastric mucosa.

Chicken Se deficiency leads to refractoriness diarrhea, because of the fact that low Se causes smooth muscle and gastrointestinal mucous membrane injury. When the addition of Se to feeds is lower than 5mg/kg, it'll cause the reduction of intestinal villi and the epithelial cell atrophy (Wang et al. 2003, Wang et al. 2009). Intestinal tract injury concerned with Se deficiency caused oxidative damage, however, intestine disfunction possesses dependency of MC (Berin et al. 1998, Santos et al. 2001, Yang et al. 2001). Furthermore, Se deficiency made MC oxidative damaged and influenced the function of intestine. No informations for MC states and their role in chicken Se deficiency have ever been reported so far. Consequently, in the present study, the levels of jejunal histamine and histamine H2 receptor (H2R) mRNA expression were measured in Se-deficient and control groups to determine the mechanism by which histamine causes jejunum damage in chickens with Se deficiency.

**Materials and Methods**

**Experimental animals**

Forty 1-day-old chickens were selected and randomly divided into 2 groups (control and trial), each with 20 chickens. The trial groups was fed with Se deficiency diet (0.032 mg/kg) (Muller et al. 2002) consisting of 5% remark (no added Se feed), 63.5% corn, 28.0% soybean meal, and 3.5% wheat bran grown in low-Se areas (Longjiang County in Heilongjiang province, China). According the Standard of Elements added to Animal diet in the People’s Republic of China, 0.44 mg/kg Na2SeO3 98% was added to the fed for the control group, corresponding to a final Se content of 0.229 mg/kg.

**Sample collection**

Five chickens in each group were randomly sacrificed at days 30, 45, 60 and 75. Blood was collected from chicken heart, serum was separated (3000 rpm effERENCE 10 min), and preserved at -20°C. Jejunum tissue pieces were stored in 2.5% glutaraldehyde (pH 7.4), and the other jejunum samples were stored at -70°C.

**Determination of Se in serum and histamine concentration in jejunum**

The concentration of Se in serum and forage was detected using a PEAA800 flame atomic absorption spectrophotometer (Perkin Elmer, Fremont, CA, United States) (Muller et al. 2002). The concentration of histamine was measured using ELISA kit (USCN LIFE, USA), according to the manufacturer’s instructions through a microplate reader at 450 nm. Standard reagents were employed to construct a calibration curve. The sample OD value and the calibration curve were used to calculate the product concentrations.

**Transmission electron microscopy**

Jejunum samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 2 h at 4°C, and then 1 mm × 2 mm samples were removed from the fixed tissue (Lazaru and Staf10w 1998). The tissue samples were rinsed 3 times in sucrose phosphate buffer for 10 min, postfixed with 1% osmic acid for 1 h at 4°C, dehydrated by a graded ethanol series, washed with propylene oxide and then embedded in Epon. Semi-thin sections (1 μm) were stained with toluidine blue (pH 8.5) were observed by a microscope, selecting the regions in which many MCs were present (Nishi and Takesue 1978). LKB ultrathin sections (0.05 μm thick) were preheated with lead citrate or uranyl acetate and lead citrate (double staining) and then examined using an electron microscope (JEM 100-CX, Japan).

**Real-time quantitative PCR (Q-PCR)**

Primers were designed using Primer Premier 5.0 based on the gallus sequences. Table 1 presents the primer sequences, expected product lengths and accession numbers in GenBank. Total RNA was extracted from endometrium samples with TRIzol reagent (Invitrogen, USA), and 1 μg of total RNA was
Table 1 The primer amplification purpose gene sequences and purpose fragment length.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer sequence</th>
<th>Reverse primer sequence</th>
<th>Fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2R</td>
<td>5’-GCACAGACCATCCCTCTA-3’</td>
<td>5’-CTTCCCATTCCTCATCTCCA-3’</td>
<td>192</td>
</tr>
<tr>
<td>β-actin</td>
<td>5’-ACCGCAAATGCTTCTTAAACC-3’</td>
<td>5’-CCAATCTCGTCTTGTGGTATGC-3’</td>
<td>93</td>
</tr>
</tbody>
</table>

subjected to reverse transcription to form cDNA with transcriptase according to the manufacturer’s instructions (Invitrogen, USA). The mRNA expression of the H2R and β-actin were quantified by real-time PCR with a LightCycler (ABI StepOne, USA) using a commercial kit (TaKaRa, Japan). The values were normalized using β-actin as the internal standard (Pfaffl et al. 2003).

Statistical analysis

Statistical analysis of all data was performed using SAS procedures (SAS Institute Inc., Cary, NC). The effect of Se deficiency on mRNA levels in chickens was assessed by Mantel-Haenszel chi square test. All values were expressed as means ± SD, and P<0.05 was considered a significant difference.

Results

Serum Se concentration

Se serum concentration in the Se-deficient group was significantly lower than that in the control group on days 30 and 45 (P<0.05) 60 and 70 (P<0.01). In the Se-deficient group, the concentration on day 45 was significantly different from the levels on days 75, 60, and 30 (P<0.05). The level of Se declined gradually in the Se-deficient group and was considerably lower than that in the control group during the entire experimental period. The differences in the Se level over time were highly significant (P<0.01), exhibiting a time-effect relation (Table 2).

Table 2. Result of serum Se.

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Se-deficient group (μg/mL)</th>
<th>Control group (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.199 ± 0.031&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.267 ±0.019&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>45</td>
<td>0.153 ± 0.020&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.248 ±0.018&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>0.093 ± 0.022&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.255 ±0.054&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>0.049 ± 0.012&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.287 ±0.036&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at 5% level of significance (P>0.05), and the capital letters denote highly significant differences (P<0.01).

MC ultrastructure

The results were of different size, homogeneously distributed and the round/oval granule were obtained in the control group (Fig. 1). Se deficiency induced MC degranulation in the jejunum in the low-Se diet group. MC granules mingled freely with the cell membrane, and the cytoplasm was filled with fused granules and vacuoles (Fig. 2).

Histamine concentration in the jejunum

The level of histamine in the Se-deficient group was significantly higher than that in the control group during the entire experimental period (Table 3). Se deficiency resulted in a highly significant difference in the jejunum histamine concentration (P<0.01).

Table 3. Result of jejunal histamine.

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Se-deficient group (ng/g)</th>
<th>Control group (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>5.950 ± 0.569&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.329 ±0.257&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>45</td>
<td>6.792 ± 0.392&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.886 ±0.172&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>10.358 ± 0.911&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.066 ±0.185&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>11.229 ± 0.592&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.865 ±0.163&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at 5% level of significance (P>0.05), and the capital letters denote highly significant differences (P<0.01).

Level of H2R mRNA expression

The level of H2R mRNA expression in the Se-deficient group was significantly higher than that in the control group (Fig. 3). The difference between the
Fig. 1. MC ultrastructure in the control group (×25000). Differently sized, homogeneously distributed, round/oval granule were found in the MC. A. MC nucleus, B. MC grains.

Fig. 2. MC ultrastructure in Se-deficient group (×25000). MC showed an excess of heterochromatin, chromatin condensation, larger grains, and denser cytoplasm and vacuoles. A. MC nucleus, C. MC grains vacuole.

Fig. 3. H₂R mRNA expression. The H₂R mRNA expression level in the Se-deficient group was significantly higher than that in the control group (P<0.01).

Discussion

These cells are the major source of granule-stored histamine. Histidine decarboxylase synthesises histamine in mammals. Histamine is one of the most important mediators involved in various physiological and pathological conditions (Church and Clough 1999), including neurotransmission and numerous brain functions, secretion of pituitary hormones, regulation of gastrointestinal and circulatory functions and...
inflammatory reactions, such as gastric acid production, intestinal motility, and mucosal ion secretion (Fujitani et al. 1996). The physiological effects of histamine are mediated through membrane histamine receptors (Entschladen et al. 2004, Akdis and Simons 2006). Se deficiency weakened the activity of GSH-Px decrease, and produced excessive free radicals, which led to the stringently oxidative state and disorganization of membrane structure. Decreased activity of GSH-Px caused MC deregulation (Gushchin et al. 1990, Ohta et al. 1997). It was voted by the jejuna MC deregulation in this study. Simultaneously, excessively released histamine resulted in digestive disorders (Katzkal 2000).

H2R is coupled positively to adenyly cyclase. Activation of these receptors also leads to mainly excitatory effects through blockade of Ca2+-dependent K+ channels and modulation of the hyperpolarization activated cation channel (Brown et al. 2001), leading to the increase in cAMP level. In turn, cAMP activates protein kinase A (PKA) which can phosphorylate various proteins that are responsible for cAMP response element binding CREB-activation and the Ca2+-dependent potassium conductance inhibition. Those proteins participate the process of regulating the gastric acid secretion, decreasing blood pressure, relaxing airway and vascular smooth muscle, adjusting fluid balance and hormonal secretion. Furthermore, stimulation of gastrointestinal secretion is the specific function of H2R. Histamine released by MC is paracrine mitogen, the effect of which is mediated by H2R (Franzen and Norrby 1980). Along with development of molecular biology, histamine has been found to regulate cell proliferation (Mitsuhashi et al. 1989, Hill 1990, Delvalle et al. 1992), be associated with cAMP, inositol triphosphate (IP3), Ca2+ signal transducting system. H2 promotes cell proliferation referred to c-fos genetic transcription (Shayo et al. 1997, Wang et al. 1997).

Therefore, histamine and H2R play a regulatory role in maintaining function of intestines and promoting enterocyte proliferation. Se deficiency increases jejunal H2R mRNA expression level, indicating that H2R protects the jejenum, thus avoiding injury during Se deficiency.

Acknowledgments

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References

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